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Biorefineries

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Editors

Biorefineries

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Biorefineries: A Short Introduction

Kurt Wagemann and Nils Tippkötter

Abstract The terms bioeconomy and biorefineries are used for a variety of processes and developments. This short introduction is intended to provide a delimitation and clarification of the terminology as well as a classification of current biorefinery concepts. The basic process diagrams of the most important biorefinery types are shown.

Keywords Bioeconomy, Biorefinery definitions, Introduction, Process schemes, Renewable resources

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1 Biorefineries: Definition

The basic concept of biorefinery is rather simple: a biorefinery plant uses some kind of biomass as feedstock which is converted – preferably completely – into a range of commercial products.

This concept is an analogy of petroleum-based refineries. Their dominance over the supply of today's fuels and chemicals relies on several factors: excellent availability of petroleum, its relatively low selling price, and the use of efficient process schemes developed over the decades following World War II. Despite the fact that petroleum from different regions differs in character and composition of the hundreds of its constituent components, more or less all petrorefineries can be described by one general scheme. In this scheme, a rectification column acts as the central processing unit, splitting the petroleum inlet into different intermediate streams. Other chemical conversion units, such as fluid catalytic crackers or catalytic reformers, modify the molecular components of some of these streams. Their aim is the greatest possible conversion of the petroleum feed into fuels such as gasoline, diesel, and jet fuel. Only about 10% of the output of these refineries is related to the production of chemicals, lubricants, or other products such as asphalt. Chemicals production depends to a very large extent on the steam cracking of naphtha, one of the above-mentioned intermediate streams, for which the conversion to fuels would require an uneconomic conversion effort. The majority of petrorefineries do not have a steamcracker available so instead they commercialize the naphtha to the petrochemicals sector. The annual output of modern refineries can exceed 10 million tons. This is only possible because of the simple and cheap design of petroleum transport from the drilling hole to the refineries, most often accomplished via pipelines or very large ocean vessels when necessary (Figs. 1 and 2).

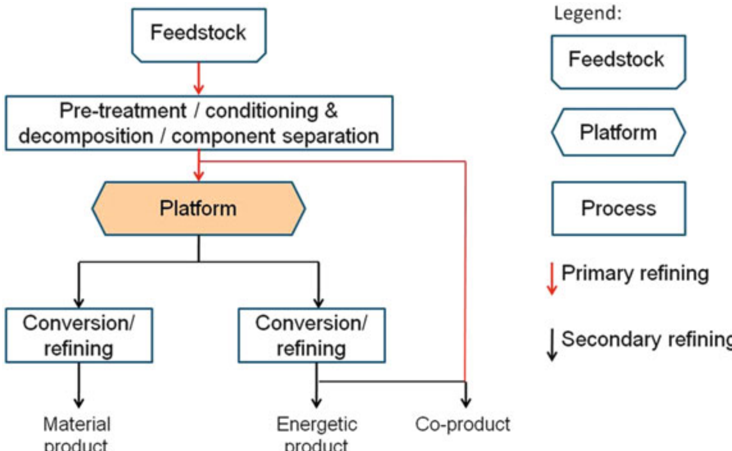

Fig. 1 General biorefinery scheme

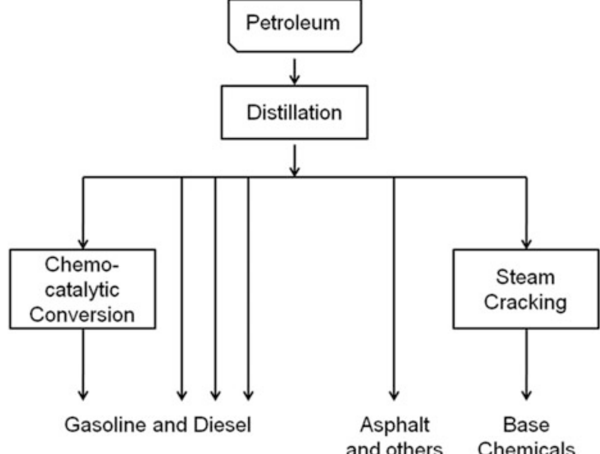

Fig. 2 General petroleum refinery scheme

The framework for biorefineries is very different to those of traditional refineries (see the chapter "Logistics of Lignocellulosic Feedstocks: Preprocessing as a Preferable Option"). It is not a set of highly productive point sources such as the drilling holes but large areas where the feedstock is produced and must be collected. This makes logistics much more challenging.

Further challenges are related to seasonal availability (and in some cases, the quality) of the feedstock. Economically viable biorefinery concepts must be able to overcome these obstacles.

This book aims to provide insight into the different biorefinery schemes, their statuses of realization, proposed strategies of implementation, and recent R&D advancements therein. Being part of the "Trends in Biotechnology" series, the rationale for this publication is obvious. Whereas in the case of petroleum refineries, all processes are of pure chemical origin, in the case of biorefineries, biotechnological processes are dominant.

2 Biorefineries: Definitions and Classifications

The first precise definition of a biorefinery was provided in 2004 by the US Department of Energy (DOE):

> A biorefinery is an overall concept of a processing plant where biomass feedstocks are converted and extracted into a spectrum of valuable products.

The German Biorefineries Roadmap published in 2012 developed a much more comprehensive description:

> A biorefinery is characterized by an explicitly integrative, multifunctional overall concept that uses biomass as a diverse source of raw materials for the sustainable generation of a spectrum of different intermediates and products (chemicals, materials, bioenergy/biofuels) allowing the fullest possible use of all raw material components. The co-products can also be food and/or feed. These objectives necessitate the integration of a range of different methods and technologies.

For the classification of the biorefineries one has to be aware that different approaches exist.

The classification can rely on

- The *raw material* (e.g., cereal crops biorefinery, oil biorefinery, grass biorefinery, straw biorefinery, wood biorefinery, algae biorefinery)
- The *process* (e.g., thermochemical biorefinery, biotechnology biorefinery)
- The *product(s)* (e.g., bio-ethanol biorefinery, fuel biorefinery)
- The *intermediate* (e.g., synthesis gas biorefinery, lignocellulosic biorefinery, vegetable oil biorefinery)

In the processing schemes one can distinguish two sections: primary and secondary refining. *Primary refining* involves the pretreatment of biomass and separation into useful intermediates. In *secondary refining*, those intermediates created from the primary refinement process – in the further description defined as platforms – are chemically or biotechnologically converted to either semi-finished or finished products (chemicals, polymers and fuels).

Sometimes an additional distinction is made between bottom up and top down biorefineries; this refers to the practical realization of a biorefinery. In the case of a *bottom up* approach, established biomass conversion facilities increase their traditional production portfolio. This can be realized either by extracting further substances from the feedstock, by utilizing or extracting waste streams, or by forward integration, when traditional products are further processed creating new products. In the case of a *top down* approach, a new, independent, highly integrated scheme with its own logistics and proprietary conversion processes is established.

3 Biorefineries: Different Types

According to the classification scheme based on intermediates, so-called "platforms", the following major types of biorefineries can be distinguished:

- Sugar biorefinery
- Starch biorefinery
- Vegetable oil biorefinery
- Algal lipid biorefinery
- Lignocellulosic biorefinery
- Synthesis gas biorefinery

In the following, each is briefly described – details can be found in the respective chapters of this volume.

3.1 Sugar Biorefinery

Sucrose, colloquially known as sugar, is the mainstay of a sugar biorefinery. There are two major sugar-producing plants providing the feedstock: sugar cane and sugar beet. The processes applied in the primary refining stage are juice production by pressing, juice purification, juice thickening, and crystallization (Fig. 3). In the case of sugar beet, the press cake is used as animal feed. In the case of sugar cane, the residues, called bagasse, are usually burned to produce process steam and electrical power.

For the secondary refining there are two options:

- Sucrose can be inverted and the resulting fructose and glucose separated and commercialized directly or further converted
- The juice itself, or molasses, the by-product of the crystallization, can be used as feedstock for fermentation; in most cases the product is bioethanol

The by-products are carbon dioxide from the fermentation and stillage from the distillation. The first can be captured and sold, for example, to the beverage industry; the second further can be processed and commercialized as animal feed. There are many plants in existence worldwide, as this is the traditional business concept of the sugar and bioethanol industry. However, there are very few examples of the generation of other products. There is one exception – the conversion of ethanol to ethylene for the synthesis of polyethylene.

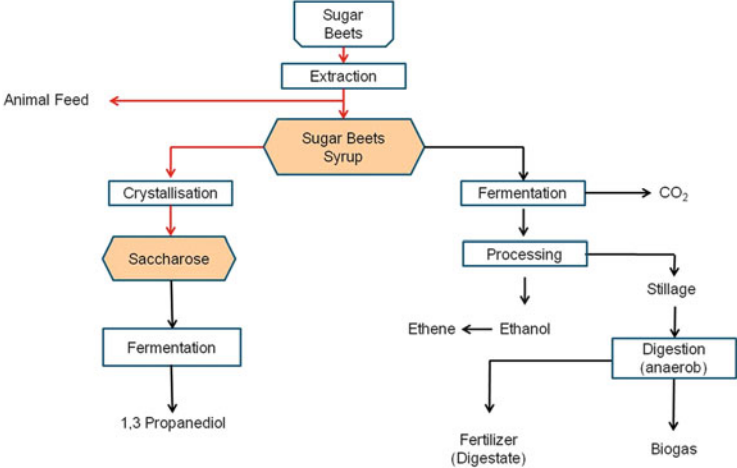

Fig. 3 Sugar biorefinery

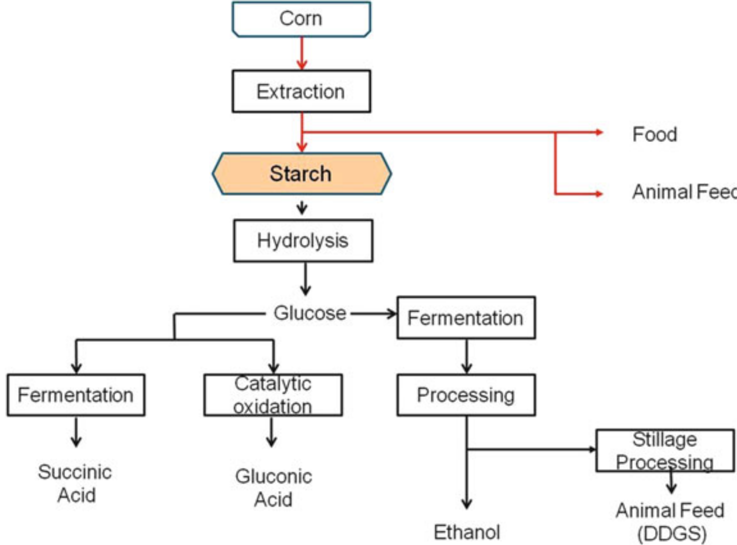

Fig. 4 Starch biorefinery

3.2 Starch Biorefinery

Major feedstocks for starch biorefineries include cereal crops such as corn, wheat, and rice, as well as potatoes and cassava. In primary refining, and after mechanical treatment, the starch is suspended in water and fibers and proteins separated; the latter by-products are usually used for feed production (Fig. 4).

The resulting starch slurry is cleaned. After drying, the pure native starch can be commercialized either directly or after chemical or physical modification for the food industry as well as other sectors such as paper or cosmetics producers.

Other options arise from the hydrolysis of the starch, resulting in dextrose or glucose solutions. They can be input either for bioethanol production or for other fermentation products such as lactic or succinic acid.

Starch production is a well-established industrial sector. There are several examples where starch producers extend their value chain downstream.

3.3 Vegetable Oil Biorefinery

The secondary refining scheme of a vegetable oil biorefinery is more or less identical to the production scheme of classical large-scale oleo-chemical plants (Fig. 5). The most important processes are the hydrolysis and the transesterification of the triglycerides and further processing of the resulting intermediates, fatty acids,

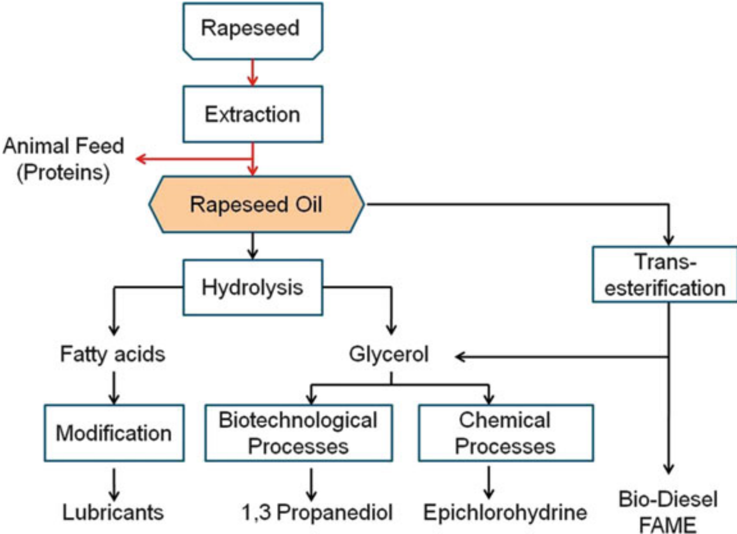

Fig. 5 Vegetable oil biorefinery

esters, and glycerol. In addition, chemical or biotechnological processes for the conversion of glycerol might be added.

The primary refining consists of the treatment of oil seeds and oil fruits by shredding, pressing, and extracting, followed by purification of the crude oil. The press cake is usually used for feed production.

3.4 Algal Lipid Biorefinery

From a general point of view, the algal oil biorefinery scheme is very similar to the scheme of a vegetable oil biorefinery as the secondary refining can be identical; additionally, valuables such as carotenoids can be extracted.

The primary refining to triglycerides, however, is completely different. The production organisms are microalgae or cyanobacteria which are cultivated either in open ponds or in closed photo-bioreactors. The downstream processing differs from that of oil plants because materials with high water content have to be handled. Centrifugation, filtration, or flocculation for the separation of water, disruption of the cells, and extraction are major steps (Fig. 6).

Large scale (open pond) cultivation plants of microalgae have existed for decades. They have usually been devoted to a single product such as beta-carotene, but no integrated biorefinery approach had been implemented. A huge number of projects and start-up companies have been established in recent years in the context of biofuels production. Large-scale breakthroughs are not in sight at present, primarily for reasons of economy and energy balance.

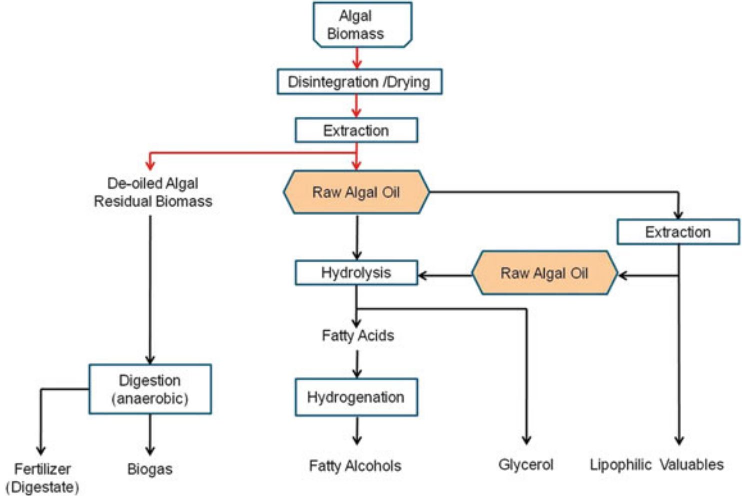

Fig. 6 Algal lipid biorefinery

3.5 Lignocellulosic Biorefineries

The high priority given to lignocellulosic biorefineries in research and development, as well as in political discussion, is related to the discussion of "fuels vs food and feed". Although other biorefinery schemes (with the exception of algal biorefineries) rely on feedstocks provided by agriculture, such biorefineries make use of lignocellulosic materials such as wood and wood residues from forestry, and straw as residues from agricultural processes. In some cases, other residues from food production or landscaping may be used as well. Two different schemes must be distinguished. There are those processes that primarily or exclusively generate bioethanol and those which try to valorize the three constituents of lignocelluloses, lignin, cellulose, and hemicelluloses, individually.

The first scheme is equivalent to the scheme of a second-generation bioethanol plant: straw or wood is pretreated before hydrolyzing cellulose and hemicelluloses either by the application of mineral acids or enzymes. The monomeric sugar solutions are fermented to produce bioethanol, which is separated and purified by thermal processing. The remaining lignin is burned for steam and electric power generation (Fig. 7).

The second scheme uses the solvation power of liquids, that is, ethanol or acetic acid. As in the classical pulping processes, cellulose remains undissolved, as in the first fraction of the separation scheme; different methods are used to separate consecutively lignin and hemicelluloses, the monomeric sugars generated in the process. Usually these sugars, as well as glucose from the hydrolysis of the cellulose, are used for fermentation (Fig. 8).

There is a subtopic: the attempts to use green – not yet completely lignified – biomass such as grass. The scientific idea behind such concepts is related to the composition of green grass and other plants. In contrast to lignified biomass such as straw, green plants contain rather high amounts of proteins. Most schemes try to extract these proteins for use as food additive or in cosmetics. Another concept

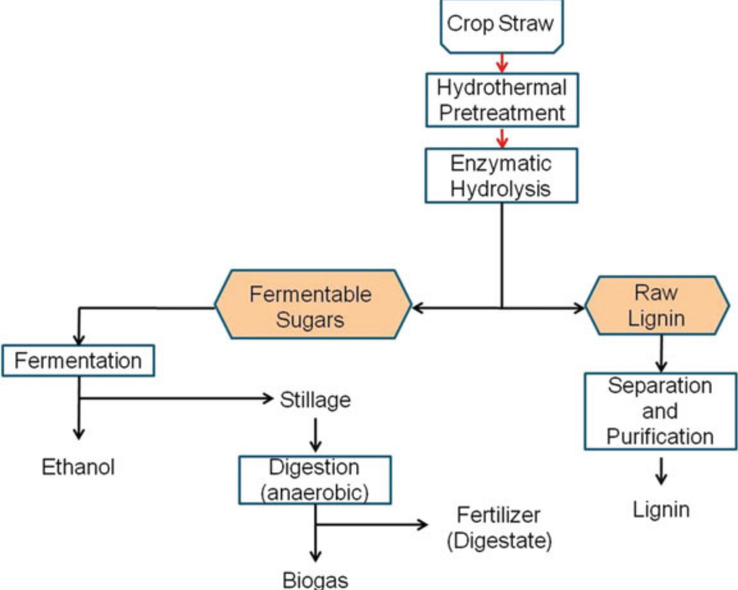

Fig. 7 Lignocellulosic biorefinery – 2G bioethanol plant

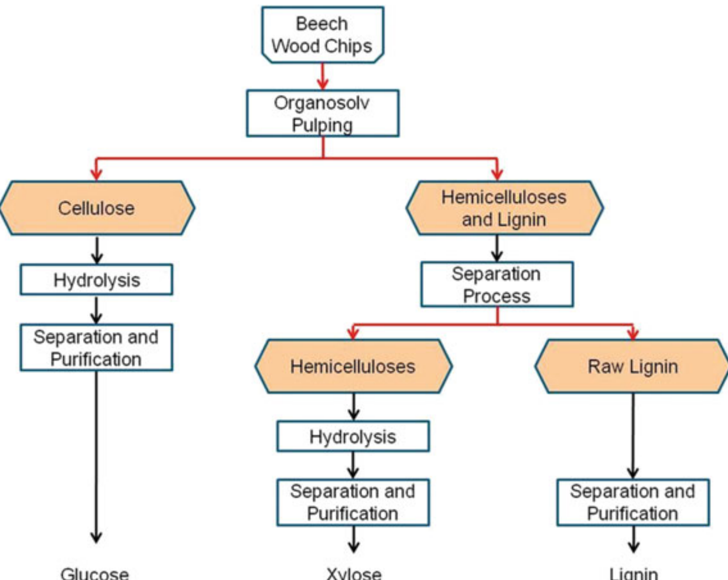

Fig. 8 Lignocellulosic biorefinery – chemicals focused

starts with the isolation of the fiber fraction. Both concepts are connected through their use of all residues in a biogas plant. The third scheme is closely related to the classical production of silage as animal feed: Pressing silage from green plants delivers a juice wherefrom lactic acid and amino acids can be isolated.

3.6 Synthesis Gas Biorefinery

The route to the platform syngas – a mixture of carbon monoxide and hydrogen – relies exclusively on classical high temperature chemical engineering. At high temperatures the equilibrium between carbon- and hydrogen-containing materials (the feedstock) together with oxygen (the gasification agent) on one hand, and syngas on the other, lies on the platform's side. Processes originally developed for coal gasification can be applied using dry or torrefied biomass. Others are very specific to biomass: the gasification of black liquor from the production of pulp, the two-step route via pyrolysis of biomass, and the subsequent gasification of pyrolysis oil and coke.

For the generation of products, there is a multitude of processes based on syngas, applied in the context of coal or natural gas conversion. The two major routes are the Fischer–Tropsch process for the production of hydrocarbons and the methanol production. Methanol itself is the starting point of a whole product tree: hydrocarbons, ethylene, propylene, aromatics, and others. A biotechnological route recently received attention as it offers a great variety of functionalized products: the fermentation of syngas (Fig. 9).

The strength of this concept is the fact that different lignocellulosic materials can be used as feedstock and all components are utilized. On the other hand, for reasons of economy, large-scale plants are necessary.

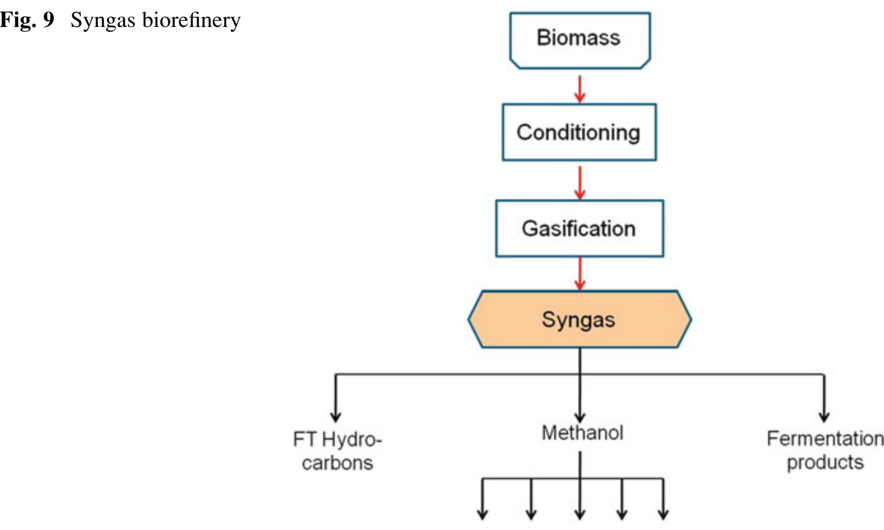

Fig. 9 Syngas biorefinery

3.7 Others

In principle, one could define further biorefinery types as follows:

- Pyrolysis oil biorefinery – extracting (aromatic) compounds
- Biogas biorefinery – converting methane (produced by the traditional biomass digestion)
- Biochar biorefinery – making use of the products coming out of the hydrothermal carbonization of biomass

For the first two examples, the relevant chemical and biotechnological process options are described in this volume. De facto, the old Degussa process for the preparation of charcoal with methanol and acetic acid as additional products could be taken as a kind of relative of the third example.

4 Concluding Remarks

Biorefineries promise the efficient and complete use of biomass for the generation of chemicals, polymers, and fuels. However, at the end of the day a detailed analysis of each scheme must prove whether this promise of a high degree of sustainability is valid. That being said, the sustainability analysis of biorefineries is an important subject.

This book takes a detailed look at the key elements of the resources available for biotechnological processing (chapters “Agriculture”, “Wood Processing Residues” and “Logistics of Lignocellulosic Feedstocks”) and the biorefinery classes currently available (chapters “Vegetable Oil-Biorefinery”, “From Current Algae Products to Future”, “Sugarcane-Biorefinery”, “Starch Biorefinery Enzymes”, “Organosolv Processes”, “Lignocellulose-Biorefineries”, “Synthesis Gas Biorefinery”, “Syngas-Utilization”, “Anaerobic Digestion” and “Pyrolysis Oil Biorefinery”). Special attention has been paid to represent chemical and biotechnological processing routes adequately. The third section of the book gives an overview of the most significant product groups that can be produced with biorefineries (chapters “Products Components: Alcohols”, “Biotechnological Production of Organic Acids from Renewable Resources”, “Hydrocarbons: Microbial hydrocarbon formation from biomass”, “Bioplastics” and “Biotechnological and Biochemical Utilization of Lignin”). In conclusion, a detailed insight into the critical aspect of sustainability is given. As known from the *Advances in Biochemical Engineering/Biotechnology* series, current aspects and future developments are in focus. Even traditional biorefinery concepts, such as the starch and oil processing, are ongoing subjects of research. New biocatalysts and biotechnological value-added processing routes are under development.

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Biomass Resources: Agriculture

Ingeborg N. Kluts, Marnix L. J. Brinkman, Sierk A. de Jong, and H. Martin Junginger

Abstract Bioenergy is the single largest source of renewable energy in the European Union (EU-28); of this, 14% was produced from agricultural feedstocks in 2012. This chapter provides an overview of the current use (for bioenergy) and future potential of agricultural feedstocks for (amongst others) biorefinery purposes in the European Union. The main application of these feedstocks is currently the production of biofuels for road transport. Biodiesel makes up 80% of the European biofuel production, mainly from rapeseed oil, and the remaining part is bioethanol from wheat and sugar beet. Dedicated woody and grassy crops (mainly miscanthus and switchgrass) are currently only used in very small quantities for heat and electricity generation. There is great potential for primary agricultural residues (mainly straw) but currently only part of this is for heat and electricity generation. Agricultural land currently in use for energy crop cultivation in the EU-28 is 4.4 Mio ha, although the land area technically available in 2030 is estimated to be 16–43 Mio ha, or 15–40% of the current arable land in the EU-28. There is, however, great uncertainty on the location and quality of that land. It is expected that woody and grassy crops together with primary agricultural residues should become more important as agricultural feedstocks.

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Keywords Agricultural feedstock, Energy crops, Energy potential, Primary agricultural residues, Straw

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1 Introduction

Two-thirds of renewable primary energy production in the European Union (EU-28) in 2012 was derived from biomass and renewable wastes [1]. In 2012, bioenergy accounted for 12.4, 4.1, and 5.3% of the renewable energy share in heat and cooling, electricity, and transport sectors, respectively [2]. The share of bioenergy produced from agricultural feedstocks is small compared to bioenergy produced from forestry feedstocks, but increased from approximately 7% in 2007 to 14% or 720 petajoule(PJ) in 2012 [3]. Agricultural feedstocks include conventional food crops such as rapeseed, wheat, and maize (i.e., first-generation feedstock), and crops specially cultivated for energy purposes, such as miscanthus, switchgrass, willow, and poplar (i.e., second-generation feedstock). In addition, agricultural residues in the form of straw, cuttings, and prunings are used for bioenergy production. Agricultural feedstocks are mostly used for the production of biofuels and biogas, whereas heat and electricity are mostly produced from forestry feedstocks, although straw and other crop residues are increasingly used as well [4, 5].

This chapter discusses the current use of agricultural feedstocks for bioenergy production and future agricultural potentials as feedstock for (amongst others) biorefineries. The chapter also considers constraints and focuses on the European Union.

This chapter is structured as follows. Section 2 covers the current use of agricultural feedstock in the EU, including energy crops (Sect. 2.1) and agricultural residues (Sect. 2.2). Section 3 focuses on the future potential in Europe. This section first gives an estimation of the land potentially available for energy crop cultivation (Sect. 3.1), and continues with the energy potential from this land and from agricultural residues (Sect. 3.2). A synthesis is provided in Sect. 4.

2 Current Bioenergy Production from Agricultural Feedstocks

2.1 Energy Crops

Currently, sugarcane, maize, oil palm, rapeseed, and soybean are globally the major crops for biofuel production [4]. Although, globally, bioethanol represents the largest share of biofuel production, biodiesel represents more than 80% of total biofuel production in Europe, mainly from rapeseed oil [6, 7]. Sugarcane and maize are the predominant crops for bioethanol production in Brazil and the USA, respectively, although wheat and sugar beet are mainly used in Europe for bioethanol production [4, 6].

European liquid biofuel production increased from 50 PJ in 2002 to 485 PJ in 2012, whereas biofuel gross consumption increased from 47 PJ in 2002 to 658 PJ in 2012 [1]. Hamelinck et al. [8] estimated the agricultural land within Europe required to meet the biofuel consumption in 2012 as approximately 4.4 Mio ha; this is 3.9% of the total arable land. An additional 3.5 Mio ha of agricultural land was required outside Europe to produce the biofuels consumed in the EU-28 in 2012. The authors consider the actual acreage required for biofuel production to be lower because conservative data were used for conversion efficiencies and yields [8].

Besides conventional crops, grassy and woody crops are used for bioenergy production. Currently, this only concerns small quantities, mainly for heat and electricity generation. A synthesis of different data sources by AEBIOM [6] shows approximately 0.16 Mio ha grassy energy crop cultivation in the EU-28 in 2014, of which 32% is switchgrass and 25% is miscanthus. Switchgrass is solely produced in Romania, whereas miscanthus is produced in various countries, including the United Kingdom (17,000 ha), Germany (15,000 ha), France (3,500 ha), and Ireland (2,200 ha). Countries with the highest cultivation of lignocellulosic energy crop cultivation are Romania, Germany, the United Kingdom, and Finland [6].

2.2 Primary Agricultural Residues

Primary agricultural residues include crop residues remaining in the field after harvest, whereas secondary agricultural residues are generated from processing the primary crops. The most important primary agricultural residue in Europe is wheat straw followed by barley straw and maize stover [9]. Conventional uses for straw include animal feed and bedding, mushroom cultivation, surface mulching in horticulture, and industrial uses, such as in the pulp and paper industry [10]. Straw can also be used to produce bioenergy, including fuels, electricity and heat, and biochemicals.

Only part of primary crop residues is potentially available for energy or biorefineries. A certain proportion of the crop residues needs to be left on the field to maintain soil quality, prevent soil erosion, and improve water retention [11]. A sustainable removal rate should therefore be considered when removing crop residues from the field. This removal rate is site-specific and is affected by crop type, farming practices, harvesting equipment, and local soil and climate conditions [9], and is estimated to be in the range of 30–70% [9, 11–16]. The yearly use of crop residues for non-energypurposes, expressed in dry matter (dm), isestimated to be around 28 Mt_{dm} in Europe (also excluding use for soil quality maintenance) [9].

Excluding the crop residues used for soil incorporation and other competitive uses, currently approximately 53–204 Mt_{dm}/year crop residues are available in Europe for energy or biorefinery purposes, equalling 960–3,700 PJ/year [5, 9, 14, 15, 17]. However, crop residue availability varies greatly from year to year [9]. Countries with high crop residue availability are France, Germany, Romania, Spain, Italy, Hungary, and Poland. The agricultural sector is large in these countries and the existing demand for crop residues is relatively low [9, 15].

Across Europe, straw is used to produce heat, power, and, more recently, biofuels. Denmark, the frontrunner in Europe, uses approximately 1.8 million tons of straw each year for energy purposes [18]. In recent years, multiple biofuel plants converting straw to ethanol have come online. European plants include the Abengoa plant in Salamanca, Spain (35,000 tonnes/year input), the Inbicon plant in Kalundborg, Denmark (30,000 tonnes/year input), Beta Renewables/Chemtex in Crescentino (180,000 tonnes/year input), and Chempolis, Oulu, Finland (25,000 tonnes/year input) [10, 19], but not all of these plants are yet operating at full capacity.

Several barriers still exist to extensive mobilization of straw for bioenergy purposes. Barriers include immature markets and lack of market information, competition with traditional uses of straw, lack of infrastructure, lack of experience with straw extraction and mobilization, and varying straw quality and availability over time because of changing weather conditions [10]. Moreover, average straw prices tend to be higher than forestry residue prices (on a mass and energy basis) [20]. Large geographical differences between straw prices also exist as prices are mainly determined by local scarcity [5]. In 2014, straw prices ranged from 14 €/tonne in Lithuania to 169 €/tonne in Greece [21]. Transport costs of straw tend to be high because of the low energy density of the feedstock.

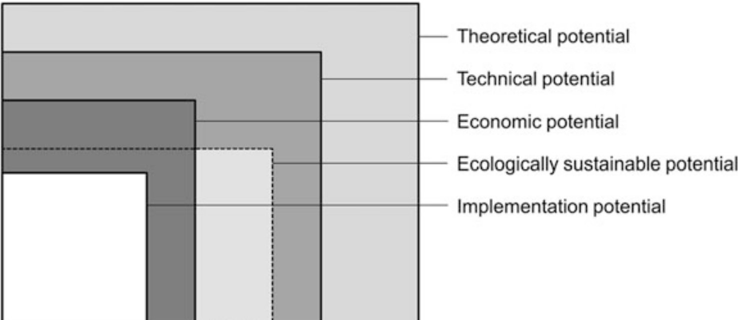

Fig. 1 Overlap between different potential types [23]

3 Future Potential of Agricultural Feedstocks

The sustainable potential from agriculture that could be utilized by, amongst others, biorefineries is constrained by the amount and suitability of the land available for energy[1] crop cultivation and various constraints related to, among others, available technologies, sustainability (e.g., greenhouse gas (GHG) emission mitigation targets, prevention of biodiversity loss), and market conditions defining economic profitability. A distinction between different types of biomass potentials is often made according to the type of constraints as shown in Fig. 1; see [22, 23]. The theoretical potential is defined as the maximum biomass supply constrained only by biophysical limits. The technical potential is the fraction of the theoretical potential available under current available technologies, and limited by other land uses including food, feed and fiber production, and urban areas. The ecologically sustainable potential is the technical potential further constrained by environmental criteria such as biodiversity conservation and soil and water preservation The share of the technical potential meeting certain economic criteria within given conditions is referred to as the market or economic potential. Some studies also estimate the implementation potential, the economic potential that can be implemented within a certain timeframe and socio-political framework.

3.1 Land Potential for Biomass Feedstock Production

Future land potentially available for energy crop cultivation is constrained by the land required for food, feed and fiber production, forests, biodiversity protection,

[1] As scientific literature mainly focuses specifically on the potential for *energy* crops, we also use this terminology throughout this chapter, although energy crops can also be used as feedstock for material/biorefinery purposes.

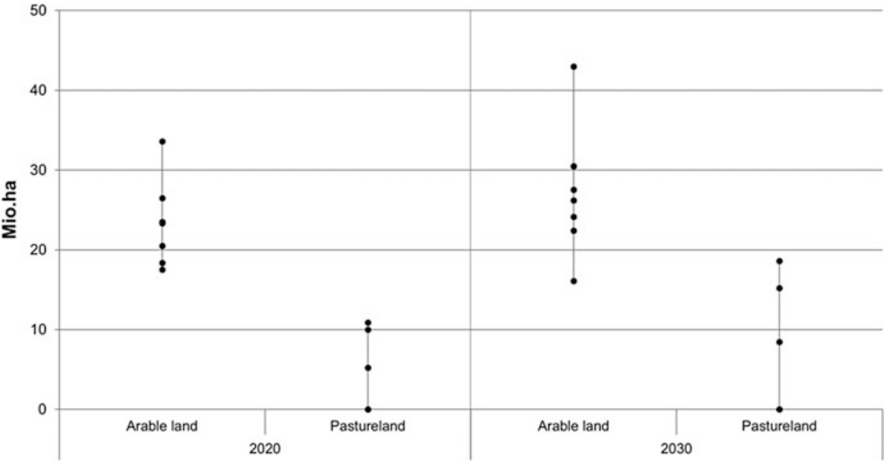

Fig. 2 Estimated land potentially available for energy cropping in the EU-27 in 2020 and 2030 based on [5, 14, 24, 25]

and urban and recreational areas. Projections on European cropland technically available for energy crop production are 6–22 Mio ha currently, 18–34 Mio ha in 2020, and 16–43 Mio ha in 2030 (Fig. 2) [5, 14, 24, 25]. Total arable land in the EU-27 was 66 Mio ha in 2012, so the above numbers correspond with 5–20%, 17–31%, and 15–40% of current arable land, respectively [5, 14, 24, 25]. In addition, pasture land technically available for lignocellulosic energy crop production in Europe is projected to be 0–4 Mio ha currently, 0–10 Mio ha in 2020, and 0–19 Mio ha in 2030 (Fig. 2), corresponding with around 0–6%, 0–16%, and 0–28% of current pasture land [14, 24].

The studies estimating the land availability for energy cropping apply a "food first" paradigm, that is, agricultural land required for food and feed production is never included in the land availability estimates for energy crops. Two key factors in determining the amount of land required for food and feed production are the projected food demand and production intensity. Production intensity is, in turn, related to the level of agricultural intensification and rationalization. Although the demand for agricultural land for food production is projected to increase globally, large differences exist between developing countries (further expansion of agricultural land) and developed countries (further decline of agricultural land) [26]. Although an increase in European agricultural output is projected, the utilized agricultural land area is projected to continue to decline; from 180 Mio ha in 2009 to 173 Mio ha in 2024 [27].

Differences in the projections of future land potential between studies are caused by different methods, approaches, and assumptions being applied. Assumptions on the interaction between land use for food and biomass feedstock production are central in different ways. First, biomass feedstock production may act as an additional driver for intensification of food and feed production as competition

for land increases [28, 29]. Assumptions on intensification rates of food and feed crops are critical in the estimation of land availability. In addition, many studies neglect the role of pastureland in biomass feedstock provision. Woods et al. [29] emphasize the role of pastureland in biomass feedstock provision. Pastureland occupies a large area of global agricultural land (i.e., twice the area of cropland) although only providing a small share of the food supply (i.e., about 3% of human dietary protein consumption) [29]. Woods et al. [29] argue that pasture intensification is likely to be larger in the presence of a robust bioenergy industry than without. Second, competition for resources may alter prices of land and therefore the competitive position of food and feed commodities [14]. Third, by-products produced during bioenergy production may substitute animal feed sources and are therefore interacting with the animal feed sector [30].

Differences in future land potentials between studies are also caused by the application of different sustainability criteria. Stricter criteria on sustainability, related to nature and biodiversity conservation and GHG emissions, lead to less land being available for biomass feedstock production as a higher share of agricultural land is reserved for nature conservation and there are less regions where the GHG mitigation requirements are reached [14, 31, 32].

3.1.1 Land Categories

In addition to land that can be made available for bioenergy production by intensification of current agricultural systems, there is also land available that is currently not used to its full potential. This under-utilized land can be divided into two types: low productive land that is not suitable for conventional crop production and unused agricultural land [22].

Low productive lands are known under various names: marginal, degraded, or contaminated lands. The amount and suitability of these lands are difficult to assess as many reasons for the low productivity exist, including economic, environmental, and agronomic limitations or a combination of these [33]. Agricultural production might no longer be economic with current agricultural practices, salinized lands might arise where the salt content has risen to a level where food production is no longer possible, and manufacturing or mining can also have detrimental effects on the quality of the soil [33, 34]. Improved management and technological development can make these lands productive again [34], although productivity could be lower than average.

Despite the resemblance in the unused lands category between fallow land and abandoned land, the reasons for the land to be out of use are very different; fallow land is set aside in the crop rotation, whereas abandoned land is land that has been used for agriculture but has fallen out of use in recent years. The amount of fallow land in Europe has for many years been connected to the requirements of the Common Agricultural Policy (CAP), in which a certain amount of fallow land was mandated. This requirement has been abolished in the CAP 2014–2020 reform, which means that fallow land has been included for agricultural production again

and the available fallow land is now diminishing rapidly [33]. In addition, fallowing of land is important in maintaining soil fertility and energy crop cultivation on fallow land should therefore be considered carefully. Abandoned crop or pastureland, on the other hand, can be used for energy crop production, as it is not in use for food, feed, or fiber production and under the condition that this land is not constrained by the sustainability criteria of the Renewable Energy Directive (RED) of the European Union [35]. See [36] on sustainability evaluation for more details on sustainability criteria in the RED. The use of pastureland for energy crop cultivation should also be carefully considered and limited to perennial crops only to minimize tillage practices and related environmental impacts.

As Allen et al. [33] note, there are no official statistics on the different land categories, which makes it difficult to estimate directly the amount of land that can be used for energy crops. A first estimate shows there can be great potential as the agricultural area in Eastern Europe (Belarus, Bulgaria, Czech Republic, Hungary, Poland, Moldova, Romania, Russia, Slovakia, and Ukraine) has declined by over 16 Mio ha in the period 1992–2012 [37]. This decline can be attributed to the decrease in demand for agricultural products from the former Soviet Union after the collapse and economic decline in the beginning of the 1990s.

However, not the whole area is available for energy crop production, as not all land complies with the current sustainability criteria for liquid biofuels. If we assume that these criteria are to apply for all future uses in a biobased economy, existing carbon stocks in particular may be a critical factor limiting land conversion to energy crops. Carbon stocks slowly increase after abandonment [38] and are released when taking the land into production for agricultural energy cropping, thereby possibly negatively affecting the carbon balance of biofuels. The effect on the biofuel's carbon balance depends on the type of crop used with lignocellulosic (perennial) crops in general performing better. Perennial crops sequester more carbon because of the deeper rooting systems and have lower tillage and fertilizer requirements compared to annual crops [39]. The FAO statistics show an increase of 3.2 Mio ha in forest areas in Eastern Europe in the period 1992–2012, the same period in which the agricultural area declined significantly. This trend was also recently confirmed by data from satellite images by Potapov et al. [40]. Schierhorn et al. [41] identified that, in the 20 years after the large-scale abandonment in European parts of the former Soviet Union, carbon stocks have increased on average by 15 tonnes/ha. These ongoing increases make abandoned agricultural land for energy crops increasingly unavailable.

3.2 *Future Feedstock Potential*

3.2.1 Energy Crops

Many studies projected the future bioenergy potential from energy crops and agricultural residues; an overview is shown in Fig. 3 for the years 2020 and 2030.

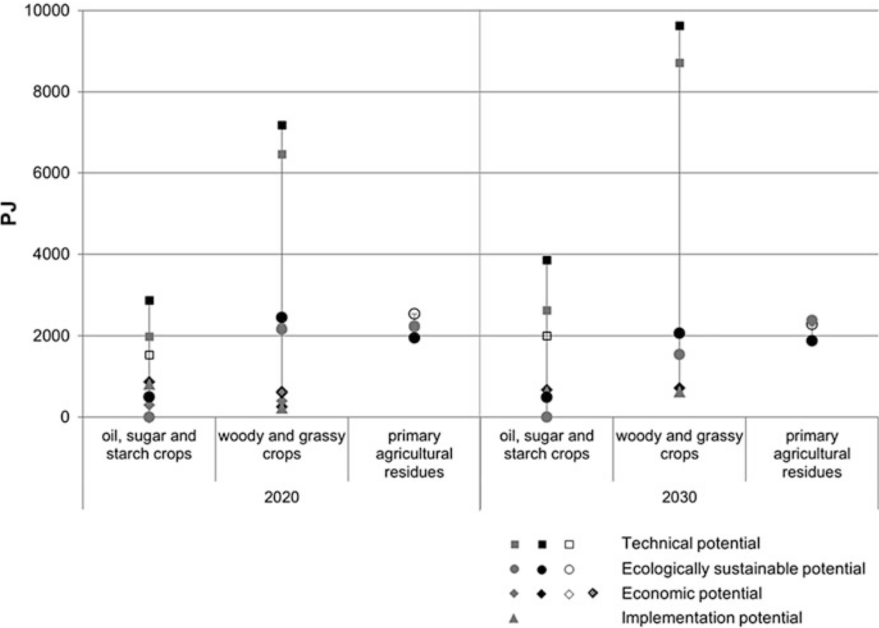

Fig. 3 Estimated bioenergy potentials from energy crops and agricultural residues in 2020 and 2030 in the EU-27 based on [5, 14, 24, 25]

The technical potential is estimated to be in the range of 1,530–2,860 PJ in 2020 and 2,000–3,860 PJ in 2030 for first-generation crops, and 6,470–7,180 PJ in 2020 and 8,720–9,630 PJ in 2030 for second-generation crops [24]. These potentials are calculated based on cropping the total available land with crops from one specific crop group (i.e., oil, sugar, starch, woody, or grassy crops). Considering sustainability criteria, other than food security, but considering both annual and perennial crops, gives a potential of 2,160–3,160 PJ/year in 2020 and 1,540–2,500 PJ/year in 2030 [5]. The economic potential of energy crops is projected to be 600–1,100 PJ in 2020 and around 1,400 PJ in 2030 [12, 31].

Sustainability constraints are considered to a varying extent in the ecologically sustainable and economic potentials. Stricter sustainability constraints lead to a lower potential from energy crops for two main reasons. First, less land is available as more land is reserved for nature protection. Second, the GHG emission mitigation requirements as set in the EU's RED [35] for the production of liquid transport fuels are not met by all energy crops for different production pathways. Considering the GHG emissions from indirect land use change (ILUC) in the GHG emission mitigation requirement lowers the energy potential from energy crops further, as is shown by, for example, Elbersen et al. [32]. However, large variations are found in land use change-related GHG emissions for the different energy crops [34] and the use of default ILUC factors is debatable. Generally, the calculated ILUC-induced GHG emissions are lowest for woody and grassy crops, followed by sugar and

starch crops, and highest for oil crops [42]. More on land use change induced by energy crops can be found in [36]. It remains to be seen whether similar sustainability criteria are also applicable for the use of biomass feedstocks in biorefineries for the production of, for example, biochemicals and plastics, but this could ultimately become a limiting factor for these applications as well.

The type of energy crops cultivated on the available land determines to a large extent the final potential (in terms of energy content/dry matter). Woody and grassy crops are expected to play a key role in the future sustainable bioenergy potential. The results of De Wit and Faaij [24] show the importance of crop selection on the total potential as they estimate the potentials by dedicating the whole land area available to one specific crop group. The highest potential is from grassy crops, followed by woody crops, because these crops reach high yields with relatively extensive agriculture management practices, leading to lower costs [24].

A shift from oil, sugar, and starch crops to woody and grassy crops is also foreseen by the European Environment Agency (EEA). The EEA [31] used a demand-driven approach to estimate the amount of land needed to reach the targets on bioenergy set in the National Renewable Energy Action Plans in 2020. They projected land demand for energy crops to be between 7 and 17 Mio ha, depending on the assumptions regarding the bioenergy mix, the use of different bioenergy feedstocks, and conversion pathways. Less land is required in the scenarios that emphasize sustainable biomass feedstock production, the avoidance of ILUC impacts, and with a higher price support. These assumptions lead to a higher availability of woody and grassy crops with higher yields and thus a more efficient use of the land. If these feedstocks are also to be used for biorefineries, the specific type and feedstock requirements of the biorefinery plays a crucial role with regard to the land availability.

3.2.2 Agricultural Residues

Agricultural residues are also expected to play a role in supplying bioenergy potential as well as woody and grassy energy crops. The sustainable potential of primary agricultural residues remains fairly constant over time and is estimated at 115–150 Mt_{dm}/year (2,000–2,500 PJ/year) and 110–135 Mt_{dm}/year (2.000–2,300 PJ/year) for the EU in 2020 and 2030, respectively [5, 14, 24]. Including non-EU Member States in the supply potential for Europe raises the sustainable potential to 4,000 PJ/year in 2020 and 4,100 PJ/year in 2030 [13]. Overall, wheat straw contributes most to the total share of primary agricultural residues, followed by barley and maize.

The amount of crop residues is affected by crop yield. Crop breeding aims at improving yields by increasing the share of the harvestable component of the crop, thereby reducing the residues to product ratio (RPR). However, as the use of straw for soil protection is proportional to land use, intensification of crop production leads to a higher sustainable supply potential as less land is required to produce the same amount of crops in intensive production systems than extensive production

systems [13]. However, when taking a global (rather than European) perspective, Daioglou et al. [13] found the residue supply to be more sensitive to developments in competitive uses, including livestock feed and fuel use for poor households, than to the rate of intensification. Bentsen et al. [17] also estimate an increase in the theoretical potential of crop residues through agricultural intensification. This increase is estimated to be high for Africa (93% of current theoretical residue availability), Oceania (155%), and Eastern Europe (61%), whereas the increase in agricultural residue supply through agricultural intensification is low (12%) for Northern, Western, and Southern Europe, because high input agriculture is already applied [17].

4 Synthesis

This chapter provided an overview of the current use and future potentials of agricultural feedstocks for energy and biomaterial purposes in the European Union. Agricultural land currently in use to produce energy crops in the European Union is 4.4 Mio ha, and land technically available in 2030 is estimated to be in the range of 16–43 Mio ha, which is 15–40% of the current arable land in the EU-28. Abandoned lands offer a good opportunity for energy crop production without competing with other uses such as food and feed production and nature protection. The availability of abandoned lands is, however, uncertain as statistics do not separately report this land type. Furthermore, it can be expected that productivity on these lands is lower than average. To add these lands to the land potential estimates, better maps are required to expand the knowledge on the location of these lands.

Agricultural feedstocks are used to produce approximately 14% of the bioenergy in the EU-28 in 2012. Oil seed biodiesel forms the majority of biofuel production in Europe, whereas wheat and sugar beet for bioethanol are used in smaller amounts. The future energy potential from crops is estimated to vary between 1,530 and 7,180 PJ in 2020 to 2,000 and 9,630 PJ in 2030, depending on crop type and sustainability constraints considered. Stricter sustainability constraints on nature protection and GHG emissions lead to an overall lower potential from crops and causes a shift from annual to perennial crops.

Primary agricultural residues are a large resource for bioenergy and biomaterial production that is not used to its fullest extent, mainly for cost and logistic reasons. The low energy density of straw makes transport costly. Besides, average straw prices are higher than forestry residues and a high variation in straw prices is observed from region to region, as prices are mainly determined by local scarcity. The availability of crop residues is estimated to stay rather stable (i.e., 115–150 Mt_{dm}/year (2,000–2,500 PJ/year) and 110–135 Mt_{dm}/year (2.000–2,300 PJ/year) in 2020 and 2030, respectively). Crop management practices, influencing crop yields and the amount of crop residues that need to be left on the land, influence the amount of crop residues bioenergy and biomaterial production available. It can be concluded

that primary agricultural residues, together with woody and grassy energy crops, should become more important as agricultural feedstocks, although the share of oil, starch, and sugar crops should decrease. This effect is reinforced if sustainability criteria become more stringent and/or if they are applied for all energy uses and material application.

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Wood Processing Residues

Ulrike Saal, Holger Weimar, and Udo Mantau

Abstract Rising demand for and scarcity of wood – together with cost savings and resource efficiency requirements – have led to a constant increase in the use of wood processing residues, where appropriate, in the production of wood-based products. This chapter presents/reviews the available information and existing knowledge of residues at various regional levels. It describes the segment of wood processing residues as an important wood resource and the availability of data on a national and on a global level for the quantification and the projection of the resource. The chapter points out the importance of empirical data (collection). Furthermore, it provides a terminology concept for a harmonised use of the diverse assortments and production stages of wood processing residues.

Keywords Assortments of wood-based residues, Data availability, Forest industry branches, Terminology of wood-based residues, Wood resource assessment

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1 Introduction

Looking at the long-term trend, the demand for wood has constantly increased over the last few decades. On the one hand this is driven by a constantly increasing demand for wood-based products and, on the other, by increased demand for wood for energy purposes. Besides traditional users of wood resources, new competitors also influence the demand for wood. The chemical industry is likely to increase the use of woody biomass for biotechnological purposes and biorefinery of wood. Consequently, the demand for wood as a raw material is also rising in parallel with the demand for (its) related products.

Basically, the demand was largely fulfilled by a rising supply of roundwood, driven by increased fellings in forests. However, given the material structure of wood as a raw material, wood-based residues which accrue during the different steps of wood processing are also suitable for further material and energetic uses.

Rising demand and scarcity of wood – and also cost savings and resource efficiency – have led to a constant increase in the use of wood processing residues, where appropriate, in the production of wood-based products. For example, the development of particle board has its origin in technological investments for a more efficient use of the available quantities of wood processing residues. This resource originates from, for example, sawmills, planing mills or the furniture industry, and would otherwise have been disposed of as waste.

It should be noted that the increase of the material use of wood processing residues moved forwards in parallel with technological developments in the panel board industry and, to a certain extent, in the pulp industry. The material use of waste wood for particle board production is also strongly related to the scarcity of available fresh wood fibres and further possibilities to reduce costs of raw material.

In fact, in many countries the use of wood processing residues for different purposes is a necessity, given the limited availability of the raw material and the cost of fresh fibres/roundwood. In this regard, recent developments should also be noticed in the chemical industry which uses wood for biotechnological and biorefinery purposes.

However, knowledge of the market structure, concerning supply and demand of wood processing residues, is surprisingly low. It seems as if the official national statistical systems of data gathering throughout the world are only focusing on the main resource flow, as long as it can be called a product. However, if there is a supply of (wood) raw material that originally is a residue from the production of a specific (wood-based) product, there is nearly no statistical interest in the recording and surveying of these quantities. Anyhow, in any case, wood processing residues are valuable raw materials which achieve revenue if sold on the market.

So far, knowledge and information concerning available quantities of wood processing residues (i.e. available on the market) and the different kinds of

assortments of wood processing residues (available) in use are not easily at hand. It is therefore the objective of this analysis to unveil these questions: what are wood processing residues, what are the different assortments and sources and which quantities are supplied? It shows the importance of empirical research and field data to answer (the question and) the demand for detailed wood resource information.

Hence, the objective of this chapter is to review available information and existing knowledge regarding the resource of wood processing residues, its origins and available supply within the structure of forest industry. Existing research results and previous literature on biomass potentials on a European and on a global scale are compared. The chapter is intended to differentiate from common biomass potential studies. It is not our objective to show potentials of the resource but to give an overview of existing data and quantities based on modelling. Because modelling is used, based on empirical research results, the German wood resource monitoring project is presented as (so far unique) periodic empirical research on supply and use of wood resources, including wood processing residues.

The chapter is structured as follows. In Sect. 2 we present results of our review of the existing literature in this regard and provide an introduction to the terminology and a definition of wood processing residues. Section 3 focuses on the analysis of existing information and data on the supply of wood processing residues. This is done on three different regional levels: we first give insight to the research which has been conducted in this regard in Germany, we then present the available knowledge gathered on a European level and finally present our results on a global level. Section 4 concludes the chapter with a discussion.

2 Literature Review, Terminology and Definitions

2.1 Literature Review

Current research on biomass resources cannot be imagined without the assessment of wood processing residues. Various studies were published in the last few years, presenting global, European or regional biomass and bio-energy potentials, either for the current situation or for future scenarios. Agricultural and forest biomass are the specific focussed resources. Resource assessment of forest biomass often includes residues from the wood industry. However, this particular segment is not well-differentiated in the literature and overall energy potentials do not give respective resource information. Moreover, because of missing harmonised terminology and units, data are not comparable between regions and countries.

Volumes of wood processing residues represent a significant share of woody biomass. However, existing literature rather focuses on theoretical forest biomass quantities. Most of the studies on potential biomass supply present scenario-based results, such as, for example, [1, 2]. Available studies on wood biomass potential mostly summarise available volumes of wood biomass other than forest biomass without introducing further assessment approaches. In addition, information and

data on wood processing residue volumes are still rare. The segment of wood processing residues is not covered as comprehensively as required by official statistics and the empirical research is exceptional. So far, the available results from some countries are only based on modelling. A first approach to assessing and modelling volumes of wood processing on a broader level (e.g. EU27) was adopted by applying the wood resource balance for European countries [3–5].

The literature on wood biomass potentials differs considerably on methodological approaches, applied scenarios, references and data units. Particular results on volumes of wood processing residues are either subordinate or mixed with volumes of forest residues. Global estimates of global fuel resources, mainly related to forest resources, are available, for example, from Parikka [6]. Smeets and Faaij [2] provide results based on a literature review and general estimation of wood processing residues by using a share of 50% of the total forest industry production. Another study on a global level is presented by Thrän et al. [7] on spatial distribution of biomass potentials based on FAO data from 64 countries. Estimates of woody biomass potential, in particular shares of wood processing residue (with a 25% share of felled wood) potentials on European level are given by Ericsson and Nilsson [1] based on rough approximation.

A study by Alderman et al. [8] investigated the available volume of wood processing residues in Virginia (USA) on the basis of company surveys and product statistics nomenclature. A study by Szostak et al. [9] on the industrial wood residue market in Poland, based on a survey in the Polish forest industry, provides differentiated results on wood processing residues in combination with country statistics. In Germany, various studies based on mail surveys have been conducted within the wood resource monitoring. Results of the EUwood study [3] on the segment of wood processing residues are based on modelling and data of the above-mentioned empirical studies in the context of the German resource monitoring project (for detailed information see Sect. 3.1). Modelling volumes of wood processing residues (on a resource-based level) is based on data of material balance and specific conversion factors. The material balance of a wood product is described by the input of the initial raw material (roundwood, sawnwood, wood-based panels) and the output of the final product (compare [10]). However, reliable data on material balance and conversion factors can only be provided based on empirical research.

In contrast to this, the segment of sawmill residues is analysed in more detail [11–14]. Studies on material recovery within the sawmill industry were conducted mainly for North America. They provide information on sawmill residues as side information. The focus of most of these studies, however, lies on the increased lumber/sawnwood output and production efficiency.

The low number of available assessment studies compared to studies mainly focusing on biomass potential, which do not further differentiate into possible assortments, shows the importance of empirical research for comprehensive results given by primary data collection. National and international statistical databases are already quite well-set with data: Eurostat and FAO provide international statistics on the main sectors of the forest industry. However, encompassing wood resources supply and demand at a sufficient level of detail is not possible for reasons of imprecise terminology and, hence, definition of the resource.

2.2 Terminology and Definition

So far, terminology and definition for wood processing residues is neither definite nor well-harmonised. As results of volumes on wood processing residues differ in the literature [15], so do terms on residual woody biomass [16]. A broad variety of terms is used in the literature as regards the segment of residual woody biomass from industrial processes. Terminology describing the assortment of residues from roundwood production and further processing of wood products is inconsistent. For the most part, the resource of wood processing residues appears in the literature with similar features but it can also be confused with forest residues or waste wood. On the other hand, existing terminology summarises the whole resource of wood processing residues and does not clearly differentiate between its particular assortments such as sawmill residues and other wood processing residues or pulp production residues, which should be done because of the large differences in shares and the quantification of the different volumes.

The estimation of volumes of wood processing residues in particular needs prior common definition of the following relevant terms:

Residue: an inevitable remainder of any production process. The term does not imply any valuation or category of desired or undesired. It has to be differentiated from waste.

Waste: an unserviceable remainder of any production process. It is considered as useless and unsalable [17].

Moreover, the terminology and definition of wood processing residues should be differentiated according to their derivation. Residues are derived from production processes. In comparison to that, by-products receive a market value and product features from the markets' resource demands.

Wood processing residues accumulate during all mechanical and chemical production processes in the forest industry. The resource has to be differentiated from forest residues and waste wood. For a long time, wood processing residues were considered to be waste or remnant biomass without further use. After the demand for woody biomass for energy use started growing as well, wood processing residues, especially sawmill residues, became a by-product with competitive product features [18]. The resource comprises residues from sawmilling, residues from other wood processing activities and black liquor as the residue from the pulping process. In this context, bark is not considered as an assortment wood processing residue. Bark accumulates before the actual wood processing (debarking prior to, e.g. sawing or pulping process). As regards its characteristics, bark is not comparable to wood fibre and the use of residual woody biomass. However, in terms of wood resource supply, bark volume is considered as a forest resource [19] of, for example, 50.9 million m^3 in the EU27 [20].

Forest products definitions of the FAO cover data on the resource of wood processing residues by differentiating in two assortments: (1) wood chips and particles and (2) wood residues [21]. The application of the terms is difficult because of ambiguous meanings and application by third parties. The segments of

wood processing residues consist of different assortments (chips, slabs, dust, edgings, trims, cores). The two terms cannot be easily allocated to a corresponding segment with more than two different assortments. Forest industry production is very highly differentiated, and so are the assortments of residues (see Fig. 1). The volume of wood processing residues in this chapter is provided in cubic metre solid wood equivalent (m^3 swe). In general, assortments of wood and wood processing residues are dealt with and measured in different units (e.g. bulk volume, solid volume, tonnes). To assess total supply of, for example, wood processing residues and to comprise assortments of different units in the wood resource balance (see, e.g. [3]), all units are converted into cubic meter solid wood equivalents (m^3 swe) so that data can be compared with, for example, statistics on removals. Conversion factors depend on the wood specific gravity. Thus, the conversion factor for 1 m^3 solid wood into tonnes dry matter can vary between 0.48 tonnes/m^3 for and 0.55 tonnes/m^3 for the different assortments [19]. According to Mantau [19] the average of 0.5 tonnes/m^3 is a good approximation. The results of our analysis in Sect. 3 are provided in both units, cubic meter solid wood equivalent and in tonnes dry matter.

Figure 1 gives an overview of forest industry branches, forest product segments, the three considered segments of wood processing residues, the end-use sectors and

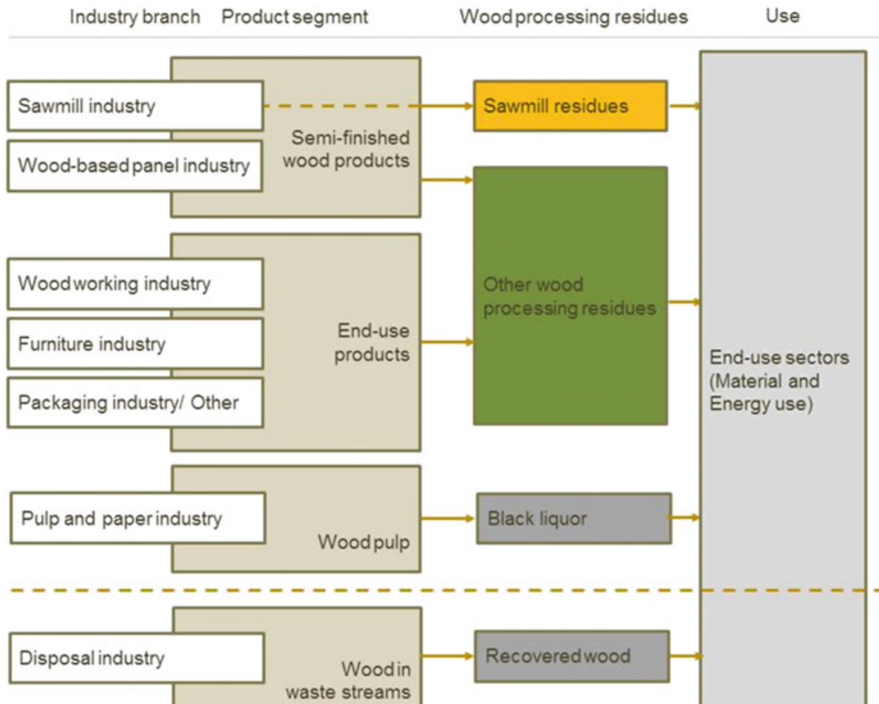

Fig. 1 Scheme of the forest industry sector and wood processing residues. Source: based on Saal [5]

the disposal industry as the sector which recovers wood from waste streams. It displays the context of the common forest industry production processes and production output (e.g. sawmill industry producer of semi-finished wood products and sawmill residues).

In the following, the particular segments of wood processing residues and their assortments are explained based on the origin of the resource.

Semi-finished wood products are produced within the sawmill industry and wood-based panel industry. They cover all sawnwood products and wood-based panels.

Sawmill residues inevitably accumulate as a side yield during production of sawnwood within the sawmill industry. The main assortments of sawmill residues are chips, sawdust and slabs. Cross-cut ends, edgings and trimmings are additional residues of sawnwood production. Sawmill residues consist of primary wood fibre. The assortments are a homogenous wood resource of constant dimensions and quality [22]. They are desirable for the production of pulp and wood-based panels and energy products, such as pellets.

Other wood processing residues (other than sawmill residues) accumulate during the production of wood-based panels, such as particle board, different fibreboard products, plywood and veneer. Residue assortments are shavings, veneer rejects and peeler cores, trimmings and edgings, wood dust and chips. Most of the residues are of fresh fibre, although wood processing residues of some fibre board products are an exception. Because of fillers and additives, these other wood processing residues do not consist exclusively of primary wood fibre.

Further amounts of other wood processing residues result from the manufacture of finished products. They cover all wooden products made of semi-finished wood products, such as furniture, packaging and applications in construction (e.g. engineered wood products). Wood processing residues which accumulate during the further processing of semi-finished wood products have to be clearly separated from sawmill residues and wood processing residues of primary fibre. Wood residues from further processing to finished products are residues such as dust and shavings from planning, milling and drilling as well as trims and clippings.

There is a huge variety of output shares of wood processing residues as it largely depends on the type of manufacturing process and the kind of wood product used as input to the respective production process. For example, sawmill residue shares range from 35% to 45% depending on wood species, log dimensions and technical processing parameters [10, 23–25]. Shares of wood processing residues from wood-based panel production also differ. Production of, for example, fibre boards or oriented strand board yields shares of 4–12% of wood processing residues. Production of, for example, plywood and veneer results in higher shares (45%) of wood processing residues because of lower material efficiency [26].

Black liquor is the residue of the pulping process within the pulp and paper industry. The residual mass mainly consists of lignin and hemicelluloses, cooking chemicals and water which are used to extract wood fibre. Approximately 40–50% of the input wood raw material is recovered as further usable fibre in the chemical pulping processes ([27], p. 38). So far, black liquor does not appear on resource

markets but is almost entirely used for industries' internal energy generation [28]. However, because of increasing demand for lignocellulosic resources, black liquor volumes are considered to be possible future chemical resources [19].

Recovered wood, also referred to as waste wood or post-consumer wood, is wood or wood products that have been disposed after a first use or after end use. It consists of wood from packaging materials, wood from construction or demolitions sites or wood which can be recovered from municipal waste (e.g. used furniture). Parts of recovered wood also originate from manufacturers of wood-based products which dispose of wood processing residues at waste management companies (e.g. [29–32]).

3 Supply of Wood-Based Residues: On Three Regional Levels

This section focuses on the analysis of existing information and data on the supply of wood processing residues. This is done on three different regional levels: first, results of wood resource monitoring research conducted in Germany is presented and compared with results of (modelling data EUwood) and available statistical data from FAO. Subsequently analysis of available data on a European and on a global level are undertaken.

3.1 Germany

The wood resource monitoring project in Germany has been continuously assessing the supply and demand of wood raw materials in the forest industry since 1999. This periodic research based on empirical surveys allows one to display the development of wood resource availability and wood flows within the forest industry. Additional information is achieved for balancing wood resources and information on conversion factors. This assessment requires comprehensive data sets. Some data are provided from national statistics. However, many parts are recorded insufficiently. Detailed information on particular wood consumers is not covered by official statistics or is only underestimated because of statistical cut off thresholds (e.g. [20, 33]). Volumes of wood processing residues are also not covered by official statistics. Based on detailed surveys on the wood resource input of the respective industry branches, the segments of sawmill residues and other wood processing residues from wood-based panel production and further processing are analysed. Thus, surveys are designed to gather information on internal and external distribution of wood processing residues. This allows one to describe the resource mix of wood biomass consumers and thus material flows. Figure 2 shows the

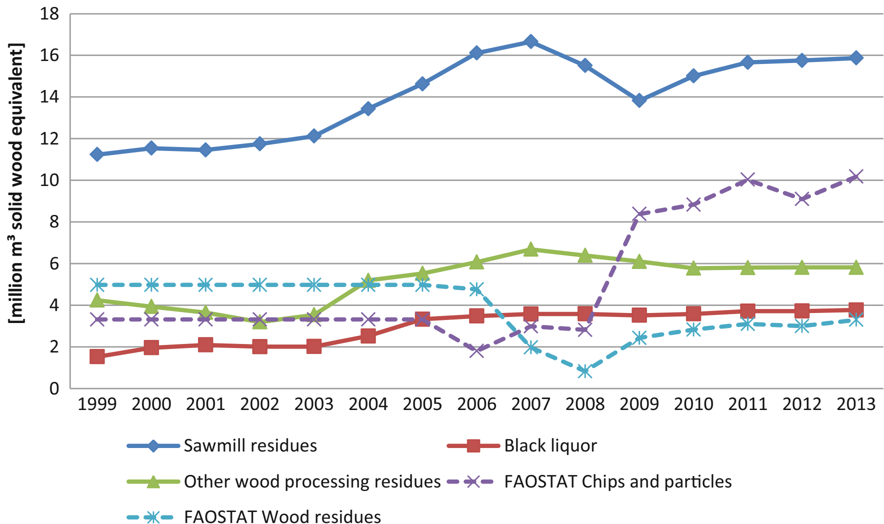

Fig. 2 Development of particular assortments of wood processing residues. Source: [19, 21]

development of the different assortments of wood processing residues in Germany. For comparison, data from FAO are also shown.

It can be seen from the graphs that, by volume, wood processing residues are an important source of wood supply in Germany. Data given by FAO differ considerably. On the other hand, because of different compositions of the assortments (1) chips and particles and (2) wood residues, the development of residue volumes can only be compared based on total volumes.

Table 1 presents current data on wood resources and wood use in Germany. Results of the latest resource monitoring of the German forest industry (2010) are shown in comparison to the resource potential calculated within the EUwood study [3] and available data by FAO for 2010.

As can be seen in Fig. 2 as well as in Table 1, data gathered by wood resource monitoring, based on empirical research, are significantly higher than data provided in international databases. A systematic underestimation of available volumes in FAO can be stated.

3.2 Europe

As described in Sect. 2.1, comparable assessment studies of wood processing residue volumes on national and European scale studies are rare. Thus, quantification of (potential) supply of wood processing residue volumes is based on modelling. Wood resource modelling depends on comprehensive datasets and feasible default values, such as material balance, industry consumption and size classes and

Table 1 Comparison of data on wood processing residues and post-consumer wood in Germany, 2010

Assortments	Resource monitoring 2010 Mantau [19] (million m^3)	EUwood, potential for 2010 Mantau et al. [3] (million m^3)	FAO, 2010 (FAO 2015) (million m^3)	
Sawmill residues	14.4	13.8		
of which chips	9.1[a]	8.9[b]	8.8	Wood chips and particles
Other wood processing residues	5.8	6.9	2.8	Wood residues
Black liquor	3.6	3.6		
Post-consumer wood	*14.0*	*8.7*		
Total (assessed)	23.8	24.3	11.6	Total
Total (incl. post-consumer wood)	37.8	33.0		
Total in tonnes dry matter [t_{dm}]	11.4	11.2	5.5	
Total (incl. post-consumer wood) [t_{dm}]	17.8	15.5		

Volumes in million m^3 solid wood equivalent
Total volume given in tonnes dry matter [t_{dm}] are based on the conversion factor of 0.47 t_{dm}/m^3 solid wood equivalent
Source: [3, 5, 19, 21, 23]
[a]Döring and Mantau [23]
[b]Calculations based on Saal [5]

particular coefficients. This information is not covered by official statistics and only partly available for some countries. As shown in Sect. 2.2, data by FAO on wood chips and particles and wood residues are not fully applicable. However, data that can be generally applied to the production of forest products, consumption and trade data for Europe (EU28/EFTA), are available from FAO.

Within the EUwood study, the modelling of wood processing residue volumes on a European scale was carried out [5] for the purpose of a European Wood Resource Balance. This included detailed quantification of the segment wood processing residues. Similar modelling based on EUwood results was used for the European Forest Sector Outlook Study 2012 [34]. The modelling approach followed the general forest industry structure (see Fig. 1) which follows a resource-based assessment structure. German data served as default data for modelling wood processing residue volumes in Europe [3, 34]. Datasets of comparable extent for other European countries are not known so far. Results of the periodic resource monitoring of the German forest industry sectors were applied as default values on FAO production and wood products consumption data (see [5]).

The comprehensive size class structure and further parameters of the German sawmill industry were applied to sawnwood production data (by FAO) to model volumes of *sawmill residue assortments* of the EU27 countries and to consider national differences in industry size and material conversion efficiency.

Volumes of *other wood processing residues* from the production of wood-based panels were estimated based on generalised parameters and material conversion factors. It is generally assumed that production processes throughout the producing countries are of similar quality and technological development. Thus, conversion factors are applied for all countries. Data on wood-based panel production volumes are given by FAO.

Residue volumes of *other wood processing residues* from production processes of finished products are estimated based on the wood consumption within the particular end-use processing sectors: construction, furniture and packaging industry and others. Country specific coefficients were applied to sawnwood and wood-based panel consumption (including import and export volumes) (FAO) to model particular wood consumption of the sectors. Again, German default values were applied to estimate respective shares of wood processing residues within the different end-use sectors.

Shares of black liquor as a residual product of the pulp industry were calculated based on pulp production data by FAO and available country specific conversion factors [10]. Further influencing parameters such as the share of coniferous roundwood input were modelled.

Table 2 shows the results of the EUwood study on the different segments of wood processing residues in comparison to available data by FAO.

As already seen in Table 1, data on wood processing residues based on the differentiated assessment [3] mainly based on German default values are significantly higher compared to statistical data provided by FAO.

3.3 Global Data

As presented in Sect. 2.1, studies on the supply of wood processing residues on a global scale are rare. Moreover, results of considered global estimates (compare Sect. 2.1) are not comparable because of different methodological approaches.

To provide the possible range of global volumes of wood processing residues, we applied the presented methodologies and compared the results with data from FAO. The following Table 3 shows the available data by FAO in comparison to calculated wood processing residue volumes based on Parikka [6]; FAOSTAT [21] and Saal [5].

FAO provides data on wood chips and particles and wood residues for 80 producing countries. The other countries do not report the respective volumes. For this study the global supply of sawmill residues and wood chips in particular, other wood processing residues from wood-based panel production and black liquor were roughly estimated based on FAO/UNECE [10]. Other wood processing residues

Table 2 Comparison of data on wood processing residues and post-consumer wood in the EU27, 2010

Assortments	EUwood, potential for 2010 Mantau et al. [3] (million m^3)	FAO, 2010 (FAO 2015) (million m^3)	
Sawmill residues	86.6		
of which chips	46.7[a]	61.2	Wood chips and particles
Other wood processing residues	29.7	47.0	Wood residues
Black liquor	60.4		
Post-consumer wood	*52.0*		
Total (assessed)	176.7	108.2	Total
Total (incl. post-consumer wood)	228.7		
Total in tonnes dry matter	83.1	50.8	
Total (incl. post-consumer wood) [tdm]	107.5		

Volumes in million m^3 solid wood equivalent
Total volume given in tonnes dry matter [t_{dm}] are based on the conversion factor of 0.47 t_{dm}/m^3 solid wood equivalent
Source: [3, 21]
[a]Calculations based on Saal [5]

Table 3 Comparison of different calculations on global data on wood processing residues, worldwide 2010

Basis	Parikka [6] (million m^3)		FAO/ UNECE [10] (million m^3)		Saal [5] (million m^3)		FAO, 2010[a] (million m^3)	
Range	From	To	From	To	From	To		
Assortments								
Sawmill residues	339.4	414.8	223.7	394.8	229.5	404.9		
of which chips	83.1	101.6	118.4	243.5	108.2	190.8	260.4	Wood chips and particles
Other wood processing residues					104.7[b]		46.7	Wood residues
Black liquor			277.8	333.3	278.5	296.2		
Total (assessed)	422.5	516.4	619.9	971.5	720.7	996.6	307.1	Total
Total (t_{dm})	198.6	242.7	291.4	456.6	338.7	468.4	144.3	

Volumes in million m^3 solid wood equivalent
Total volume given in tonnes dry matter [t_{dm}] are based on the conversion factor of 0.47 t_{dm}/m^3 solid wood equivalent
Source: [5, 6, 10, 21]
[a]Data are based on FAO country data, available/provided for 80 countries
[b]Data based on coefficients of wood processing residue shares of wood-based panel production – only one value calculated

from further processing, such as from the furniture industry, were not estimated as the modelling approach developed for the EUwood study [5] was/is not applicable on a global scale. The estimation of sawmill residues and chips is based on general assumptions on material recovery [6] and country data [10]. The estimations of black liquor volumes are rough shares based on conversion factors [10] and more specific estimations which consider shares of wood species input in global pulp production given by, for example, Goetzl [35]. Minimum and maximum ranges are presented.

As Tables 1–3 show, the statistical data provided by FAO also underestimates the volume of wood processing residues in total on the global level. This is partly because of the low coverage of only 80 reporting countries. Moreover, the given values for wood chips and particles are not clearly defined. They may also include reported residue assortments of different origin. However, underestimation is also through lack of statistical coverage of the volumes of wood processing residues, even if the quantities imply significant global volumes of wood assortments.

4 Discussion

Wood processing residues contribute to wood supply by around one-fifth of the total wood biomass. In general, supply and available volumes of wood processing residues are dependent on the processing of roundwood. The efficiency of roundwood utilisation influences the supply of wood processing residues. It is assumed that the production of semi-finished and finished wood products increases [3, 34, 36]. Thus, an increasing supply of residues is expected in connection with increased roundwood processing and the increasing demand for wood and wood products. Further, an increase in demand and scarcity of wood resources probably leads to a more efficient use of wood processing residues.

However, as the results show, there is a huge discrepancy between officially reported data on wood processing residues and empirical (or modelled) data. Discrepancy may be because of terminology deficits and little reported data. Wood processing residues have a significant impact on sustainable wood supply. Their occurrence depends completely on the wood processing industry. The variety of assortments and sources is as poorly addressed in the literature as is the calculation of the quantity. In some cases the quantities may be calculated fairly well because of the unique technical relationship. Residues are an inevitable remainder of any production process. If conversion factors are well-known, the quantities can be calculated based on the underlying production statistics. This applies mainly to the semi-finished sector (e.g. sawmill and pulp industry). However, the further processing of wood (e.g. construction, furniture) is very diverse and research has not paid much attention to this issue so far. Aside from unknown available quantities, the question of utilisation should be analysed because it is not known to what extent residues are consumed internally or are available on the market. Most likely, most of the material is used for power and heat but only a few

empirical studies on residue utilisation are available. This chapter clarifies the terminology of wood residues and summarises existing data on quantities. However, as official statistics focus on products, analyses in this area probably always rely on empirical studies. It is strongly recommended to intensify such studies and possibly apply the results on international statistics in order to provide more realistic data. These data are needed for a better estimation of sustainable use of wood as well as, for example, for the quantification of cascades in circular economy because residues are the main source of cascading use of wood.

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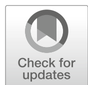
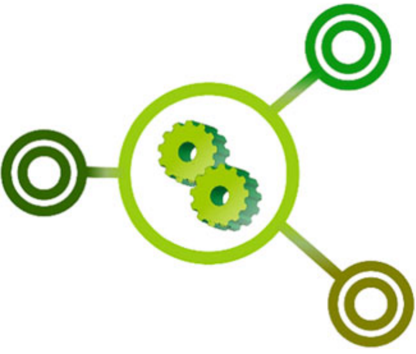
Logistics of Lignocellulosic Feedstocks: Preprocessing as a Preferable Option



Nils Tippkötter, Sophie Möhring, Jasmine Roth, and Helene Wulfhorst

Abstract In comparison to crude oil, biorefinery raw materials are challenging in concerns of transport and storage. The plant raw materials are more voluminous, so that shredding and compacting usually are necessary before transport. These mechanical processes can have a negative influence on the subsequent biotechnological processing and shelf life of the raw materials. Various approaches and their effects on renewable raw materials are shown. In addition, aspects of decentralized pretreatment steps are discussed. Another important aspect of pretreatment is the varying composition of the raw materials depending on the growth conditions. This problem can be solved with advanced on-site spectrometric analysis of the material.

Graphical Abstract



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Keywords Analytics, Decentral, Mechanical, On-site, Pre-treatment, Renewable raw materials, Storage

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1 Introduction

The availability of lignocellulosic biomass is a current challenge for biorefineries. Seasonal growth and harvesting, as well as the deterioration of biomass during storage, affect potential biorefineries. Furthermore, transportation and storage costs are crucial factors for a plant's economy. Therefore, a prerequisite for all biorefinery processing stages is mechanical pretreatment of the raw material, which reduces transport and storage volume. Ideally, conditioning the feedstock after harvest can also enhance the stability of the biomass during storage.

In lignocellulose-based biorefineries, fermentable structure carbohydrates amount to 10–55% cellulose and 5–65% hemicellulose, depending on the feedstock (numbers refer to dry weight) [1]. The water content of fresh biomass also varies greatly. Herbaceous and annual plants seem to have great advantages for use in a biorefinery because they are fast-growing and tend to accumulate little lignin, facilitating enzymatic hydrolysis. However, their water content reaches approximately 70% of fresh weight. This leads to a relatively high demand for fresh biomass for the efficient operation of a biorefinery based on such feedstock. As an example, in an Austrian pilot-scale grass-based biorefinery (now out of service) that produced mainly lactic acid, amino acids, and proteins; 3.3 tons of ensiled biomass were required per ton of product, with reported processing of up to 500 kg of biomass per hour [2, 3]. Thus, biorefineries, based on feedstock, such as fresh or ensiled grass or similar biomass, require special efforts to solve transport and storage logistics.

Transportation costs amount to approximately 13–28% of the total costs, thus representing one of the most important factors in the overall costs in a biorefinery [4]. Usually, harvested feedstock is transported to a centralized biorefinery treatment within a 100-km distance, as been reported by previous studies [4, 5]. Another study proposed decentralized pretreatment of the biomass. Following that approach, the harvested biomass can be being prepared for storage and further conversion to value-added products near its harvesting location using satellite storage facilities. Thus, the

biomass volume for transportation to a central biorefinery could be reduced in comparison to the feedstock after harvest [6].

2 Feedstock Allocation

Deciding where to locate a new biorefinery plant or storage and distribution facilities is one of the core aspects of biorefinery establishment. This chapter provides an overview of different approaches and their results in the use of assessment of feedstock availability. Decision making depends on feedstock allocation and customer demands. In addition, it should take into account uncertainties with regard to transportation costs from the feedstock location to the storage facilities or centralized plant. Depending on the site selected for the biorefinery, the costs of transportation and operations vary and influence the capital investment.

First, potentially interesting construction sites and the required capacity should be identified taking into account the feedstock (and the products). Basic knowledge about the geospatial distribution of feedstock and the demand on target products in the selected area is essential [7]. Moreover, it will be necessary to make initial assumptions concerning the envisaged process and to establish important variables, such as target prices, operation time, and process capacity. To this end, Geographical Information Systems (GIS) can be introduced into the modeling and design of the supply chain [8]. Once the potential region is selected, the locations that will receive deliveries from the selected site should be established. Consequently, in order to compare the suitability of various allocation sites, different location scenarios should be developed that can be described by a facility-dependent model [9, 10]. The functionality of these models is determined by their incorporation within the supply chain management (SCM) [11].

The SCM comprises all the significant supply chain aspects, such as procurement, transferment and storage of raw materials, maintaining a process inventory, production, distribution, and routing, and should be applicable throughout the considerable operating life of the biorefinery during which the parameters can change. In particular, the analysis should include the calculation of transportation costs for various distances in the selected area, taking into account detours, deleterious road conditions, and indirect routes from the biomass production field to the satellite storage facility or the centralized plant. The combination of facility-dependent models and the SCM allows a comprehensive problem analysis in finding the best allocation site with an optimal supply chain configuration. Within this approach, the uncertainty surrounding specific input variables can be modelled using multiple probability distributions or discrete scenarios with a stochastic model. The predictable time-dependent unknown parameters such as demand levels or costs can be implemented using specific forecast functions and combined with the stochasticity model if the probabilistic behavior changes over time. Finally, environmental performance should be managed as profit maximization is not always accompanied by a good

environmental performance, as is shown in the approach to mathematical modelling by Grossmann and Guillén-Gosálbez [12].

Several authors have described the supply chain design and the procurement of biomass sources [13–15]. Large amounts of biomass are located in forests. Nevertheless, the future demand for wood for, for example, heating will continue to increase [16]. Currently, the merchantable tree components are predominantly used in established conventional processes and are not available for biorefineries. Consequently, the focus today is on the forest biomass that traditionally remains in the forest, such as logging residues, stumps, and trees with small diameters. Up to 47 million dry tons of currently unused lignocellulosic biomass are potentially available in Germany [17]. In Europe, Sweden is the leading wood producer, where 24.0–53.2 TWh of non-harvested forest residues are available for biofuel production [18]. However, the sustainability of additional extraction of forest residues should be critically investigated, because the woody residues that are usually left on-site play an important role in the forest ecosystems and their removal could negatively affect these systems.

The location of biofuel production processes and the corresponding supply chain network of the forestry resources in the Southeastern region of the USA were investigated by Kim et al. [19]. Their study covered candidate sites and capacities for two conversion processes: fast pyrolysis and a Fischer Tropsch bio-diesel process. The most profit-relevant parameters were identified and combined into scenarios in which to analyze them using a stochastic two-stage model, where the first model stage manages the capital investment, including the size and location of the processing plants, and the second model manages the biomass and product flows of each scenario [20]. Here the biomass availability, maximum demands, sale price of product, yield of intermediate product, and yield of final product were identified as the most dominant parameters, and the optimization of 33 scenarios was carried out to maximize profitability of the process. This model example demonstrates how combination of the facility location model and SCM can support the decision of where to locate new forest biomass processing plants on a national or regional level.

Alternatively, to supply greater amounts of woody biomass for bio-based products, the acreage of fast-growing, intensively managed trees can be increased. Short-rotation woody crops or short-rotation coppice are promising alternatives to conventional forest biomass due to their fast-growing high biomass yields. Here, a variety of species can be used (willow, poplar, mallees [20], etc.). The harvest times vary from tree species to tree species and can be as short as 3 years. However, physical properties such as the density, composition, form, and geographic distribution of various species influence the supply chain design and the required harvesting costs. Often specific machinery and tailor-made pre-transport and storage strategies are needed due to the different densities and configurations of crops of wood compared to conventional biomass [20]. Moreover, the quality and the quantity of yield as well as the tolerance to environmental stresses vary between individual plants and influence the suitability of plant material as a potential feedstock.

Further promising biomass sources are the energy crops. In addition to woody crops, energy crops include perennial grasses such as Switchgrass [21–25] or

Miscanthus [26–28] and annual energy crops, such as high-yield sorghum [29]. Growing these energy crops can provide a high biomass yield per hectare of land with low energy inputs. These plant species are usually grown specifically for use as a fuel supplement. Their cultivation is attractive for farmers and landowners because of the prospect of additional profit. However, it must be borne in mind that the energy crops in general compete with food crops for agricultural land. In the USA, Switchgrass (and to a lesser extent Miscanthus) is a promising cellulosic energy crop, while in the EU and Japan Miscanthus is the plant of choice. Miscanthus can be cultivated in cold temperate climates and on various land areas. This is extremely advantageous because it can also be grown in areas that cannot be used for cultivation of food plants. Moreover, unlike other short-rotation crops Miscanthus is harvested annually with conventional harvesting equipment. It grows very rapidly and delivers a high biomass yield. Moreover, Miscanthus species are viewed as relatively environmentally friendly crops due to the low amount of fertilizer and pesticide needed during their cultivation.

Bomberg et al. [30] analyzed the Miscanthus supply for an ethanol fermentation process using an optimization framework to minimize production costs. The developed integrated optimization model consists of a stochastic sub-model for land conversion and combines it with a base fermentation scenario in considering several relevant parameters such as market prices, farm-specific inputs (price, production costs, and crop yields), transport costs, and capital inputs. This study included an area of 777 counties that included Iowa, Illinois, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin with an average Miscanthus yield of 19 dry ton/ha. The authors analyzed various refinery scales using Monte Carlo simulations in order to identify optimal plant capacity, and suggested a risk of oversizing cellulosic plants in this area by forcing their increase.

Conventional agricultural crops have additional potential for use as energy crops. For instance, sugarcane is one of the most promising energy crops in tropical and subtropical regions. In Brazil, 9 million hectares of land are used to produce 31% of global sugar cane. Large amounts (82.4 tons/ha) are used to provide sugar for bioethanol. Corn is a popular starch source with large amounts of corn produced worldwide. In 2015, about 24% of pasturelands in the USA are used for corn cultivation. Wheat cultivation claimed a further 15% of the available pastureland, including idle land and pasture areas [31, 32]. In accordance with the 2007 Renewable Fuels Standard (RFS), by 2022 36 billion gallons of biofuels should be produced in the USA. These comprise 15 billion gallons of corn-based ethanol and 16 billion gallons of cellulose-based fuels. The former goal has already been reached and an increase in the production of cellulose-based ethanol is to be expected [33]. Sugar and starch crops are extensively grown in Europe as energy crops for biofuel production [34]. In Germany, mainly starch from potatoes is exported to other countries; in contrast, starch from corn and wheat is imported. In most of the European countries sugar-beet crops are grown to produce sugar for food, which can also potentially be used as a feedstock for a variety of chemical and biochemical processes.

However, the use of conventional crops for biofuel as well as the cultivation of the cellulosic energy crops is increasingly critically viewed due to competition with the food chain. Naturally, the highest biomass yields can be achieved on high-grade lands that are co-located to food plants [35], but the use of low-grade land can provide considerable biomass yields from energy plants. For instance, widely unused land areas in Ireland have been successfully planted with Miscanthus for biofuel [36]. Consequently, the conflict between food and energy plants could be solved by careful selection of energy crop species and innovative agricultural approaches or technologies that might enable the cultivation of crops in currently undeveloped areas [37, 38]. It should be kept in mind that the additional extraction of agricultural residues should be done without significantly affecting the soil fertility. Usually residues are left on the field or are given back to the field to be incorporated into the soil for improved soil quality. Therefore, alternative methods should be developed to guarantee sufficient soil fertility before the residues can be extensively removed from the field.

To achieve considerable biomass amounts and to allow optimal land and nutrient use, double- or even multiple-cropping systems are becoming increasingly important. Here, the growing season is extended by the cultivation of two complementary plants on the same land. High biomass yields have been achieved in the past using this agricultural approach [38]. Basically, all winter cereals such as winter barley, winter rye, triticale, and winter wheat can be used as first crops due to their early harvesting in May–June. Sorghum or sorghum × sudangrass, as well as a variety of other summer grains, can be cultivated as a second grain in double-crop systems.

Lignocellulosic agricultural residues and waste materials provide a suitable alternative raw material source for biorefineries [39]. Rice and corn stover (stalks and leaves from corn) are among the most plentiful agricultural residues, followed by straw and stubble from other small grains such as wheat, barley, oats, and sorghum. The amount of residues depends on the crop yield itself. For instance, high amounts of sugarcane bagasse are produced in Brazil due to the predominant cultivation of sugarcane in this region for the production of sugar and ethanol [40]. In areas where rice production is dominant, rice straw is the most plentifully available waste source. 731 million tons per year of rice straw are accrued worldwide, with 667.6 million tons in Asia alone, where rice straw, wheat straw, and corn stover may be the most promising future bioethanol feedstocks. In Europe most ethanol from residues comes from wheat straw and in the USA from corn stover [41].

In the USA, corn is the most accessible feedstock due to the historic development of the US agricultural industry. Hence, corn-based bioethanol production in the USA and its feedstock supplies are predominantly located in the Midwestern states. In the future, higher expected corn yields for expanding biofuel production will result in higher amounts of agriculture residues. More than 300 million tons of combined forestry, agricultural residues, and waste are currently available annually in the USA for fuel production. By 2040, an increase to more than a billion tons of these residues is expected [32]. The lignocellulosic residue and waste materials provide an alternative raw material source for a relatively inexpensive fuel production without competing with food production [42–44]. Examples of lignocellulosic residues and

waste materials include agriculture residues and non-harvested forest residues (tops and branches, not including stumps). In the future they can potentially play a significant role in the production of second-generation biofuels and platform chemicals, but economical processes require suitable supply chain solutions.

Marvin et al. [45] presented a net present value optimization approach to calculating the supply chain of biomass-to-ethanol production from lignocellulosic residues in a nine-state region in the Midwestern USA. Mixed integer linear programming (MILP) was used to determine optimal locations and capacities of biomass-to-bioethanol plants simultaneously with biomass harvest and distribution. The study includes biomass sources from an area of 100 miles for 69 candidate biorefinery locations. Favorable biorefinery locations were identified using Monte Carlo-based random sampling of the parameter space and recalculation of the economics. The model was optimized for 200 independently drawn parameters taking into consideration feedstock and product costs, transportation cost investment, and lifetime operation cost of various sizes, amount of biomass harvestable at various production locations, and biorefinery conversion of biomass to product. Additionally, a sensitivity analysis was performed to describe how possible price changes and their effect on the robustness of the supply chain may influence the profitability of proposed biorefineries during their lifetime. The results show that the locations chosen least frequently by the analyst in the studies are not surrounded by biomass-producing counties. Moreover, in 21.5% of the trials it was shown that it is not economical at all to construct any biorefineries, even though large amounts of biomass are available in the region. The authors state that an ethanol sale price stabilization at higher levels and lower capital investment costs could increase the attractiveness of the process for investors.

In summary, due to the increase in biomass demand and the competition with current food production processes [44], lignocellulose is seen to be the next major raw material for the production of bioethanol and other biorefinery products. However, additional extensive research is necessary to guarantee the compatibility of the sustainable feedstock with biorefineries and profitability of the process.

3 Regional and Seasonal Feedstock Diversity

3.1 Component Variations

With biorefineries based on renewable plant resources, the biomass composition, fiber structure, and molecular weight of the components differ from species to species [46]. For instance, the lignin content decreases in the order of softwoods, hardwoods, and grasses [47]. Furthermore, variations can also be found within the same biomass category depending on the species and genotype, the plant part, the physiology of the plant, its location, cultivation conditions such as different fertilizer treatments, and the harvesting time [29, 48–51]. This diversity influences both the suitability of plants as a feedstock for bioenergy conversion, as well as the process

and supply chain design [49, 51, 52]. For instance, the structural analysis of nine crops (*Miscanthus*, switchgrass, fiber sorghum, fiber corn, spelt, tall fescue, cocksfoot, hemp, and Jerusalem artichoke) showed that *Miscanthus* has the highest content of cellulose, hemicellulose, and lignin, while the fiber corn shows the highest starch content. Moreover, the later the harvesting date, the lower the content of proteins and mineral compounds in the crops. This effect is caused by a decrease of the non-carbohydrate-rich part of the plant leaf with age, while the related amount of the carbohydrate-rich stem increases. Consequently, late winter harvesting provides a biomass that has a higher content of structural components (cellulose, hemicelluloses, and lignin) compared to an early harvested crop. In fact, the higher total carbohydrate content makes this feedstock more suitable for biorefineries. Nevertheless, harvesting in autumn is recommended more strongly due to the higher total dry matter yield, which outweighs the effect of the carbohydrate content [48].

3.2 On-Site Measurements of Biomass

To implement optimal pretreatment and value-adding processes, easy and rapid analytical methods are needed to characterize the physical and chemical composition of diverse feedstock – preferably on-site. The chemical composition of the native and pretreated biomass is nowadays usually analyzed according to established laboratory analytical procedures, so-called National Renewable Energy Laboratory (NREL) Analytical Procedures (LAP), and American Society for Testing and Material (ASTM) procedures. Many pretreated feedstocks have already been characterized using these procedures and are available on the Biomass Feedstock Composition and Property Database [53]. The most frequently used method was developed by Sluiter and Sluiter [54]. It is based on the total acid hydrolysis of the biomass sample and allows the quantification of the hemicellulose, cellulose, and lignin amount. However, these reactions typically require harsh conditions and analysis takes place only on a laboratory-based scale. To supply biorefineries with suitable feedstock, easier analytical methods need to be established for feedstock characterization.

Infrared spectrometry is widely used to characterize agricultural products. Unlike conventional techniques, near- and mid-infrared spectrometry allows the fast analysis of biomass composition and provides quantitative information within a short time period without any previous sample preparation or degradation. These techniques can be used in off-line and on-line modes and are suitable tools for feedstock characterization. In general, spectrometry has often been applied to characterize the morphological and chemical composition of biomass [55–66].

However, most spectrometric devices are only designed for laboratory use since they are sensitive to vibrations. For on-site measurements, the spectrometric instruments should be integrated, for example in a harvester. This enables the evaluation of transportation aspects and possible performance degradations of the raw materials at an early processing state. Various technical solutions already exist on the market,

where only the measuring head containing the light source and the receiver are in close contact to the measured feedstock, while the evaluation unit and the wavelength separator are placed in the driver's cab of the harvester [67, 68]. The measuring head is most commonly connected to the control and evaluation unit by electric or fiberoptic cables. It can also be integrated in a bus system of the harvester [69]. During the on-site measurements, the either crop moves along the sensor or a sensor is moved along the crop. The collected measuring results and the internal calibration allow the calculation of various biomass components such as proteins, starch, oil content, moisture, or feedstock properties such as cutting length, fiber state, and temperature of the measured materials without the need for sample pretreatment. Additional sensors allow simultaneous detection of the crop throughput and the current position (using GPS) so that all collected values can be stored as a georeferenced data set [70]. The measured values are processed by a computer and can be analyzed.

If near-infrared spectrometry is applied to analyze a biomass composition, the calibration should be qualitatively or quantitatively modelled by multivariate data analysis. The chemometric analysis can be done based on principal component analysis (PCA) and various regression algorithms using specific software. The PCA is usually applied to preliminarily decrease the high data density. Through its application, orthogonal directions of maximal variance and the relationship between variables and objects can be identified [71]. The focus of the PCA is on the determination of qualitative information and identification of the relationship between the absorption and concentration of components in multicomponent samples. To correlate the concentration of individual components with the measured absorption and to quantify the single components, multiple linear regression (MLR) and multiple regression (MR) or a partial least squares regression (PLS2) algorithm can be used afterwards. The advantage of the PLS2 algorithm compared to the other regression methods is that it can utilize all of the information of the whole spectral data set. It accounts for all correlations and can describe them using only a few components [72]. Using this method, multiple variables can be calculated simultaneously. Additionally, the wavelength regions where analytes of interest absorb can be identified by the use of specific algorithms [73]. This method simplifies the analysis and improves the calibration quality [74].

4 Harvest and Pre-Transport Treatments

Despite their abundant supply, readily available biomass streams are rarely allocated homogenously and uniformly. A lignocellulosic biomass from different feedstock origins, such as agricultural and forest residues and dedicated energy crops, often cannot be transported and further processed in an efficient and cost-effective way due to the physical characteristics and complexity of cellulosic biomass [75]. Thus, the supply chain for raw biomass distribution requires several preliminary steps in order to maintain low overall costs. In Fig. 1 the delivery system of feedstock to

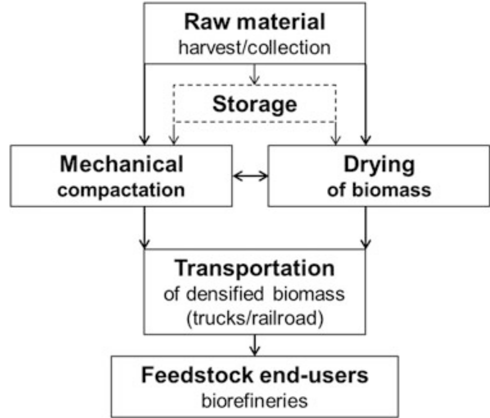

Fig. 1 Graphic representation of the common supply chain from raw biomass to biorefineries

biorefineries as the intended end-users is depicted. These include the collection and harvest of biomass and the subsequent storage or pre-processing of the raw material. Treating the biomass for transport is necessary in order to improve the flowability and stability of the raw material.

Depending on the infrastructural conditions and the distribution policy, different strategies for the handling and transportation of biomass can be applied. General pre-processing methods are readily carried out on-field or after transportation into storage facilities, conversion plants, or in small satellite processing facilities. The main approach for the transportation of the provided feedstock takes place by using existing distribution channels, such as highways or railroads. Depending on the local conditions and the type of transported material, waterways or multi-link transport chains are also used as routes for transportation [76]. Spatial proximity of the location of harvest to the processing facility is preferable since it reduces both the cost of handling (e.g., loading and unloading) and transportation. As the transportation costs of biomass contribute the major costs in logistics, a reasonable strategy for the supply must be worked out [6, 77]. In novel approaches, two logistic scenarios have been distinguished: the conventional bale system (CBS) and the advanced uniform supply system (AUS) [78–80]. The CBS can be designated as a local depot of field-dried and baled biomass, which supplies biorefineries, without changing the properties or stability of the biomass. In contrast, AUS pre-processing depots lead to a final uniform material that can be easily stored and transported into biorefineries. Although the latter involves pre-processing, it still remains more cost-effective for larger biorefineries (up to 10,000 dry metric tons per day) in comparison with the conventional system, and higher biomass volumes can be employed. In CBS, the capacity per hour of loading and unloading of biomass from vehicles into depots is limited [81].

Regardless of the selected facilities and transportation modes for the supply and delivery of feedstock, a pre-transport treatment remains essential for most lignocellulosic biomass, with the possible exception of grass and herbaceous plants that have a very low lignin content. Residual lignocellulosic materials are usually found

Table 1 Raw materials with corresponding bulk densities

Raw material	Bulk density (kg/m^3)	Reference
Grass and crop residues (loose)	70	[84]
Hardwood chips	230, 402.5	[87, 88]
Miscanthus	350	[87]
Softwood chips	180–190	[85]
Straw (loose)	20, 36.1, 24–111[a]	[90–92]
Switchgrass (loose)	49–3,231	[90]

[a]Depending on the water content [95]

to have low bulk and energy densities and high moisture content [77, 82, 83]. The limiting factor of transportation and shipping is therefore often restricted by the large volume of a given biomass as opposed to weight alone. Depending on the type of feedstock used, the volume may alter. Accordingly, an excerpt of reported bulk densities found in the literature is given in Table 1.

Using pre-processing steps, the inhomogeneous structure and size of available biomass can be normalized, which increases the bulk density and simplifies handling and transportation. These steps include cutting and drying of plant material, collection, and mechanical compactation [4, 86, 91].

Mechanical Compactation

In order to make raw biomass transportable and reduce the overall costs, feedstock should be densified before loading [92]. Mechanical compactation of bulky, uneven, or fluffy biomass especially simplifies handling, increases bulk density, and leads to a product with uniform properties. Biomass packing can be distinguished by applied forces by means of briquetting, extrusion, palletization, or tabletizing, resulting in increased bulk densities and characteristic shapes of the densified biomass (e.g., cylindrical, cuboid, pillow-shaped, round). Biomass is mechanically compressed between a roller press, by a screw/piston, or with the aid of a perforated and rotating hard steel die [93–95]. These methods are usually additionally combined with techniques for size reduction such as cutting, grinding, or milling [96]. Common on-field processing includes balling of grasses and crop residues by balers, or using mulchers for woody biomass [87, 97]. In Table 2 bulk densities of raw biomass after compression are given.

Nevertheless, depending on the actual biomass properties (bulk density, size, weight) and external circumstances (harvest season, location, moisture), the achievable densification levels vary [100]. For instance, Chevanan tested the compactation of switchgrass, wheat straw, and corn stover, and determined changes in compressibility from 64–174%, 22–51%, and 42–118%, due to variations in size and pressure levels (5–120 kPa) of chopped biomass [89].

In contrast, woody biomass is often directly treated in sawmills, and is thus available as chips or saw dust. When compacted, pellet density changes adversely with particle size, owing to the larger surface area of smaller particles [101]. However, compactation facilitates the ensuing steps within the transportation chain and

Table 2 Bulk densities of selected raw biomass after densification

Treatment	Straw	Switchgrass
	Bulk density (kg/m^3)	
Baled	110–200 [85], 81–158 [91]	149 [86], 142–186 [98]
Briquettes	–	480–530 [99]
Chopped	20–80 [85]	–
Cubed	320–670 [85]	–
Hammer milled	20–110 [85]	115–182 [91]
Pelleted	560–710 [85]	
Tapped	68–323 [90]	34–130 [90]

might be additionally combined with further preconditioning of biomass located in the vicinity (e.g., by ammonia fiber expansion) (AFEX) [102].

Drying

The drying of biomass is a common practice in on-field handling and harvest of biomass, and makes storage and transportation more convenient. In agriculture, this procedure has been successfully approved in order to prevent degeneration and microbial attacks of biomass [103]. The operation involves different procedural steps, which include mowing of biomass, windrowing, balling, collection, staging, and finally storage of biomass [104]. Typically, stored dry bales must have a moisture content of less than 20% in order to prevent biological degeneration [105]. Depending on the chosen bale shapes (rectangular or round) and the duration of storage, different amounts of dry matter losses might occur, which will have a significant influence on feedstock quantity and quality [106, 107]. Moreover, field-drying is associated with some problems, including harvest timeline, seasonal changes, and risk of rehydration, which influence the overall costs and the degree of dryness [108]. Over-intensive drying might result in irreversible shrinkage of pores and reduction of accessible surface area for enzymatic degradation [109]. Mild drying at room temperature only leads to a decline of small pores, which are already inaccessible for enzyme deconstruction and do not hamper sugar conversion [110]. The concept of drying is often directly combined with mechanical compactation of biomass in the form of pelleting or briquetting. Thus, there are different recommendations for optimal moisture content for the densification procedure: for wood residues an optimal moisture content of 8% was determined, where high-density and long-term performance was seen during compactation [93]. In the case of straw, usually a dry moisture content of 15% or less is used [97], while recently Tumuluru determined that briquettes produced at a low moisture content of 9% yielded the maximum densities of 700 kg/m^3 for wheat, oat, canola, and barley straw [111]. In a parametric study, Rudolfsson found that the pelletizing temperature (125–180°C) and size, as well as the moisture content (0–10%) had an influence on the pellet strength and dimensions of compacted spruce. Using a pressure of 300 MPa for 5 s, the biomass density could be increased to 1,000–1,260 kg/m^3 [112]. Additionally, dry biomass is also appropriate for the production of pellets in

combination with thermal pretreatment such as torrefaction [113]. It has been shown that dry torrefaction of woody biomass leads to a more thorough removal of hemicellulose and to more char combustion reactivity than wet torrefaction [114].

5 Influence of Transportation Cost of Biotechnological Processed Feedstocks

In general, the feedstock transportation costs decrease with increasing feedstock density [115]. Therefore, pre-transport treatment is usually applied to allow for preparation of biomass for optimal transportation. Recent research demonstrates that decentralized biomass processing has a positive effect on transportation costs. The target points of the decentralization process are the transportation of the feedstock from the production place to the central facility, the transportation of the accumulated side products back to the farm, and the capital costs [116].

Decentralization of the storage facilities can have a positive effect on the transportation costs. The transport of biomass from farm sites to the storage facility is usually carried out with the farmer's equipment, which is less efficient compared to a conventional tractor trailer truck. Hence, the introduction of mobile storage facilities into the production process can minimize this distance and improve transport. As a result, savings of 14.8% can be achieved compared to permanently placed storage facilities [117].

Furthermore, the reduction of side and waste product movements due to decentralized processing reduces the transportation costs. Bruins and Sanders [116] suggested a hypothetical decentralized process to produce ethanol from sugar beet, where sugar beet is converted into the crystalline sugar directly at the farm. Based on this proposed process, Kolfschoten et al. [118] developed a three-step small-scale biorefining process for the treatment of sugar beet, also hypothetical. This approach includes firstly the decentralized synthesis of crystallized sugar, secondly sucrose fermentation to ethanol using pulp from extraction and bleed stream from the sugar production, and thirdly the final anaerobic fermentation of all accumulated residues to biogas. The back-transport costs of the side and waste products are lower compared to the traditional centralized facilities, as the residues can simply be left on field. Moreover, the authors reported improvements in economy, energy utilization, and environmental effects by using decentralized preprocessing, and predicted an increase of farmer profits due to the implementation of new agriculture operation areas.

On the other hand, however, decentralized processing can result in additional energy and capital cost, which negatively affects the economy of the process. Therefore, to develop an optimal production process, explicit techno-economic calculations are necessary to define the allocation of required pretreatment steps and to calculate the effect of their decentralization on total costs. For instance, analysis of the decentralization of the currently operating biogas plants and the

biomass logistic (wooden biomass and straw) in the Netherlands showed that decentralization increases the total production costs because the increase in conversion costs was higher than the decrease in transport costs [119].

The decentralization of sweet sorghum conversion to ethanol was investigated by Caffrey et al. [120]. In contrast to the previous examples, in this study the complete techno-economic and LCA analysis of five different scenarios was conducted. The scenarios differ from each other in the number of centralized and local operations. The authors assume the process capacity of 1,683 MT/day. The distance between the farm and the centralized production plant was realistically set to 80.5 km, corresponding to a 100 m^2 collection area. The entire process was divided into four sections: farm, transportation, biorefinery, and by-product utilization. Each of these processes involves significant operations. However, several important technical barriers were not considered. Nevertheless, the primary energy and environmental factors were taken into account and a sensitivity analysis has been done. The results showed that the process decentralization results in a moderate increase of the breakeven sales price of ethanol (0.08 $/L). As a positive effect, an increase in the farmer's profit due to the implementation of the alternative agriculture practices and lower environmental impact was predicted.

In the process that is currently in development at our group, several types of biomass and various possible process options to pretreat biomass for biofuel production are being compared. This study covers only freely available residual lignocellulosic feedstocks not used for further value addition in an area around the city of Kaiserslautern (Germany). Feedstocks in a catchment radius of around 50 km include 30,000 kg of wood, 15,800 kg of straw, and 18,900 kg of garden waste per year. The lignocellulose waste is first pretreated locally and then centrally by Organosolv or hot-water methods, depending on the raw material composition. As a value-adding step, subsequent enzymatic and microbial conversion of the material into biobutanol is performed. The side and waste products are fermented to biogas and fertilizer. Local pre-treatment is required to standardize the heterogeneous feedstock with respect to the moisture content, density, and particle size for optimal transport, storage, and further processing. Decentralized pretreatment using pressing, pre-drying, and shredding results in an increase in feedstock density. To analyze the effect of the local pre-transport treatment and to identify the optimal number of local operations and the plant capacity, different scenarios have been developed in silico. Here, the energy demand, investment, and transportation costs are considered. As expected, an increase in the processing capacity improves the profitability due to the economy of scale. It was shown that a significant reduction of transportation costs (by a factor of 10) and an improved process economy can be achieved if all three pre-treatment steps are carried out locally.

6 Decentralized Value-Adding Options

The efficiency of lignocellulosic conversion plants is highly dependent on the logistics of the biomass distribution. Lignocellulosic biomass, including agricultural residues (wheat straw, corn stover) and forestry residues, often appears locally in limited quantities and with low bulk densities, which adversely affects the cost effectiveness of the process [102]. Furthermore, merging and processing of different feedstocks requires several preliminary stages, which include harvest, transportation, preprocessing, and the creation of value-added products out of biomass. Additionally, it has to be considered that the delivery of biomass in sufficient quantities at modest costs into a conversion plant is one of the major challenges [14]. In order to overcome this problem, economical handling of the biomass is necessary, which includes the reduction of dry matter losses during storage, utilization of available pipelines or distribution paths for the supply of feedstock, as well as novel approaches for pretreatment of biomass. A sustainable option is provided by the implementation of several decentralized process-units in existing biomass-processing facilities (farms, co-operatives, forestry industry), which increases the efficiency of biomass usage [121]. Processing of biomass on a regional level might also allow local economic opportunities by providing a uniform and consistent feedstock supply at a modest investment cost [120]. Transformation of harvested biomass into valuable products consists of four major steps: pretreatment, hydrolysis, fermentation, and product recovery. Consequently, advanced conversion technologies must be developed that allow efficient biomass transformation at a regional level.

Bag-Hydrolysis

One of the major obstacles in using lignocellulosic biomass as a source for product generation is the recalcitrance of biomass followed by the necessity of a pretreatment step. Currently, most lignocellulosic raw materials are therefore mechanically or thermochemically pretreated, followed by enzymatic hydrolysis [122, 123]. The available carbohydrate content of raw materials is between 56% and 74% and can be converted into mono- and disaccharides by enzymatic hydrolysis [124]. Typically, enzymatic hydrolysis is conducted in stirred tank reactors, which might not be feasible for use in decentral processing units, since higher investments costs are necessarily paired with high operating costs due to stirrer power consumption. Enzymatic hydrolysis at high solid loadings (>15%) increases the process economics, but leads to higher viscosities and yield stress coupled with poor rheology [125]. The prerequisite for a cost-effective biomass conversion is a reactor design that allows a maximal conversion of cellulose with a minimal amount of enzymes at high solid loadings [126]. On a laboratory scale, different modes of saccharification such as shaking, gravitational tumbling, and hand stirring have been investigated and have shown that effective initial mixing facilitates high conversion rates, due to sufficient enzyme distribution [127]. As part of this, new reactor concepts are currently developed that focus mainly on the employment of new or combined

stirrer models [128–130] or the use of existing solid-state bioreactors for high solid loadings with maximal working volumes of 3–4 kg [131].

For an on-site hydrolysis biomass operation, single-use bag reaction systems have been considered. These are relatively simple reaction vessels made of polyethylene (0.2 mm thick) that can be mixed by simple rocking movements. As a substrate, beech wood or crude beech wood cellulose fractions are used up to solid concentrations of 10% (w/w) with low enzyme dosages. In Fig. 2, the temporal change of liquefication of pretreated biomass during enzymatic hydrolysis in a single-use bag system is depicted.

After only 8 h of hydrolysis, degradation of previous fiber structures is already apparent. With increasing hydrolysis time, full liquefication and visible loss of solid matter is reached. The rocking motion of the bags in combination with enzymatic conversion leads to homogenization of the slurry. Figure 3 shows the course of hydrolysis during enzymatic conversion of the pretreated biomass.

The utilization of a single-use bag system allows the enzymatic conversion of pretreated beech wood into glucose and xylose, reaching maximum concentrations of 48 g/L glucose from cellulose and 13 g/L xylose from the remaining hemicellulose, respectively. Direct application on-farm seems to be possible, since a low-cost polyethylene foil can be easily prepared and welded for use with simple and minor equipment costs. Moreover, further truck transportation while enzymatic conversion takes place is conceivable if the trucks are equipped with systems comparable to

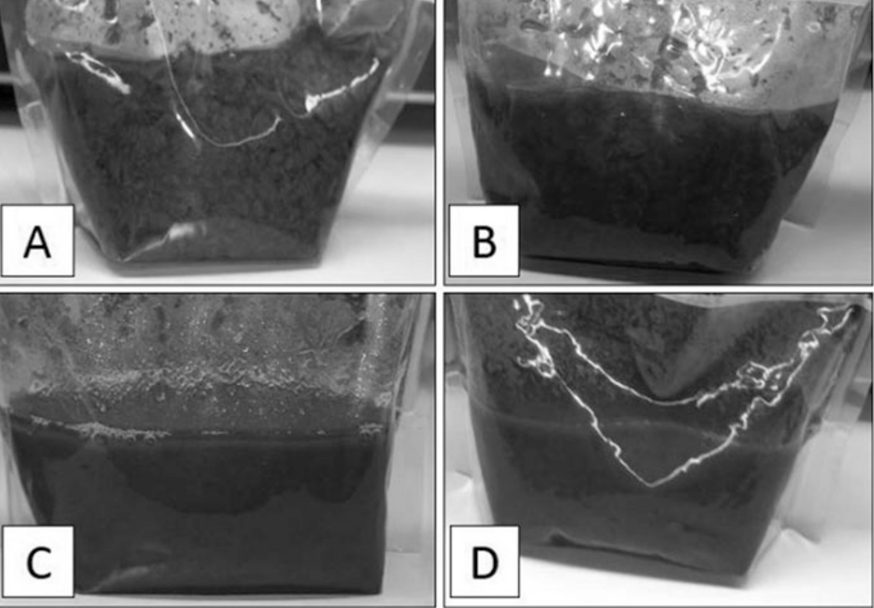

Fig. 2 Enzymatic hydrolysis in single-use bag systems, with a crude cellulose solid loading of 100 g/L. Duration of hydrolysis: (**a**) 3 h, (**b**) 8 h, (**c**) 20 h, (**d**) 30 h. Temperature: 50°C

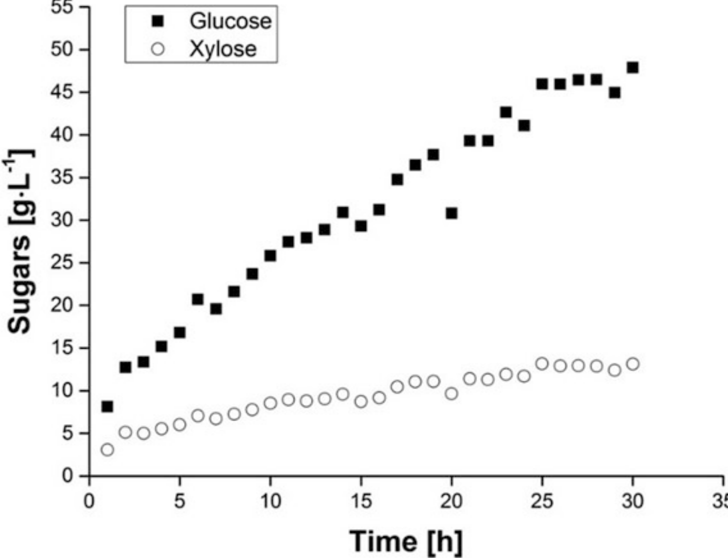

Fig. 3 Glucose and xylose concentrations during enzymatic hydrolysis at a crude cellulose (with Hemicellulose Impurities) solid loading of 100 g/L using single-use bags. Enzymes: 6% cellulase, 6% xylanase. Rocking-motion: 30 rpm. Temperature: 50°C

concrete mixers. Mixing by stirring has been shown to be an adequate way of mixing in enzymatic hydrolyses. Hence, it needs to be further examined whether this approach is economically competitive. This concept leads to further conversion potentials such as simultaneous transportation and saccharification, which has already been discussed for direct pipeline application [132]. First findings indicated that transport of biomass slurries from wood residues and wheat straw in pipelines is possible at approximately 30% biomass loadings with regard to rheology [133–135].

Ensiling of Biomass and Product Generation

A common method for long-term preservation of medium moisture content lignocellulosic plant material, such as from grassland, can be provided by ensiling the biomass. The resulting silage is the product of solid-state lactic acid fermentation, which can be used as a high-quality animal feed the entire year [136]. During storage of fresh plant material under anaerobic conditions, the production of organic acids, as well as a pH shift, takes place. This prevents the growth of other microorganisms and the decomposition of the plant biomass [137]. Other additional benefits compared to dry storage are that biomass loss during handling is reduced and there is no need for costly pre-drying of the biomass. Additionally, ensiling can be considered as a combination of storage and pretreatment of biomass [138]. Direct on-farm realization of both storage and pretreatment makes handling considerably easier, since pretreatment is conducted at ambient temperature and pressure without the need for chemical or thermal pretreatment, leading to cost and energy savings.

Nowadays, ensiling is also suggested as a biological pretreatment for lignocellulosic biomass and has been reported to improve the enzymatic saccharification of biomass [139]. In several studies using pretreated silage biomass, anaerobic fermentation using the yeast *Saccharomyces cerevisiae* or *Kluyveromyces marxianus* was conducted separately from the ensiling process allowing for conversion of available sugars into ethanol [140–143]. On a laboratory scale, a combined approach of ensiling and simultaneous saccharification and fermentation with the addition of cellulase and glucoamylase for 20 days resulted in 6.5 wt % ethanol (a yield of 169 g/kg dry mass) using 250 g non-sterile forage paddy rice plants [144]. This indicates that a reaction of lactic acid fermentation and subsequent ethanol fermentation coupled with the enzymatic deconstruction of plant material is possible. Thus, a decentralized ethanol production system that can be directly applied in the on-farm level was proven on a laboratory scale. In a further study of Horatio et al., this system was extended and directly applied on-farm [145]. For this purpose, rice plants were chopped, harvested, and baled, resulting in 0.8 m tall round bales of 1-m diameter with a weight of 273–283 kg (48–50% dry weight). Before baling, a mixture of commercially available enzymes and microorganisms for lactic acid fermentation and ethanol production was dissolved in 40 kg of distilled water and added to the feedstock. In detail, cellulase (0.74–0.77 FPU/g DM) of *A. celluloslyticus* and a glucoamylase from *Aspergillus niger* (0.29 g/g DM) was employed as well as freeze-dried lactic acid bacteria (2×10^5 cfu/g DM) and freeze-dried *S. cerevisiae* (3×10^6 cfu/g DM). The bales were then wrapped with a plastic film for ensiling. The resulting effluent was collected by further entrapment of the bale system with a water-impermeable polyethylene plastic foil. The entire solid-state fermentation was monitored directly on-field without temperature control by sample collection from different locations of the bales. The effluent collected at the bottom of the bales was recovered monthly and ethanol was recovered with a vacuum distiller. During the operating period of 1–6 months, 90.9–139.6 g/kg DM of ethanol was produced during Simultaneous Saccharification and Fermentation (SSF) in whole round bales, which corresponds to a maximum yield of 14 wt % ethanol.

7 Conclusion

In recent decades, various studies have been conducted worldwide focusing on developing strategies for feedstock supply for lignocellulose-based biorefineries. Numerous plant residues have been evaluated by researchers, including abundant residues from forestry and agriculture, but also certain residues available in smaller quantities regionally. To date, the decentral approach is a promising model, since transportation costs of biorefinery feedstock contribute vastly to the overall costs of the process. Further, excessive biomass conveyance is extremely likely to lead to high climate-damaging gas output and therefore to contravene the goal regarding eco-friendly biorefineries. Transferring intermediate products instead will lead to a

reduced transport volume and is therefore preferable to long-distance transportation of raw plant residues.

The degree of on-site pretreatment should be determined with consideration of local and regional feedstock types, their availability, and the applicability of pretreatment methods. The benefits of a reduction of transport volume by mechanical methods has been studied intensively by various researchers in recent years. Even though the positive effects of a reduction of transportation are evident, the exclusive use of mechanical methods is rarely adequate to achieve low transportation costs. The introduction of rot fungi during a storage period has been shown to improve the feedstock convertibility while requiring neither extensive equipment nor training. The improvement of transportation efficiency needs to remain a central aspect of research concerning the implementation of lignocellulose-based biorefinery plants.

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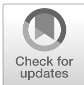
Vegetable Oil-Biorefinery



Frank Pudel and Sebastian Wiesen

Abstract Conventional vegetable oil mills are complex plants, processing oil, fruits, or seeds to vegetable fats and oils of high quality and predefined properties. Nearly all by-products are used. However, most of the high valuable plant substances occurring in oil fruits or seeds besides the oil are used only in low price applications (proteins as animal feeding material) or not at all (e.g., phenolics). This chapter describes the state-of-the-art of extraction and use of oilseed/oil fruit proteins and phyto-nutrients in order to move from a conventional vegetable oil processing plant to a proper vegetable oil-biorefinery producing a wide range of different high value bio-based products.

Keywords Glycerol, Phyto-nutrients, Plant protein, Processing, Refining, Vegetable oil

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1 Introduction

Conventional vegetable oil processing plants, consisting of oil mill, refinery, and modification unit, process oil fruits or seeds to vegetable fats and oils of high quality and predefined properties. Nearly all by-products are used: meal as protein-rich animal feeding material, lecithin as a food additive, free fatty acids in chemistry and deodorizer distillates as sources for the recovery of valuable phyto-nutrients such as phytosterols or as feed for biodiesel plants.

The extension of such plants by a biodiesel plant is often already called a vegetable oil-biorefinery. However, most of the high value plant substances occurring in oil fruits or seeds, besides the oil, are used only in low-price applications (proteins in meal) or not at all (e.g., phenolics). This chapter shows the potential to move from a conventional vegetable oil processing plant to a proper vegetable oil-biorefinery producing a wide range of different high value bio-based products.

2 Biomass Usable

Vegetable fats and oils can be divided into fruit and seed (kernel) oils. Typical fruit oils are palm oil, olive oil, and avocado oil. They are obtained from the pulp of these fruits. Because of the rapid enzymatic hydrolysis of ripe fruits, which can be accelerated by mechanical damage, they have to be harvested and processed as quickly as possible after reaching ripeness. In contrast to that, oilseeds are more resistant and can be stored after drying over a long period. The globally most

Table 1 Global production of fruit and seed oils [1]

Vegetable oil	Global production (million tons per year)
Palm oil	56
Soybean oil	43
Rapeseed oil	25
Sunflower oil	14
Palm kernel oil	6
Cotton seed oil	5
Peanut oil	4
Coconut oil	3
Maize oil	2.93
Olive oil	2.85
Sesame oil	0.87
Castor oil	0.68
Flaxseed oil	0.6

important seed oils are soybean oil, rapeseed oil, sunflower oil, peanut oil, cottonseed oil, and palm kernel oil. In addition, flax oil, hemp oil, safflower oil, grape seed oil, and others are produced in smaller amounts. Castor oil, tung oil, and jojoba oil are inedible and used only for chemical purposes. Particularly as bio-energy source, jatropha and camelina are upcoming new oil crops (Table 1).

3 Schematic and Principals of the Biorefinery

3.1 Biodiesel Process

The extension of an existing oil mill by a biodiesel plant is often called a (simplest kind of) biorefinery.

Transesterification of triglycerides using methanol, or sometimes ethanol, leads to fatty acid methyl/ethyl esters, known as biodiesel. Transesterification is a catalytic reaction and base catalysts, acids or, enzymes (lipases) are used.

The main by-product of biodiesel production is glycerol. There are many technologies for purification and transformation into valuable chemicals available or under development, biotechnological ones particularly [2].

3.2 Enzymatic (Rapeseed) Biorefinery Concept

The aim of the enzymatic biorefinery concept is an environmental friendly processing of oilseeds with the comprehensive fractionation of the pre-treated crop into oil, protein, and valuable bio-active compounds at the end of the process, usable in the food, non-food, and feed industries. Another characteristic feature of

the enzymatic biorefinery concept is economic activities in closed circles, for example, short routes of transport. The key step is enzymatic treatment of the raw material, which allows a gentle extraction and fractionation of the different components without the use of organic solvents and without changing the functional properties of the resulting products.

The enzymatic biorefinery process for rapeseed which was initially developed by Novo Nordisk and the Chemistry Department of the Royal Veterinary and Agricultural University, Copenhagen, Denmark in the late 1980s is well investigated. The concept involves (1) the inactivation of enzymes such as lipoxygenase, myrosinase, and lipases which would adversely affect the final products, (2) degradation of cell wall constituents by enzymes in milled seed material suspended in water, and (3) separation of the different fractions, oil, protein-rich meal, syrup, and hulls by centrifugation. The physicochemical properties of this extraction system and the good water solubility of many high value compounds, such as glucosinolates, usable as natural pesticides and some proteins, permit the simultaneous extraction of oil and these products from cruciferous oilseed meals [3].

After cleaning the seeds and milling in a hammer mill, inactivation of enzymes takes place by heating at 85–90 °C for 20 min with water [4]. Then cold water has to be added before the treatment with cell wall-degrading enzymes based on *Aspergillus niger* is performed for about 4 h at 50 °C. In the following, the hulls are separated by decantation, oil, protein and other valuable compounds are obtained by three washing and centrifugation steps, and finally the fractions with protein-rich meal and syrup are spray-dried.

The oil yield from an enzymatic biorefinery is about 35% based on the seed dry matter, which is distinctively lower than for conventional solvent extraction [5]. Because of the higher content of antioxidants, the oil shows a better oxidative stability, and during biorefinery processing less phospholipids go into the oil, resulting in amounts of 0.03% compared to 1.8% for conventional processing [6]. The content of other unwanted substances is also very low, and thus further oil purification by refining is not necessary [7].

Another advantage of the enzymatic biorefining concept is the mild treatment of the raw material which allows the isolation of valuable bio-active compounds from the meal. This may improve the quality of the meal with regard to suitability as animal feedstuff. An example is the isolation of pure glucosinolates which adversely affect the usability of the meal and limit the use of rapeseed meal as feedstuff. The isolated glucosinolates can be used as natural pesticides in crop management [8]. In the same way, pure myrosinases can be isolated directly from the crop [5].

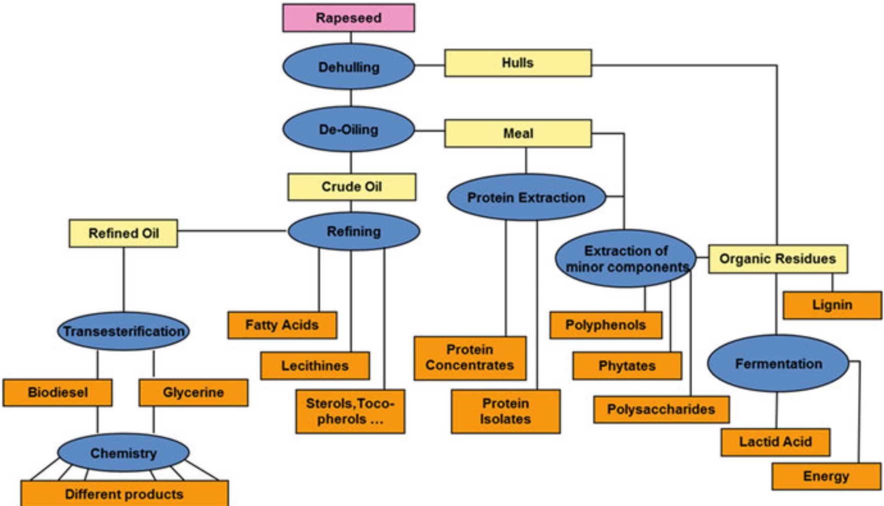

Fig. 1 Rapeseed biorefinery concept

Table 2 Protein content of different oilseed products [9]

Source	Crude protein (% of fresh weight)
Soybean meal	44.88
Rapeseed meal	35.51
Sunflower meal	28.51
Flaxseed meal	34.27
Peanut meal	33.67
Cotton seed	32.22
Sesame meal	43.32

3.3 Subsequent Extraction of Oil, Proteins, and Phyto-Nutrients

Figure 1 shows exemplarily a rapeseed biorefinery concept based on the subsequent extraction of oil, protein, and phyto-nutrients.

Besides the oil, most of the oilseeds also contain considerable amounts of proteins, particularly storage proteins; see Table 2. In the form of protein-rich meal, the by-product of oil extraction, these are commonly used as animal feeding material. Because of both their high nutritional potential and their manifold functional properties, a wide range of new applications in human nutrition as well as in non-food use could be developed. To meet the specific requested quality, a concentration to 50–60% protein in the matter (flours), 65–80% (concentrates), or >85% (isolates) is required. This is often connected with a decrease of undesired secondary plant substances.

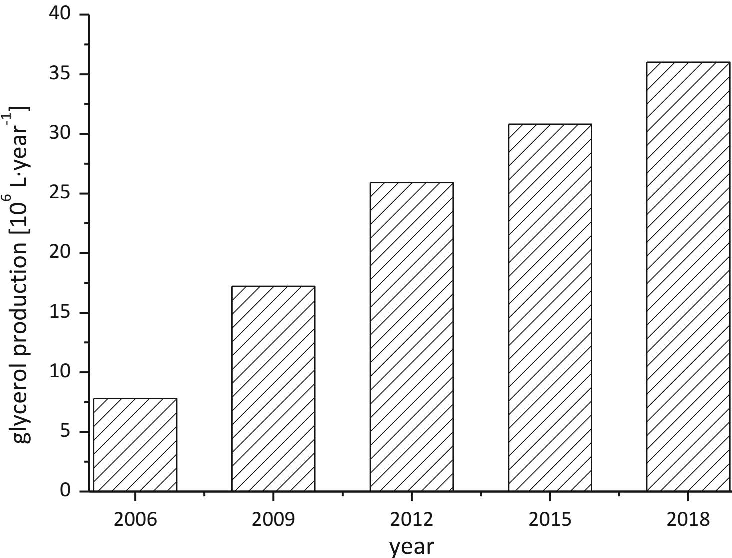

Fig. 2 Scenario for the worldwide potential of crude glycerol according to [11]

Additionally, most oil fruits and seeds contain several phyto-nutrients, which can be used in the pharmacy, food, feed, and cosmetics industries. Typically, they remain in the oil or meal fraction after oil mill processing.

3.4 Biotechnological Utilization of Crude Glycerol

3.4.1 Potential of Crude Glycerol

The production of biodiesel by transesterification of plant oil, animal fat, and oil-containing microorganisms is a continuously growing industrial application. During the biodiesel process, for 1 mol of fatty acid methyl ester, 1 mol of glycerol is generated as a side product. This accounts for almost 10 wt% of the product stream [10]. Additionally, crude glycerol is generated as a side product during the production of fatty acids and soap. Recent numbers for the forecasted development of the annual glycerol production have been publicized by Nanda et al. and can be found in Fig. 2.

After the transesterification process, the glycerol phase is separated from the fatty acid methyl ester phase, when the crude glycerol can be purified by removing water and methanol. Depending on feedstock and transesterification process, the crude glycerol has a purity of 75–90% and contains different impurities such as water (5–14.2%), methanol (up to 1.7%), remaining fatty acids, esters, and partial

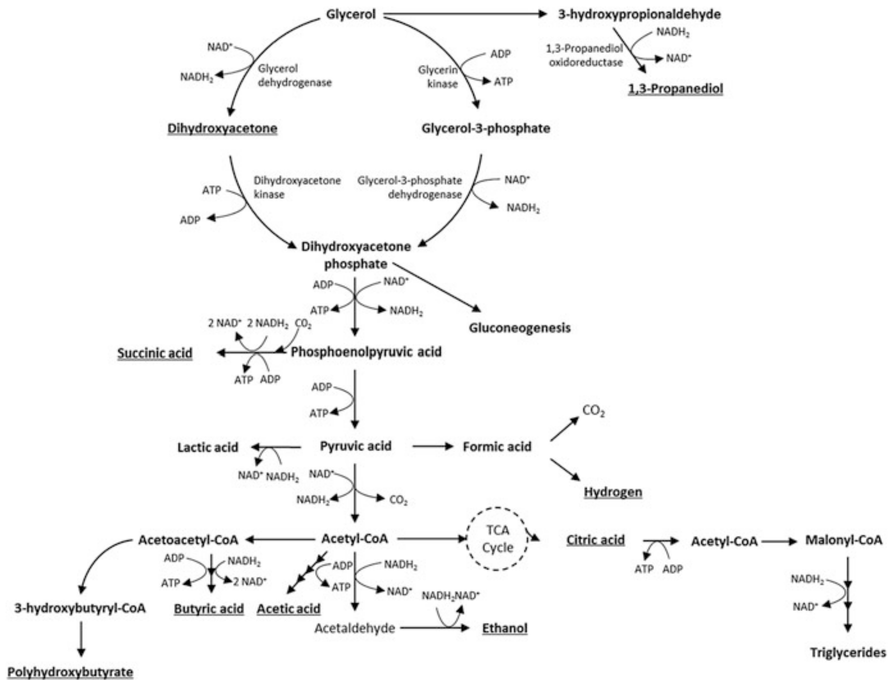

Fig. 3 Biochemical pathways for the generation of value-added chemicals based on glycerol by prokaryotes and eukaryotes. Adapted from [40]

glycerides, and, depending on which catalyst has been used, 4.2–5.5% NaCl or 0.8–6.6% K_2SO_4 [12, 13].

The growing supply of glycerol has led to a drastic reduction of its market price. In 2007 the price was in the range of 0.50 €/kg in comparison to 1.15 €/kg before the expansion of biodiesel production. At the same time, the crude glycerol price has fallen from 0.41 € to 0.082 €/kg [14].

The upgradation of biofuel side products falls under the fourth generation biofuel strategy of minimum waste production. By using this cheap and abundant feedstock, biodiesel plants could develop to versatile biorefineries by adding chemicals, materials, and energy to their portfolio. This could be an important step in improving the economic feasibility of the plants [15].

3.4.2 Utilization of Crude Glycerol

Even though there are more than 1,500 known applications for glycerol [16], the classical glycerol market is unable to utilize the huge amounts generated nowadays. This surplus of raw material leads to an increased interest in processes which can raise the value of glycerol. Its value can be increased by chemical or biological processing. Most chemical conversions are based on oxidation or reduction of the

Table 3 Selected bioconversions of crude glycerol to different products

Species	Strain	Product/yield (mol/mol glycerol)	Reference
Clostridium	*C. pasteurianum*	*n*-Butanol/0.43	Jensen et al. [24]
	C. butyricum AKR102a	1,3-PD/0.63	Wilkens et al. [25]
	C. diolis DSM15410	1,3-PD/0.67	Wiesen et al. [26]
Escherichia	Engineered *E. coli* SY03	Ethanol/0.95	Yazdani et al. [27]
	E. coli AC521	Lactic acid/0.9	Hong et al. [28]
	Engineered *E. coli*	Succinic acid/0.8	Zhang et al. [29]
Citrobacter	*C. freundii* FMCC-B294	1,3-PD/0.48	Matsoviti et al. [30]
	C. freundii H3	H_2/0.94	Maru et al. [31]
Gluconobacter	*G. frateurii* CGMCC 5397	DHA/0.89	Zheng et al. [32]
Klebsiella	*K. pneumoniae*	Ethanol/0.89	Oh et al. [33]
	K. pneumonia (encapsulated)	1,3-PD/0.65	Zhao et al. [34]
Propionibacterium	*P. freudenreichii*	Propionic acid/0.68	Kośmider et al. [35]
Fungus	*A. niger* strains	SCO/0.41 g/g BM	André et al. [36]
Yeast	*Y. lipolytica* Wratislavia AWG7	Citric acid/0.67	Rywińska et al. [37]
	P. tannophilus CBS4044	Ethanol/0.56	Liu et al. [38]
Microalgae	*A. limacinum*	Docosahexaenoic acid	Abad et al. [39]

Adapted from [15]

glycerol or a connection with another molecule. The most important reduced products of glycerol are acrolein and propanediol. By oxidation of glycerol, glyceric acid (chelating agent) and tartronic acid (food additive) can be produced. Glycerol carbonate is an example of an important product from a reaction with another molecule [17].

In comparison to chemical reactions, bioconversions show an increased reaction specificity, lower requirements for temperature and pressure, and a lower demand for toxic chemicals. Therefore, value adding with enzymes or microorganisms is usually preferred [8]. In comparison to sugars, glycerol has a higher degree of chemical reduction. The transformation of glycerol to intermediates of the glycolysis generates double the amount of reduction equivalents compared to glucose and xylose [18], which leads to higher yields in the production of fuels and reduced

chemicals compared to monosaccharides [19]. The availability and the additional benefits of the substrate have led to an increased interest in processes for the production of chemicals, biopolymers, and fuels based on glycerol. Such processes have been investigated recently with wild type as well as with genetic modified organisms. Potential products are, for example, polytrimethylene terephthalate, made from 1,3-propanediol, succinic acid, 2,3-butanediol, polyhydroxyalkanoates, single cell oil, ethanol, butanol, citric acid, polyols, itaconic acid, and dihydroxyacetone [13, 20–22], whereas the production of 1,3-propanediol tends to be the most promising application for glycerol [23]. Figure 3 gives an overview of the products which can be produced in biotechnological processes utilizing glycerol as substrate.

The diversity of products, which can be produced from glycerol via bioconversion technologies, has been recently summarized by Garlapati et al. [6]. In this chapter the focus is given to 1,3-propanediol, which is seen as the most promising secondary product of glycerol [23]. Table 3 gives some examples of bioconversions of crude glycerol by different microorganisms.

When utilizing cheap crude glycerol as substrate for fermentations, the quality of the glycerol and the tolerance of the production organism against impurities play a crucial role. Some studies have shown that crude glycerol exhibits a growth inhibition effect on some microorganism. This effect can even vary a lot within the same species [41]. The type of impurities present in the crude glycerol strongly depends on the production process, for example, the type of catalyst, whereas the purity of the crude glycerol varies between 75 wt% and 90 wt%. Further components of crude glycerol are mainly water, but also methanol, free fatty acids, and salts (catalysts or buffering salts). These impurities can often be found in concentrations at which they also have an inhibitory influence to the growth of microorganisms [42]. For this reason, it might be necessary to pretreat the glycerol before it can be efficiently used as a substrate in a fermentation process. The effects of different impurities are described in the literature [13, 43]. Methanol and NaCl both show no inhibitory influence in well-mixed systems at concentrations up to 2 g/L and 6 g/L, respectively. However, free fatty acids such as oleic acid lead to considerable inhibition of the growth of *Clostridium butyricum*, beginning at a concentration of 0.25 g/L.

3.4.3 Production of 1,3-Propanediol

1,3-Propanediol (1,3-PD) is a molecule with two endstanding hydroxyl groups, and thus offers a great potential for applications in synthetic chemistry, for example, as a monomer for polycondensation. Products of this syntheses can be polyesters, polyethers, and polyurethanes, but a multiplicity of other applications are possible with 1,3-PD [44]. The 1,3-PD-based polymer polytrimethylene terephthalate (PTT) is even more durable and colorfast than its ethylene glycol-based counterpart polyethylene terephthalate (PET). It also shows superior stretching and stretch recovery characteristics [45]. Because of the superior characteristics of PTT, its production has led to an increased demand for 1,3-PD [46]. PTT is especially useful

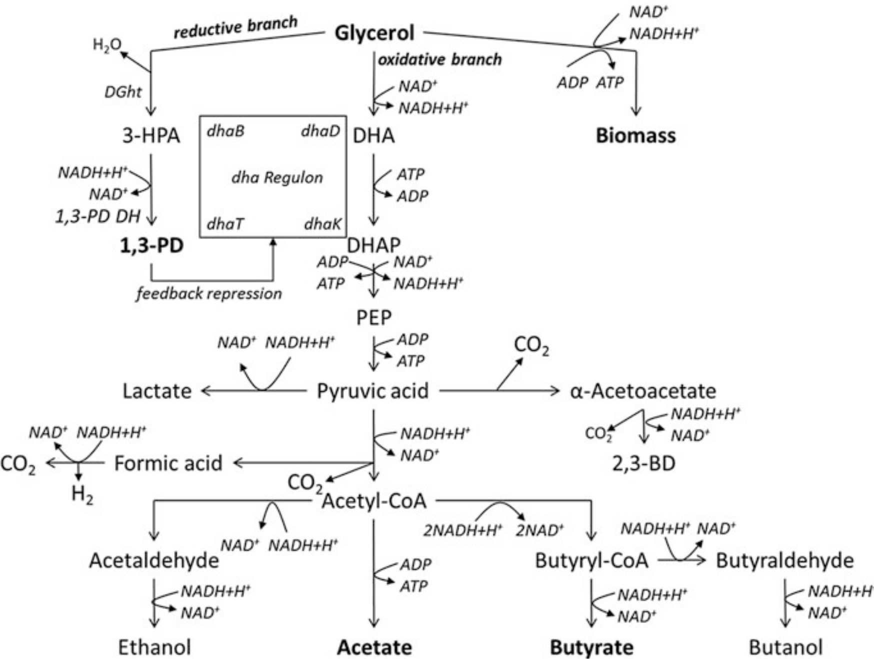

Fig. 4 Glycerol metabolism of 1,3-PD-producing organisms. *Rectangle*: key genes of the *dha* regulons. *GDHt* glyceroldehydratase, *1,3-PD DH* 1,3-propanedioldehydrogenase, *PEP* phosphoenolpyruvic acid, *2,3-BD* 2,3-butandiol, *DHA* dihydroxyacetone, *DHAP* dihydroxyacetone phosphate, *3-HPA* 3-hydroxypropanaldehyde, *1,3-PD* 1,3-propanediol. Adjusted according to [52]

for the production of carpets and textiles, and PTT is also biodegradable. Because of the high production costs, the utilization of 1,3-PD has been restricted in the past. However, in the mid-1990s this situation changed. The companies Shell and DuPont announced the commercialization of the 1,3-PD-based polyester PTT. According to Shell, the price of PTT is competitive with PET (about 0.75 €/kg). This development has led to a massive boost in the production of 1,3-PD [47]. In 2012 the market demand for 1,3-PD was over 60,000 tons. By 2019 it is expected that the demand should increase to approximately 150,000 tons [48].

Traditionally, 1,3-PD is produced chemically and there are two main processes. The first is based on acrolein, followed by two hydrogenation steps. The second involves hydroformylation of ethylene oxide followed by hydrogenation. The yield of both processes is 40–80%, and high pressures and temperatures are involved. Additionally, toxic side products accumulate in both cases [49].

Fermentative Production of 1,3-Propanediol

By biological means it is possible to produce 1,3-PD via fermentation. 1,3-PD is one of the oldest known fermentation products, discovered in 1881 by August Freund in a fermentation utilizing *Clostridium pasteurianum* amongst others [50]. Other microorganisms capable of producing 1,3-PD come from the genera of *Citrobacter, Clostridium, Enterobacter, Ilyobacter, Klebsiella, Lactbacillus,* and *Pelobacter*. All these organisms have in common that they produce 1,3-PD by a two-step enzyme-catalyzed reaction sequence. In the first step, glycerol is converted to 3-hydroxypropionaldehyde (3-HP) and water by a dehydratase. In the second step, 3-HP is reduced to 1,3PD by the action of an NAD^+-dependent oxidoreductase. 1,3-PD is not further metabolized and accumulates in the medium. The whole reaction uses a reduction equivalent in the form of the cofactor nicotinamide adenine dinucleotide ($NADH+H^+$), which is oxidized to NAD^+ [51]. In the case of *Clostridium pasteurianum*, butanol is generated as a side product, which is converted from butyryl-CoA via butyraldehyde. Side products such as ethanol, lactic acid, succinic acid, and 2,3-butanediol appear in the metabolism of *Enterobacteria* [52]. Figure 4 gives an overview of the metabolism of 1,3-PD-producing organisms.

4 Current Process Technologies

4.1 Palm Oil Processing

As an example for the processing of fruit oils, palm oil processing is briefly described. Ripe fruit bunches are harvested and transported as quickly as possible to the oil mill, which is mostly situated in the heart of the oil palm plantation. The first processing step is sterilization of the fresh fruit bunches (FFB) to inactivate the enzymes and to decompose the material. Sterilization is carried out with direct steam (3 bar, 130 °C, 2 h). After that, the single fruits are stripped from the bunch stalks. The fruits are digested and pressed to separate the kernels. The crude palm oil (CPO) is separated and purified from the residual pulp by different steps of sieving and separation. Finally it is vacuum dried. The palm kernel oil (PKO) is obtained from the kernels by mechanical pressing. From 100 tons of FFB about 20 tons of CPO and 6 tons of PKO can be produced. The empty bunches, kernel fibers, and the sludge are used as compost, as burning material, or for irrigation purposes.

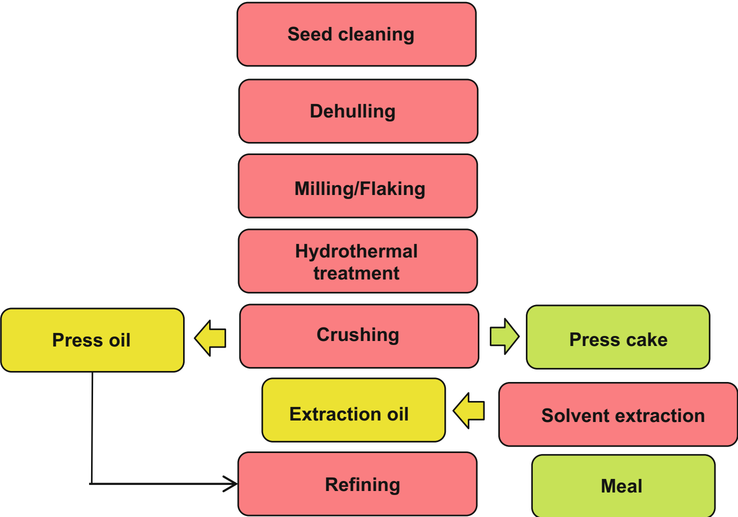

Fig. 5 Conventional seed oil production

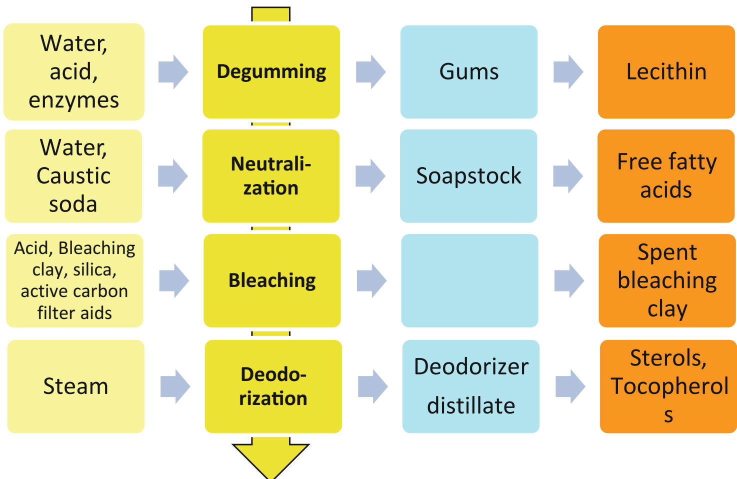

Fig. 6 Conventional refining process

4.2 *Oilseed Processing*

Oilseeds are processed completely differently from oil fruits. Figure 5 shows the typical process scheme of a seed oil mill. At first the seeds (or kernels) have to be cleaned. Impurities, sand, stones, and pieces of metal have to be removed. Some kinds of oilseeds are dehulled, such as peanuts, cotton seeds, soybeans, and sunflower kernels. Dehulling of rapeseed is only done at Teutoburger oil mill

(Germany). To improve oil extractability, the seeds are milled, flaked, and sometimes structured (e.g., by expanders). Subsequently, a hydrothermal treatment is necessary to adjust optimal temperature and moisture for de-oiling, to inactivate enzymes, and to degrade undesired minor components influencing the feeding quality of the meal negatively. Sometimes the seeds are roasted to influence the sensory properties of the oil. De-oiling itself is done by mechanical and/or solvent extraction. It can be distinguished as one- or two-step mechanical extraction with screw presses, which is done by small- or medium-sized oil mills and combined mechanical and solvent extraction, using oil mills of large capacity (>500,000 tons/year). Because of their low oil content, soybeans are only solvent extracted. Pre-treatment and the kind of extraction technology determine the oil yield. After mechanical pressing, the residual oil content in the meal is usually not lower than about 7%, whereas it can be reduced to about 1–2% by solvent extraction. Industrial solvent extraction plants use *n*-hexane as solvent.

4.3 Refining

More than 90% of all produced crude oil is refined to ensure the highest quality over a long period. Refining removes oil soluble secondary plant substances and both environmental and processing contaminants. Because not only undesired but also valuable components are removed, the refining technology is optimized for each kind of oil. Figure 6 shows the typical refining process which consists of degumming, neutralization, bleaching, and deodorization. Modern refining plants have a capacity of 200–2,000 tons/day and work continuously.

In the degumming step, phospholipids are removed by water, citric or phosphoric acid, or enzymatically, and can be used as lecithin in several food and other applications. Neutralization removes the free fatty acids (ffa) by saponification (chemical refining). The soaps are washed out. By soapstock splitting, ffa can be produced for chemical applications. Because of its environmental advantages, more and more oils are neutralized by physical refining. In this case, ffa are removed by distillation within the deodorization step. Bleaching removes not only colors but also different pro-oxidative substances, non-volatile polycyclic aromatic hydrocarbons (PAH), phosphorus- and nitrogen-containing pesticides, and mycotoxins. Bleaching is an adsorption process using natural or acid activated bleaching clays, silica materials, or activated carbon. The spent adsorbent has to be removed from the oil and is commonly disposed of. Deodorization removes undesired odors and flavors, but also ffa, hydrocarbons, volatile PAH, and chloride-containing pesticides. In the deodorizer the oil is heated to 240 °C (palm oil up to 270 °C) under a vacuum in the pressure range of 1–5 mbar. A certain amount of direct steam is led through the oil, stripping the volatile substances out. These are collected after condensing and sold as deodorizer distillate.

4.4 Alternative Technologies

In the literature, a lot of alternatives to the commercial technologies, especially for cell disruption and de-oiling, are described. Enzymatic pre-treatment prior to oil extraction can enhance the oil yield of mechanical pressing, but it is cost intensive, particularly because of the additional drying which is needed because the available enzymes need a certain amount of water to act.

Enzyme-assisted aqueous extraction (EAAE) is an emerging technology for simultaneous oil and protein extraction from oilseeds, and it may offer many advantages compared to conventional extraction [53]. Degumming and refining processing steps can be eliminated and it may allow the removal of some toxins or antinutritional compounds from oilseeds. Enzymatic processes have been tried on various oilseeds to facilitate oil and protein extraction [54–63]. Investigations on enzyme-assisted aqueous extraction of rapeseed has been described in more detail; see Sect. 3.2.

Other means for better cell disruption are the use of microwaves, radio frequencies, or pulsed electric fields. In contrast to some published data, it could not be confirmed that the use of pulsed electric fields has a positive influence on oil (or protein) extraction rate [64].

Supercritical fluids are used as solvent for high value natural products such as high quality vegetable oils. Supercritical fluids, similar to CO_2, are non-toxic, non-flammable, have good solvent power under mild conditions, and are easy to remove from the product. The biggest disadvantage lies in the high investment costs to install such a plant.

A new crushing alternative is gas-assisted pressing, proposed as HIPLEX® by companies such as Crown Iron Works or Harburg-Freudenberger, where CO_2 is led into a conventional screw press. This leads to higher oil yield and better oil and protein quality because of the lower temperature stress in the press [65, 66].

4.5 Fat Modification

In their native form, most edible oils have only limited application, particularly in food products. They are therefore often modified, chemically and/or physically to alter their textural properties.

Oil modification is part of the activities of modern oil processing plants. In the industry, three principal modification processes are used. Fractionation separates the fat into a more solid and a more liquid fraction, for example, to produce palm stearate and palm palmitate. Mostly crystallization is used to separate a fat into two fractions. Interesterification imposes a redistribution of the fatty acids of one or more oils and fats. Both chemical and enzymatic interesterification are used industrially. Hydrogenation saturates the double bonds in the fat, leading to a much harder fat. Because partial hydrogenation causes the formation of *trans* fatty acids, which are undesirable for food applications, mostly total hydrogenation and subsequent interesterification with oils is used.

5 Extraction and Use of Oilseed Proteins

5.1 *Soy Protein*

Soybeans contain mainly the globulin-type proteins glycinin and beta-conglycinin, and small amount of albumins. Because of the high amounts of proteins in soybeans and the simple technology, soy protein concentrates and isolates have been produced industrially for a long time.

Soy protein concentrates are processed by removing soluble sugars from soy flakes or flours. There are three common processes. Washing with aqueous alcohol (50–70% alcohol) removes soluble sugars with a small amount of soluble proteins. Most of the proteins are denaturated by aqueous alcohol and remain with the insoluble polysaccharides. The soy globulins are insoluble in water near the iso-electric point of pH 4–5. Therefore, washing with acid can also be used to remove soluble sugars. After that, the remaining material is adjusted to neutrality and spray-dried. The last alternative is thermal denaturation of the proteins by hot water leaching. The proteins become insoluble in water and remain within the extracted material.

To manufacture soy protein isolate, soybean flour is added to ionized water. The temperature of the mixture is kept at 55 °C and pH at 8.5–9.5 using NaOH. After 1 h extraction time, the mixture is separated into the solids and the liquids using a centrifuge. The solid residue is re-extracted under the same conditions. The proteins are precipitated by shifting the pH to 4.55 using HCl. The separated solids are neutralized, pasteurized, and spray-dried to produce protein isolate [67].

5.2 *Hemp Protein*

Hemp seeds consist of about 20–25% proteins. They contain all essential amino acids which are necessary for humans and is moreover rich in branched chained amino acids, for example, particularly L-arginin [68]. Hemp proteins consist mainly of the globulin edestin (about 65%) and a smaller portion of albumins (about 35%). Edestin can be obtained efficiently from defatted hemp meal by alkaline solubilization or acid precipitation. Within hemp protein isolate, edestin usually forms 70–75% of the total protein [69]. The hemp albumin is a high value protein, similar to egg white but of vegetarian origin. It is extremely easy to digest and an important source of antioxidants. Hemp is free of trypsin inhibitors.

Hemp protein is obtained by pounding or milling the hemp fruits or milling the de-oiled meal. By removal of the remaining fruit shell chunks by sieving, gray greenish protein-rich flour can be obtained, which contains about 50% protein. Additionally, Manitoba Harvest Corp. (Canada) offers a hemp protein isolate with 70% protein. These hemp protein products are used as drink additives and for sports nutrition.

5.3 Sunflower Protein

Sunflower meal contains about 40% protein, which consists of two major fractions. The globulins (11 S) and the albumins (2 S) represent more than 80% of the storage proteins. The globulin fraction, called helianthinin, has a molecular weight of 300–350 kDa, whereas the albumin is a dimer with a molecular weight of 10–18 kDa [70].

Investigations on the production of protein-rich material from sunflower meal indicate a strong influence of salt concentration on extractability. Additionally, phenolic compounds (e.g., chlorogenic acid) which react with the proteins cause a decrease of extractability and alter the functionality and the optical properties (undesired color change) of the proteins [71–73].

Isolated sunflower protein has great potential regarding its emulsifying capacity. Despite its limited solubility, it shows good foaming, film-forming, and gel-forming properties, depending on the respective application [70, 74]. De-hulled sunflower kernels contain about 50% protein after de-oiling. Sunflower protein products with higher protein content are currently unavailable on the market.

5.4 Flax Protein

The protein content of flaxseed varies from 20% to 30%, constituting approximately 80% 11–12 S, defined on the basis of their sedimentation coefficient and globulins (linin and conlinin) and 20% 1,6–2 S albumins (glutelin) [75, 76]. Flaxseed globulin has an overall molecular mass of ~320 kDa, an isoelectric point of ~4.75 [77], and is comprised of at least five subunits having molecular masses of 11–61 kDa, held together by disulfide linkages [78]. In contrast, flaxseed albumin is a basic protein containing a single polypeptide chain with a molecular mass of 16–18 kDa [76, 79]. Flaxseed has an amino acid profile comparable to that of soybean and contains no gluten. Flax protein is not considered to be a complete protein because of the presence of limiting amino acid lysine [79]. However, it also contains peptides with bioactivities related to the decrease in risk factors of cardiovascular disease [80]. Whole flaxseed, flaxseed meals, and isolated proteins are rich sources of glutamic acid/glutamine, arginine [78], branched-chain amino acids (valine and leucine), and aromatic amino acids (tyrosine and phenylalanine). The total nitrogen content in flaxseed is 3.25 g/100 g of seed [81]. The results of studies showed that flaxseed proteins have an inhibitory activity on bacteria, especially against *Entrococcus foecalis*, *Salmonella typhimurium*, and *Escherichia coli* [82]. Flaxseed contains a considerable amount of mucilage in its seed coat which interferes with the process of protein extraction from flaxseed.

After de-mucilaging and de-oiling, flaxseed powder can be extracted in water when stirring, with the pH value adjusted to 8.5 with 0.1 M sodium hydroxide [83]. Press cake of flaxseed with a protein content up to 50% is used as an additive

for baked goods and cakes in the eastern parts of Europe. Protein products with higher protein contents are not actually being manufactured industrially.

5.5 Jatropha curcas *Protein*

The proteins in the Jatropha seed are concentrated in the kernels (22–28% crude protein). The shell contains 4–6% crude protein [84]. The main storage proteins founded in Jatropha are glutelins, globulins, and albumins, accounting for 56.9%, 27.4%, and 10.8%, respectively, of the total recovered protein from defatted kernel meals of three genotypes of *J. curcas*. Slightly lower (39.8%) glutelin and higher (44.4%) globulin contents in *J. curcas* meal are also reported. Glutelins constituted two gel electrophoresis bands of 33 kDa and 27 kDa. The globulins had six major bands: four between 30 kDa and 70 kDa and two <20 kDa. The albumin fraction consisted of four main components with molecular masses <30 kDa [85].

Jatropha seed proteins have the highest solubility under alkaline conditions (above pH 9) [86]. At acidic pH (2.0–6.0), the protein solubility is low. The minimum solubility was observed at pH 4.0, which is the isoelectric point [87]. Therefore, most extraction procedures described in the literature are based on protein extraction in an alkaline medium (at pH 11 or 12) followed by isoelectric precipitation at pH 4 or 5. The resulting protein extracts have a purity of 70–90% or more [87–89]. The yield of the extracted proteins was around 40% [45]. The protein recovery obtained by [89] was 53%. Saetae et al. [87] reported an extraction yield and isolated protein of 84.6% and 19.9%, respectively. To improve the protein recovery, a multistage countercurrent extraction was suggested. The authors point out that the highest protein recovery of 82% was achieved by using 0.055 M NaOH after four-stage countercurrent extraction [86]. Devappa and Swamylingappa [90] reported a protein recovery of about 70–77% with a purity of 95–97% by alkaline extraction (pH 10.5) using steam injection and following precipitation at pH 5.5.

The isolated Jatropha protein fractions have unique functional properties in water-binding capacity, emulsion activity, and emulsion stability [87]. Emulsifying properties of Jatropha protein extracts are comparable to sodium caseinates. Jatropha protein films have a low tensile strength in comparison to soy protein or wheat-gluten but a rather high elongation. The proteins of Jatropha showed higher foam expansion and foam volume stability than sodium caseinate at basic pH. Furthermore, they have better adhesive properties than casein adhesives. The high functional properties of Jatropha proteins signify an enormous potential for various technical or non-food applications. For example, their excellent adhesive and emulsifying properties indicate the potential of Jatropha proteins as emulsifier or paper adhesive [91]. Hamarneh et al. [88] investigated their application in polyketone-based wood adhesives. Jatropha proteins have a better performance as wood adhesive in comparison with soy.

From the nutritional point of view, Jatropha proteins demonstrate high quality. They have a good amino acid composition and a high digestibility (90%). The

levels of essential amino acids, except lysine, are higher than or similar to those of the FAO reference protein or soybean protein. However, the Jatropha meal or protein isolate should be detoxified before they are incorporated into animal diets as Jatropha is non-edible because of the toxic compound phorbol ester. Detoxified Jatropha kernel meal and detoxified protein isolate show a high potential for use in livestock and aquaculture feeds, and in diets for rainbow trout (*Oncorhinchus mykiss*), common carp (*Cyprinus carpio*) or turkeys, pigs, and broilers [85].

5.6 Rapeseed Protein

The protein content in rapeseed varies depending on the variety and conditions of cultivation and climate. Raw protein contents of 19–22% in rapeseeds and 31–36% in rapeseed meals can be obtained. Compared with other protein sources and with the FAO/WHO/UNO-suggested pattern of amino acid requirements for adults, schoolchildren, and pre-school children, rapeseed protein isolates exhibit favorable amino acid composition [92].

Rapeseed contains two major storage proteins: the 2 S albumin napin with a molar weight of 12–17 kDa and the 12 S globulin cruciferin with a molar weight of about 300 kDa. The napin cruciferin ratio depends on the rapeseed variety and is for 00 quality (low in erucic acid, low in glucosinolates) about 1.1–1.3. This is significantly different to soybean, which contains about 90% globulin [92, 93].

Additionally, rapeseed contains different specific secondary plant substances. The best known are the glucosinolates. If a rapeseed cell is damaged, the enzyme system myrosinase immediately begins to decompose the glucosinolates to more or less volatile degradation products. These breakdown products have mostly negative nutritional effects. Mainly isothiocyanates are formed, being very reactive substances which react under mild conditions with some functional groups of the proteins changing their solubility, isoelectric point, and ratio of hydrophilic/hydrophobic properties as well as their molar weight. Polyphenols, particularly *trans* sinapic acid, create a dark color and bitter taste and also react with proteins, in a similar way to glucosinolate breakdown products. Finally, phytic acid forms complexes both with trace metals, which lowers their bioavailability, and with globulins, which change their isoelectric point to low pH values [94]. Therefore these secondary plant substances should be removed or reduced before or during protein extraction to secure high yield and quality of rapeseed proteins.

For a longer period, a lot of work has been done to develop simultaneously aqueous (or aqueous alcoholic or enzymatic-assisted) extraction of oil and protein from specific pre-treated seeds. An overview is given by Natsch [93]. What we know now is that both oil and protein yield are at a maximum of about 80% because of insufficient cell disruption, emulsification, and interaction between the secondary plant substances and proteins, which is too low to be economic.

In the case of protein extraction, after de-oiling two problems appear. If press cake is used as raw material, the residual oil in it (7% or more) causes emulsion

forming during the following aqueous protein extraction. If hexane-extracted meal is used as raw material its protein dispersibility index (PDI) is very low because of the required desolventizing which is done in conventional DTDC (desolventizer toaster dryer cooler) systems. During this process step, the proteins within the meal are partially damaged, leading to losses of functionality [93, 95, 96]. However, a basic requirement for the material from which the proteins are to be extracted is a high PDI. Commercial rapeseed meal often has PDIs lower than 30%. Such meal is unsuitable for subsequent protein extraction because only low yields can be achieved. Therefore, a new gentle meal desolventizing process based on fluidized bed technology was developed [97]. The use of that process leads to meals which possess PDIs of 70% and higher.

Since the 1970s, a lot of different technologies to recover rapeseed protein concentrates and isolates have been described and several processes have been patented. A good overview is given by Wanasundara [98]. In 2013 the European Food Safety Authority (EFSA) confirmed the safety of the rapeseed protein isolate Isolexx™ (Co. BioExx) for food purposes [99] and since July 2014 it is authorized as a novel food ingredient by the EU.

Based on their manifold functionalities, rapeseed proteins can be used in both nutritional and technical applications, for example, Schweizer et al. [100] describe the use of rapeseed protein isolates for human nutrition, Slawski [101] and Adem et al. [102] the use of rapeseed protein concentrates in fish feeding, and Palomino et al. [103] their use in paperboard coating.

Pure napin with a purity of >98% and pure cruciferin with a purity of >95% can be produced with a new process consisting of gentle oilseed processing, aqueous protein extraction, precipitation of cruciferin, and EBA (expanded bed adsorption) IEX (ion exchange) chromatography for isolation of pure napin. Protein separation is reproducible and can be scaled up [104]. The resulting protein products possess interesting functional properties, enabling a wide range of possible uses in both food and non-food applications (cosmetics, biochemistry, pharmaceutical). In particular, napin is comparable or even better than egg albumin and could therefore replace animal albumins, for example, in vegan foods.

6 Extraction and Use of Oilseed/Oil Fruits Phyto-Nutrients

6.1 Rice Phyto-Nutrients

Unpolished rice is rich in phytic acid, 8.9×10^{-3} g/g [105]. Additionally, rice contains phenolic acids and flavonoids, occurring especially within the outer aleuron layers. Of the phenolic acids, 62% exist in bounded form as ferulic acid. Specific for rice is γ-oryzanol, a mixture of phytosterols esterified with ferulic acids [106]. The main components of γ-oryzanol are cycloartenylferulate, 24-methylencycloartanylferulate, and campesterylferulate. Crude rice oil contains

1–2% γ-oryzanol [107]. It is described as constitutional, it has antioxidative properties, and has positive impact on cholesterol levels and cardiovascular systems.

Oryzanol extracts are sold as capsules [106]. It is isolated from the physical refined oil or from the soapstock of chemical refining. It can also be extracted by organic solvents or supercritical CO_2 directly from rice meal [108], but that technology is very expensive [107, 109]. To recover it from rice oil, γ-oryzanol is hydrolyzed by hydrochloric acid or precipitated using methanol, acetone, or isopropanol. Purification is carried out by treatment with ketones and/or alcohols followed by crystallizing the pure oryzanol [107].

6.2 Sunflower Phyto-Nutrients

Next to the presence of healthy unsaturated fats, proteins, and fiber, sunflower seeds contain about 3.3% minerals and about 1% secondary plant ingredients. Vitamin E (e.g., tocopherols) content is interesting, even if it is not high. Although γ-tocopherol is the actually plant-protecting tocopherol, sunflower tocopherols contain more than 90% of the α-tocopherol, which shows a high potential for use in cosmetics because of its antioxidant and anti-inflammatory effect. It is situated in the oil and could be obtained during the refining of the oil. Sunflower oil also contains a small amount of lecithin, carotenoids (mainly all-E-lutein), and phytosterols. The lecithin can be separated during degumming and is an interesting GMO-free alternative to soybean lecithin. In sunflower, 6×10^{-4} g/g β-sitosterol, 8×10^{-5} g/g stigmasterol, and 9×10^{-5} g/g campesterol are contained, but the concentration and sterol composition is strictly dependent on the genotype and environmental conditions. A higher temperature during seed formation induces a general increase in total sterol concentration by up to 35%. However, sunflower can contain up to 0.15% cholesterol, which is different to other plants [107]. Sunflower also contains small amounts of pectin (low-methoxyl sunflower head pectin), for example, for edible coatings [108], lignin (73×10^{-3} g/g press cake) and monoterpene glycosides. Some of these monoterpene glycosides show cell-protective effects and could be used in medicine [109]. In the hulls of the kernels, up to 80% of the sunflower wax is concentrated. It consists of fatty esters, free fatty alcohols, and free fatty acids, and has been recognized as an excellent organogelator for edible oil [110, 111].

Several phenolic compounds, especially caffeic, chlorogenic, and ferulic acids, can be found in sunflower. Dry matter of sunflower meal show a total phenolic content of 42×10^{-3} g/g. Phenolic compounds, such as caffeic acid derivatives, show a high antioxidant potential, which could be used in food, pharma, and cosmetics [112].

Dicaffeoylquinic acids (DCQAs) are one of the few known substances, barring a specific enzyme (viral integrase), which is needed for the reproduction of, for example, the human immuno-deficiency virus. In contrast to other integrase blockers, DCQAs show very few side effects. Sunflower seed contains DCQAs in

higher amounts than other plants and it can be extracted from the press cake or the meal. The extraction is strongly influenced by the choice of solvent and temperature [113].

6.3 Jatropha curcas *Phyto-Nutrients*

Many secondary metabolites identified and/or isolated from various parts of the plant body of Jatropha species have so far been reported, including terpenes, phytosterols, enzymes, alkaloids, and flavonoids. The latex of Jatrophacontains alkaloids including jatrophine and jatropham with anti-cancerous properties. Curcain (a proteolytic enzyme), isolated from latex, has wound healing properties. The leaves contain flavonoids such as apigenin, vitexin, and isovitexin, which contribute to the use of leaves against malaria and rheumatic and muscular pains [114]. Sterols and triterpenes are also found in the leaves of *Jatropha curcas*. In the stem bark extract of *Jatropha curcas*, many phytochemicals have been detected such as saponins, tannins, steroids, glycosides, alkaloids, and flavonoids of a phenolic nature [115].

The seeds contain many phytochemicals with different biological activities. Saponins (triterpene plant glycosides) in the seed of Jatropha possess physiological activities. Polyphenolic substances reported in *Jatropha curcas*, such as flavanols, cinnamic acid, coumarins, and caffeic acid, can scavenge free radicals and inhibit peroxidation. The use of phenolic compounds of *Jatropha curcas* as natural anti-oxidants for the protection of oils and corresponding biodiesel in order to prevent their oxidative deterioration is discussed in the literature [115].

The tetracyclic diterpenes, phorbol esters (PEs), found in Jatropha seeds, are the most toxic secondary plant constituents in Jatropha. The PEs are located mainly in the kernel portion of the seed and their concentration in *J. curcas* varies with different genotypes, ranging from 0.8 to 3.3×10^{-3} g/g kernel. As phorbol esters are lipophilic, during oil extraction the majority of PEs (~70%) present in the seed is extracted with the oil fraction, having a concentration in the oil of $2–8 \times 10^{-3}$ g/g. Therefore, most phorbol ester extraction procedures described in the literature are based on solvent extraction with methanol, ethyl acetate, etc., from the oil [115, 116]. Idakiev et al. [117] reported an extraction process including a step in which Jatropha crude oil is mixed with methanol to extract the phorbol esters, and a purification step in which the obtained fraction is subjected to a methanol extraction. Using this method, a very high concentration of phorbol esters in the produced extract can be achieved (up to 270×10^{-3} g/g) which is 38 times higher than that of crude oil used in this study. Moreover, it must be pointed out that these results are based on pilot-scale trials. The PE-rich extracts exhibited high biological activity – fungicidal, molluscicidal, insecticidal, etc. – suggesting the potential for use as biopesticides [118].

Most secondary compounds reported here are related to the therapeutic and medicinal or insecticidal properties of *Jatropha curcas*. However, to use them in

therapeutic or agricultural applications as well as to bring them to a marketable stage, further research effort is needed.

6.4 Rapeseed Phyto-Nutrients

Rapeseed contains comparable high amounts of phenolic compounds in free, esterified, or insoluble bound form, mainly derivatives of sinapic acid. During oil extraction most of the phenolic compounds remain in the press cake or meal, but Koski et al. [119] and Wakamatsu et al. [120] showed that in crude rapeseed oil the decarboxylation product of sinapic acid, 2,6-dimethoxy-4-vinylphenol (4-vinylsyringol or canolol) can be found when initiated by heat treatment. In contrast to the hardly oil-soluble sinapic acid, canolol is soluble in oil. For canolol a high antioxidant activity is described in the literature [121, 122], making it promising to isolate this compounds for use in pharmaceuticals or food.

Pudel et al. [123] developed a two-step method to isolate canolol-enriched extract from rapeseed meal or cake, involving heat treatment of the material at 165 °C in a fluidized bed followed by extraction with supercritical CO_2. The advantage of this approach is that the conventional oil mill process keeps the material untouched for the isolation of the canolol-enriched extract. The fluidized bed treatment allows a very high and consistent heat and mass transfer to the meal or cake, which results in a temperature load for the material as low as possible. The optimal temperature for the treatment was found to be 165 °C and, after reaching this temperature, the fluidized bed treatment has to be interrupted immediately by cooling down the roasted material. Longer heating reduced the canolol content of the extract. The fluidized bed treatment achieves about 500×10^{-3} g/g canolol in rapeseed meal, whereas in cake more than 700×10^{-3} g/g were reached. On the other hand, the use of meal for the fluidized bed treatment has the advantage of less oil finally extracted by supercritical CO_2, leading to higher canolol contents in the oily extract. The canolol concentration in the canolol-enriched extract was about 3%, and the extract additionally contained 68% triacylglycerols, 11% diacylglycerols, 10% free fatty acids, 2.5% phytosterols, and about 5% other components. It was found that canolol-enriched extracts obtained from smaller heat treated particles (<0.8 mm) showed a higher content of canolol because of an averaged shorter residence time within the fluidized bed reactor and the improved heat and mass transfer because of faster drying [123]. It was not possible to increase the canolol formation and yield of the extract by use of additional heat exchangers immersed in the fluidized bed or by using superheated steam as the fluidization medium, although these treatments improved the effectiveness of the process.

In a frying experiment over a period of 30 h, oil fortified with canolol-enriched extract (200, 500, or 750×10^{-6} g/g) showed a two- to threefold better frying stability in comparison to oils fortified with commonly used antioxidants such as TBHQ (200×10^{-6} g/g) or rosemary extract (40 or 200×10^{-6} g/g) [124].

Thiel et al. [125] developed a technology for the separation of rapeseed protein, phytic acid, and sinapic acid from rapeseed meal in one process, involving the aqueous extraction of the proteins combined with enzymatic or chemical hydrolysis of sinapine and subsequent adsorption of the formed sinapic acid at zeolites as well as binding of phytic acid at an ion exchanger.

6.5 Palm Phyto-Nutrients

Crude palm oil contains 1% minor components. Although small in amount compared to the major lipid components, these minor components impart major health benefits to the oil. These phytonutrients include carotenes ($500–700 \times 10^{-6}$ g/g), tocopherols and tocotrienols ($600–1,000 \times 10^{-6}$ g/g), squalene ($200–500 \times 10^{-6}$ g/g), sterols ($326–527 \times 10^{-6}$ g/g), phospholipids ($5–130 \times 10^{-6}$ g/g), coenzyme Q10 ($10–80 \times 10^{-6}$ g/g), and more [126].

6.6 Flax Phyto-Nutrients

In flaxseed the following phyto-nutrients can be found: polyphenols, phytosterols, phytic acid, resveratrol, and lignans. The high concentration of lignans is one of the most important characteristics of flaxseed; $3.5–6 \times 10^{-3}$ g/g can be found [127]. The most remarkable one is secoisolariciresinol (SDG), although isolariciresinol, pinoresinol, matairesinol, and other derivatives of ferulic acid are also present. Lignan consumption reduces cardiovascular risk and inhibits the development of some types of diabetes. Health benefits of flax lignans reside in their antioxidant capacity and as an estrogenic compound because of their structural similarity to 17-β-estradiol [83]. The lignans are located in the hulls from which they can be extracted by polar solvents, requiring a previous separation of the hulls [128–130, 132–134]. There are lignan extracts of different companies as dietary supplements on the market.

7 Residues Processing: Use of Fibrous By-Products

Essential to make a vegetable oil-biorefinery economic is how the residual fiber-rich material after extraction of oils, proteins, and secondary plant substances is used. In most cases it is not suitable as an animal feeding material. Besides its use as an energy source, there are different possibilities described in the literature, such as various biotechnology applications [131, 135], the production of lactic acid by fermentation of rapeseed residues [136], and the production of levulinic acid and its

use in thermosetting plastics [137]. Additionally, the extraction of high molecular lignin is discussed.

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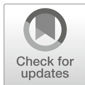
From Current Algae Products to Future Biorefinery Practices: A Review



Michel H.M. Eppink, Giuseppe Olivieri, Hans Reith, Corjan van den Berg, Maria J. Barbosa, and Rene H. Wijffels

Abstract Microalgae are considered to be one of the most promising next generation bio-based/food feedstocks with a unique lipid composition, high protein content, and an almost unlimited amount of other bio-active molecules. High-value components such as the soluble proteins, (poly) unsaturated fatty acids, pigments, and carbohydrates can be used as an important ingredient for several markets, such as the food/feed/chemical/cosmetics and health industries. Although cultivation costs have decreased significantly in the last few decades, large microalgae production processes become economically viable if all complex compounds are optimally valorized in their functional state. To isolate these functional compounds from the biomass, cost-effective, mild, and energy-efficient biorefinery techniques need to be developed and applied. In this review we describe current microalgae biorefinery strategies and the derived products, followed by new technological developments and an outlook toward future products and the biorefinery philosophy.

Keywords Biorefinery, Cell disruption, Cell wall composition, Cellular structure, Extraction, Fractionation, Harvesting, Microalgae

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1 Introduction

The depletion of fossil fuels, ecological problems associated with CO_2 and nutrient emissions, and the challenges of feeding over 9 billion people sustainably demand a fundamental rethink of our agricultural systems. The FAO predicts that food production must increase by 70% by 2050 (http://www.fao.org/news/story/en/item/35571/icode/); at the same time, the livestock sector alone is currently already responsible for 18% of global GHG emissions in CO_2 equivalent (ftp://ftp.fao.org/docrep/fao/010/a0701e/a0701e00.pdf). Moreover, there is also a need to become less dependent on fossil-based resources and increase usage of bio-based feedstocks. These goals can already be addressed by changing to a more plant-based diet, increasing agricultural yields, and converting agricultural waste-streams into bio-based chemicals/fuels. However, when trying to achieve all these goals, the classic food vs fuel dilemma arises because of an increased demand for arable land.

The most important feedstocks for biobased products are shown in Table 1 [1]. There are different generations of biomass for biofuel production (e.g., biogas, biodiesel, bioethanol) as categorized first (cereal crops), second (lignocellulosic crops), and third (marine crops). Whereas biofuels from cereal crops compete with food supply on land use and water use, for lignocellulosic crops harsh conditions are needed for conversion into a biofuel and marine crops are considered as promising because these problems do not apply. Of the marine crops, microalgae are the most important with lipid concentrations ranging from 20% to 60% [2–4].

Microalgae can play an enabling role in all these goals because of the high areal yields that can be achieved and the possibility of growing algae on non-arable land. Moreover, the unique lipid composition and the high protein content make algae a unique next generation bio-based/food feedstock. Since the beginning of this century, microalgae have received an increasing amount of attention for being a

Table 1 Feedstocks categorized in different biomass streams [1]

Biomass	Feedstock	Products	Remark
Cereal crops (first)[a]	Maize, wheat, straw, corn	Monomers, polymers	R&D phase
Oil crops	Rape seed, soy bean, oil palm	Chemicals, monomers/lipids	Full or close to full commercialization
Lignocellulosic crops (second)[a]	Lignocellulose products, wood	Monomers, sugars	R&D and pilot plant phase
Green crops	Grass, leaves (e.g. sugar beet, tea)	Proteins, sugars, fibers	R&D and pilot plant phase
Marine crops (third)[a]	Microalgae, macroalgae	Proteins, sugars, lipids	R&D phase
Others	Yeast, fungi, bacteria, mammalian	Proteins, sugars, lipids	Commercialization, R&D phase

[a]First, second, and third generation biofuels

feedstock for next generation biofuels [5–10]. However, for biofuels only, microalgae production appears to be too costly [11–13] because one of the main bottlenecks in algae production is the amount of energy used in the whole cultivation process combined with the investment costs [12, 14, 15]. Although microalgae cultivation costs have dropped significantly over the last decade, no commercially viable algae fuel plant is currently running to our knowledge.

Although there are numerous algae cultivation aspects that still need to be tackled, the valorization of micro-algal biomass also poses major hurdles. Even though initially the lipid fraction was the main focus for micro-algal biomass (as biofuels were the main foreseen application), algae also have the capacity to synthesize high-value molecules, such as carotenoids, fatty acids, antioxidants, proteins, anti-inflammatory, and other organic compounds, which can be used in the food, feed, cosmetic, biomaterials, nanostructures, and pharmaceutical industries [16–22].

When exploiting the full potential of microalgae ingredients, many different products can be obtained simultaneously and the market value can therefore be higher than the production costs [16]. Consequently, focus should be put on maximal exploitation of the microalgal biomass while minimizing energy and capital expenditure costs [21]. Lipids and proteins are the largest valuable fractions of the microalgae and globally the need for these products is rising. However, most algal biorefinery studies tend to focus on obtaining one specific component from the biomass and therefore downstream processing methods have been tailored to the purification of one specific ingredient (e.g., TAGs, PUFAs, pigments). This indicates that the other available and valuable components in the microalgae were discarded as waste and/or not valorized optimally. To achieve an economically viable large-scale algae cultivation site, all major ingredient fractions need to be valorized in an optimal way. Such a process would be performed in a biorefinery.

Considering today's petroleum refinery, in biorefineries multiple fuel and chemical products are produced from crude petroleum under harsh conditions. However,

a modern dairy plant might be more comparable with an algal biorefinery then a petroleum refinery as the dairy industry is processing a protein/carbohydrate/fat mixture. However, there is less complexity compared to an algal biorefinery as harvesting and cell-disruption are not necessary for isolating products from a complex cellular structure (Fig. 3). Biorefinery of microalgae includes the selective isolation of a large range of similar ingredients from crude biomass. In order to do so, the biorefinery process needs to be mild, efficient, and at the same time maintain functionality of the products (proteins, lipids, carbohydrates) and thus value. In this review we first describe current microalgae products followed by the different biorefinery technologies ranging from harvesting toward component fractionation and finalizing by integration toward a continuous scalable biorefinery concept, which could be the final goal of microalgae biorefinery.

2 Product Portfolio from Current Process

Several reviews have been written on the current status of microalgae products [23–30]. Therefore, we mainly focus in this review on providing up-to-date information on biorefinery practices for current products and providing future perspectives on possible new applications for autotrophically grown algae and cyanobacteria.

No reliable information on the commercial production of microalgae has been published, and only very approximate estimates of worldwide production volumes and plant gate costs for bulk biomass are possible: roughly 10,000 tons/year and $10,000/ton for *Spirulina*, 4,000 tons/year and $20,000/ton for *Chlorella*, 1,000 tons/year and $20,000/ton for *Dunaliella*, and 200 tons/year and $100,000/ton for *Haematococcus* [31]. Other species such as *Tetraselmis* and *Nannochloropsis* are also cultivated by commercial parties but have not reached the large scale of several hundreds of tons yet.

The majority of algae biomass is currently sold as dried whole biomass and has a wide range of applications and claimed activities. Dried supplements are currently sold for human health applications and claim to have immune-enhancing effects, but also prevent heart diseases, obesity, manic depression, and even have anti-tumor effects [5, 24]. However, human consumption of microalgal/cyanobacterial biomass is still restricted to a very few taxa, for example, *Spirulina*, *Chlorella*, *Dunaliella*, *Tetraselmis*, *Nostoc*, and *Aphanizomenon* [24, 30, 32, 33] (Fig. 1). The incorporation of whole dried microalgal biomass into commodity food products has not happened yet because algae species tend to have a strong green color, fishy taste and odor, and a powdery consistency [34]. Therefore, only minor amounts of algae powder (<1%) are currently blended with existing food products [33].

Most algae ingredients currently sold commercially are for the food, health and hygiene, and (aqua-)feed sectors. These ingredients tend to represent high-value compounds, being colorants/pigments and PUFAs. Currently, the only pigments on the market are β-carotene from *Dunaliella salina* and astaxanthin from *Haematococcus pluvialis* [22, 30, 35]. The extracted carotenoids are used in a

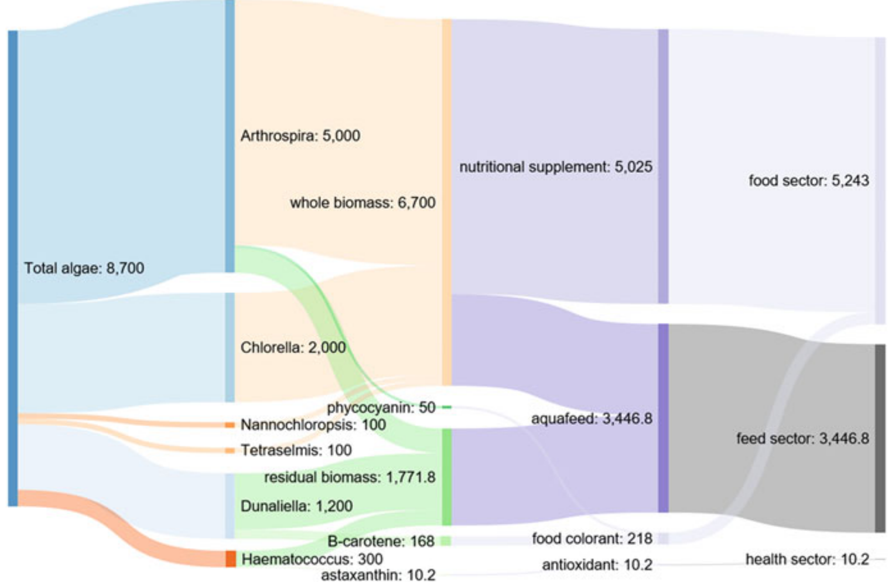

Fig. 1 Application areas of most commonly cultivated algae species

variety of applications, varying from being a natural pigmentation source for aquaculture to food coloring purposes. Another food colorant (blue) is Phycocyanin and is currently commercially obtained from *Arthrospira*. Phycocyanin is used as colorant in food (chewing gums, dairy products, jellies, etc.) and cosmetics such as lipstick and eyeliners in Japan, Thailand, and China [36]. Other major fractions include lipids, which can be used as a source for biofuels, as building blocks in the chemical industry, as edible oils for the food industry, and as antioxidants/glycolipids/phospholipids/PUFAs for the cosmetics and health market [19, 20, 30, 37]. Carbohydrates are normally a minor part except for a few algal species [2–4]. The complex (excreted) polysaccharides might have health beneficial properties [38–42], an interesting niche in the health market, whereas the carbohydrates in the cell (e.g., starch and (hemi-)cellulose) can be used for producing ethanol and chemicals [43].

3 Current Biorefinery Process

The vast majority of the annual algae biomass production (*Arthrospira* and *Chlorella*) is currently dedicated to the manufacture of powders, pills, and tablets [34]. Therefore, most algae processing only consist of a (pre-)concentration step and a subsequent drying step, which tends to be performed by spray drying (websites of earthrise (http://earthrise.com/about/our-farm/), cyanotech (http://

www.cyanotech.com/company/process05.html), and sunchlorella (http://www.sunchlorella.com/corporate-activity/manufacturing-process.html)). Other products on the market are anti-oxidants/colorants such as β-carotene and astaxanthin, which are produced from *Dunaliella salina* and *Haematococcus pluvialis*, respectively. The recovery of both compounds happens in a similar manner, with the exception that *Dunaliella* lacks a strong cell wall, preventing the need for a cell-disruption step. *Haematococcus* has a sporopollenin cell wall, making necessary to perform a cell disruption step prior to the extraction of astaxanthin. On an industrial scale this cell disruption step tends to be done mechanically by bead milling or expeller pressing. The recovery of the hydrophobic fraction (carotenoids and/or lipids) is performed using conventional or supercritical solvents. Industrially there is a preference for supercritical CO_2 extraction. This extraction technology gives the highest carotenoid recovery yields, but has the drawback that it has a high CAPEX and OPEX because of the high pressures which the process requires [44]. For high-value applications such as nutraceutical applications, this technology seems to be the most economical solution. However, the scalability beyond the nutraceutical market seems rather limited. After supercritical CO_2 extraction, 0.05% of the 2% astaxanthin remained in the dried biomass. Shrimp feeding trials have been performed with the defatted *Haematoccocus pluvialis* to replace partially fish meals in aquaculture, which did not have adverse effects on growth performance and nutritional composition, and gave significant pigmentation of the shrimp [45].

Several approaches can be taken to purify C-phycocyanin, depending on the desired purity [46–48]. It usually involves a combination of ammonium sulfate precipitation, ion exchange chromatography, and gel filtration chromatography (depending on the required purity). No literature could be found on the applicability of the remaining biomass, although it is still rich in lipids (e.g., γ-linolenic acid), proteins (membrane proteins), and carbohydrates.

4 Current Process Technologies

For the implementation of new biorefinery techniques it is important to know the biomass characteristics. These characteristics may differ among the different algal species. Most algae have thick cell walls, which make cell disruption hard. Some algal species, for example, *Dunaliella salina*, do not have a cell wall, which makes cell disruption less energy intensive, and the focus of attention shifts toward harvesting without causing cell lysis. These differences need to be taken into account when selecting the cell disruption techniques. Thus knowledge of the cellular structure (including cell wall composition and strength, localization of different products in the cell) is required to be able to select suitable processing technologies and process conditions. The algal cell contains different organelles, such as lysosomes, pyrenoids, mitochondria, and endoplasmatic reticulum. Each type of organelle is enriched in specific components, which can be used to our advantage to fractionate the different components, such as pyrenoids containing

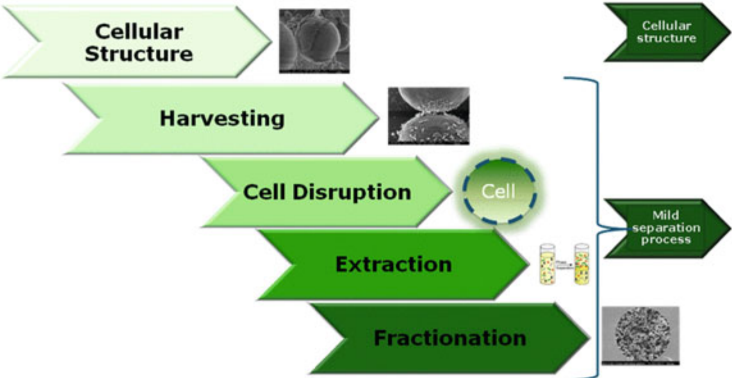

Fig. 2 Algae biorefinery strategy overview

RuBisCO (ribulose-1,5-biphosphate carboxylase oxygenase). First the cells need to be disrupted to release the lipids, proteins, pigments, and carbohydrates from the cytoplasm if feasible in a fractionated way [49, 50], after which the various larger cell compartments or organelles could be separated to obtain specific components by disruption of the organelles [4].

Proposed biorefinery strategy for high-value products (see also Fig. 2):

1. Establish composition/strength of the cell wall and product localization in cytoplasm.
2. Gentle harvesting by scalable concentration/dewatering methods possibly combined with suitable cell disruption technology.
3. Controlled cell disruption using mild technology. Initially the whole cell wall is disrupted followed by organelle disruption, if feasible.
4. Selective separation of hydrophobic (lipids, pigments) from hydrophilic (proteins, carbohydrates) components in the soluble fraction while keeping full functionality.
5. Fractionation of the hydrophilic and hydrophobic component mixtures. These highly enriched fractions can be used for different market applications:
   (a) Separation of the proteins from the carbohydrates and if needed further isolation of these compounds for specific applications
   (b) Separation of the hydrophobic components (lipids, pigments) to obtain specific products (e.g., omega 3 fatty acids, (un)saturated fatty acids, TAGs, pigments)

As a follow-up, the implementation of a continuous biorefinery process to connect the different biorefinery steps into a process chain would be the next step, which is briefly addressed below in the future perspectives part of this review chapter.

4.1 Cellular Structure

Before any biorefinery operation is started, cellular characterization is the first task to be performed with respect to cell wall strength, composition, and the localization of cellular components in the cell, such as in the chloroplasts or other organelles such as oil bodies, as shown in Fig. 3.

Recently, studies have investigated the cellular structure [51–54] of microalgae strains, showing that the cell wall is constructed of different layers and that the cellular content consists of different organelles, making it an area which is almost unexplored.

4.1.1 Cell Wall

The cell wall is the crucial barrier to separate the cellular content from the aqueous phase, both in fresh water and the marine environment. Microalgae cell walls are complex and poorly understood [55]. Microalgae species vary in cell walls and there are variations observed in a single strain grown under different conditions, which can be dramatic, and thus it is difficult to predict which of the compounds noted below is present in any other strain. For example, some *Chlorella* species have only a single microfibrillar layer, whereas others have two layers with the microfibrillar layer proximal to the cytoplasmic membrane and a mono- or trilaminar outer layer [56]. The cell walls of *Chlorella* and other green microalgae

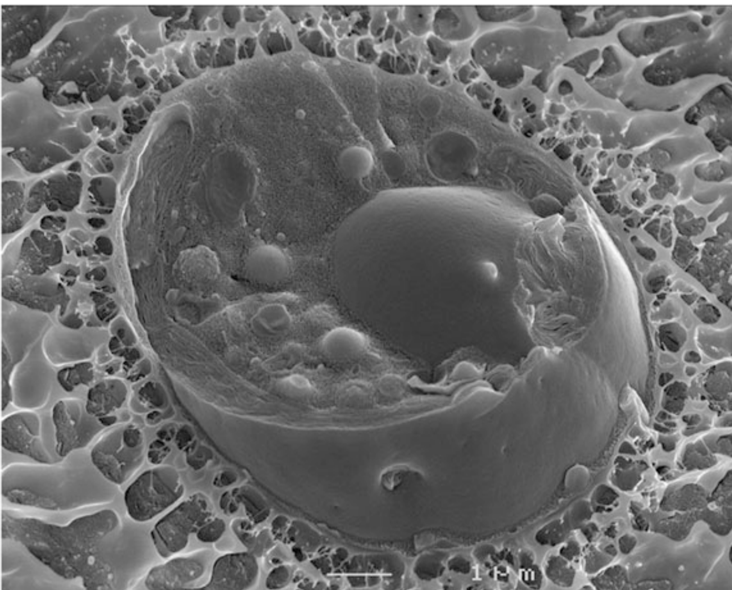

Fig. 3 Microalgae cross-section with some organelles embedded in the cytoplasm

are known to have rigid cell wall components embedded within a more "plastic-like" polymeric matrix [57, 58]. Later it became clear that these different layers consist of polysaccharides (e.g., hemicellulose and cellulose), lipids, and membrane proteins [51, 53, 54, 59]. As the strength of the cell wall depends on the microalgae strain, it is important to investigate the cell wall composition and its strength in order to establish the forces needed to break the cell wall. Furthermore, the influence of cultivation conditions on the composition and strength of the cell wall needs to be established.

4.1.2 Cytoplasm

The different cellular components may be located in distinct organelles with specific functions in the cell. Lipids (neutral) and polysaccharides may be distributed in lipid bodies or starch granules in the cytoplasm/chloroplast, whereas polar lipids (e.g., phospho-/glycolipids) are located in cellular membranes. Oil bodies and starch granules are especially formed under stress conditions. A literature search showed that only a very limited amount of research has been devoted to the localization of different components in the cell, showing a field that is still unexplored although some work performed in the past was focused on chloroplast fractionation [60]. Recently a new proteomics tool was developed for subcellular localizations of proteins in green algae [61].

4.2 Harvesting

Harvesting is the primary step in the biorefinery for concentrating the biomass to remove large water volumes as biomass concentrations in microalgal cultures are usually low, ranging from 0.5 g/L in open pond systems to 5 g/L in closed photobioreactors. Quite a few possibilities are available for concentrating microalgae such as centrifugation, membrane filtration, flocculation, (electro-) flotation, coagulation, and sedimentation to concentrate the biomass in a mild and efficient way [62–65]. Microalgae are small organisms usually ranging in size from 2 μm to 20 μm, so not all concentrating techniques are feasible for different microalgae strains. In this review the most common methods and breakthrough applications are discussed, focusing on centrifugation, cross-flow membranes, and flocculation. Overviews of harvesting of microalgae are given [64, 66], explaining the good scalability potential for sedimentation and flocculation, as these can offer the lowest energy input for micro-algal harvesting even though the concentrating factors are low. Overall, it appears to be no universal method or combination of harvesting methods suited to all microalgae. Often two or more processes in a sequence are used. On the other hand, technologies are being developed combining harvesting with cell disruption in one step such as electroflotation [67] or the use of centrifugal shear forces.

4.2.1 Centrifugation

The most robust technology in the field of harvesting algal biomass is centrifugation because it is scalable from laboratory- to large-scale operations for a broad range of biomass streams [64], with disc stack centrifugation as most preferred in microalgae harvesting. The drawbacks of centrifugation are the high investment costs and energy requirements when operating this technology for microalgae dewatering, especially the smaller sized (<10 μm) organisms for which high speed operations are needed. On the other hand, high concentrations up to 25% dry weight of biomass can be reached, which remains impossible with other harvesting technologies. In addition, recent studies [68] have shown that energy consumptions can be lowered by 82% by increasing the flow rate on the one hand and only slightly lowering harvesting efficiencies on the other (<90%).

4.2.2 Membranes

Next to centrifugation, membranes or cross-flow filtration are commonly used as scalable, robust, and mild technologies. The application of membrane technologies for algae harvesting is not new as more than a decade ago different membranes were evaluated for harvesting of two marine microalgae. Results showed a good velocity and low trans-membrane pressure under continuous use [69]. Recently, some work was devoted to cross-flow filtration [70–72], specific for concentrating microalgae, and was extended toward the reuse of nutrients and filtration of valuable components [70, 73]. Although the energy consumption of membrane filtration is much lower in comparison to centrifugation, it still is not a very low cost technology with respect to reuse of filters and/or cleaning. Moreover, the majority of current algae products are pills/powders, therefore making it favorable to use a concentration method that results in the highest amounts of dry-matter content and thereby minimizing evaporation costs. New technologies such as vibrating membrane filtration [74] and submerged microfiltration [75–77] have been developed for improving microalgae dewatering, showing its benefits over the conventional cross-flow filtration by increasing flux properties and lowering energy use (0.2–0.6 kJ/kg), but the robustness and scalability of these new technologies still need to be established.

4.2.3 Flocculation

The final considered technology in the area of harvesting is flocculation (e.g., chemical-flocculation, auto-flocculation, physical-flocculation, electro-flocculation, bio-flocculation). Various forms of flocculation have gained a lot of attention in the past few years because of ease of operation, mildness, and limited costs [62, 78–81]. The cost and energy demand for harvesting microalgae could be

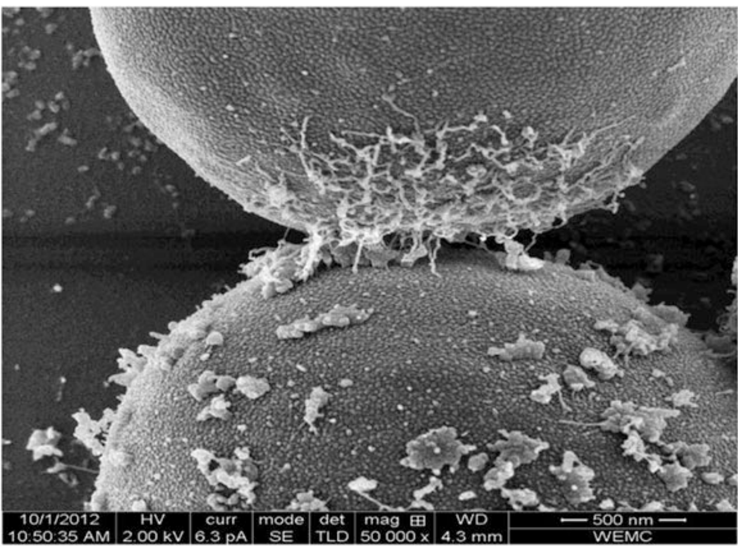

Fig. 4 Example of auto-flocculation of the microalgae *Ettlia texensis* showing the exopolysaccharides connecting the microalgae

significantly reduced if the cells could be pre-concentrated by flocculation [82, 83]. During flocculation, single cells form larger aggregates that can be separated from the medium by simple gravitational sedimentation (Fig. 4). When flocculation is used for harvesting microalgae, it is usually part of a two-step harvesting process. Flocculation is used during the first step to concentrate a dilute suspension of typically 0.5 g microalgae/L dry matter a 20–100-fold to a slurry suspension of 10–50 g microalgae/L. Further dewatering using a mechanical method such as centrifugation is then required to obtain an algal paste with 25% dry matter content [84]. The energy requirements for this final mechanical dewatering step are acceptable because the particles are relatively large and the volumes of water to be processed small [85]. However, drawbacks include the relatively low total concentrating efficiency as compared to cross-flow filtration and centrifugation, quality of the flocculants in relation to the final application of products, and interference of the flocculants with the products during cell disruption. On the other hand, flocculation might be used as a first pre-concentration step with food grade flocculants prior to use of membranes or a centrifuge. Recently, novel flocculants were developed which have an ampholytic character and change charge upon pH adjustment, which might be very useful as recyclable flocculants as, after concentrating the cells, the flocculants can be collected by changing the pH conditions [86] (Fig. 4).

4.3 Cell Disruption

To keep all components intact, mild breakage of the cell wall is a prerequisite. First breakage of the outer cell wall is necessary to make the cellular components available for fractionation and concentration. Currently, most available cell disruption techniques cause complete disruption of the cells and are focused on obtaining one specific product. Other available components which could be fragile and native can be denatured in this process. A variety of methods is currently available for cell disruption and an extensive review explains in detail the different methods [87]. In general, these techniques are divided into two main groups based on the working mechanism of microalgal cellular disintegration, that is, (1) mechanical and (2) -non-mechanical methods [87–91]. In this section, microalgal cell disruption methods are briefly discussed and divided into mechanical and non-mechanical methods, including a view on new technologies.

4.3.1 Mechanical Methods

Disruption of the cell wall by mechanical forces such as liquid or solid shear forces (e.g., bead mill, high speed homogenization, high pressure homogenization, microfluidization) occurs in a non-specific manner [50, 73, 87, 92]. Other cell disruption technologies use energy transfer through waves (e.g., ultrasonication, microwaves, light, pulsed electric fields) [87] and might become more promising once more control on the gradual disintegration of the microalgal cells is achieved. Moreover, with the latter techniques, cell disruption can be applied to a diluted stream in a continuous flow so that perforating the outer cell wall might theoretically occur in a controlled manner [4]. The challenge for microalgae biorefinery is to apply these principles at large scale and at low costs. An advantage of these types of techniques is that they can handle streams with low biomass concentrations, and thus the costly second biomass concentration step can be dispensed with. However, it requires larger volume processing in the continuous flow mode. A limited number of techniques under investigation are briefly described below.

The Pulsed Electric Field (PEF) proved to be a promising technology for controlled cell wall perforation [17, 92–94]. The energy consumption of PEF seems to be much lower than that of conventional techniques such as bead milling [93, 95] so the technology is gaining popularity in the field of cell disruption [92, 96, 97]. However, more research is necessary into the efficiency of cell wall perforation in different cell density cultures; the proper equipment design, the optimal peak voltage, and pulse time need to be established as release of products is still not satisfactory [95, 98].

Ultrasound utilizes the process of cavitation to disrupt the cell wall [17]. For microalgae, ultrasound has been used to improve oil extraction from the cells, and was recently patented [99]. Ultrasound has a very low energy requirement

compared to the conventional cell disruption techniques as explained in earlier papers [17, 87, 100, 101], but the process is still in its infancy.

Other continuous flow techniques are the microfluidizer [87], supersonic fluid flow processing [4, 17], pulsed arc technology [87, 102], and explosive decompression [87, 103]. These continuous flow technologies seem promising depending on the type of energy waves or pulses used. However, equipment design, energy use, and mildness need to be optimized further before these can be applied on an industrial scale.

4.3.2 Non-mechanical Methods

Non-mechanical methods often involve cells lysis by using temperature (e.g., thermolysis, steam, autoclaving), chemical agents, enzymes, or osmotic shock [17, 87, 88, 91, 103–105], which might have potential for biorefinery as a relatively mild and selective extraction/cell disruption step. However, hurdles concerning the harsh conditions, efficiency, toxicity, and economic feasibility are still there, and knowledge on cell disruption characteristics is still incomplete.

4.4 Extraction

With respect to extraction technologies, rapid developments in lipid/pigment extractions with organic solvents (e.g., ethanol, hexane) have evolved in the past few years [20, 91, 105–108] and more recently in the field of supercritical fluids [108, 109] mainly focusing on fuel/lipid production and neglecting the other high-value products (e.g., proteins, carbohydrates) [20]. Mild extraction technologies for proteins are currently in their infancy for microalgae and are mainly developed for, for example, therapeutic proteins [110–112] and have led to major efforts toward different extraction techniques for the more fragile proteins that are scalable and economically attractive.

Different extraction methods mainly for lipids and to a lesser extent for proteins are under development at present. Overviews have presented the possibilities for mild extraction of the different components from microalgae [2, 17]. These extraction methods use polymers (current standard), ionic liquids, and/or surfactants (not discussed in this review), the main objective here being to separate all fractions without losing any product and retaining functionality.

4.4.1 Polymers

Aqueous Two-Phase System (ATPS) separation based on the use of polymers has been widely studied for protein extraction and purification [110–114]. ATPS combines the early processing steps of concentration and purification and serves as an

alternative to traditional purification steps such as chromatography. ATPS is formed by mixing a polymer–polymer, polymer-salt beyond a certain critical concentration to form two distinct phases [115–118]. Both phases being water rich (~80–90 wt%) provide a mild and gentle environment for protein separations without affecting their nativity. On the other hand, separating a hydrophilic phase (e.g., proteins, carbohydrates) from a hydrophobic phase (e.g., lipids, pigments) at the interface by polymers is not yet established and also needs to be developed.

4.4.2 Ionic Liquids

Ionic liquids have been widely considered as alternatives to classic organic solvents and can be applied in organic synthesis, liquid-phase extraction, and catalysis for clean technology and separations [119, 120]. Ionic liquids are composed of cations and anions as they are simple molten salts. The advantage and novelty of ionic liquids are their low melting temperature, below 100 °C, combined with a negligible vapor pressure. Ionic liquids can be an alternative to classical organic solvents because of their ability to extract and separate hydrophilic and hydrophobic compounds. Recycling is feasible [121] and able to produce minimum pollution compared to organic solvents. The range of different ionic liquids is enormous and therefore the range of specific applications is correspondingly large. Interestingly, separation of hydrophilic compounds (e.g., proteins) can occur by selecting the right ionic liquids, as was also recently studied in detail [122, 123] (Fig. 5). Overall, the ideal concept would be first to rupture the cell wall in a mild way, ensuring that the lipids and proteins are available for extraction and subsequently to apply ionic liquids as an additive to the biomass mixture, separating it into hydrophilic (e.g., proteins, carbohydrates) and hydrophobic (e.g., lipids, pigments) phases. Further separation of both phases can take place with fractionation as described in Sect. 4.5, after removal of the solid phase (cell debris).

A novel approach was recently developed by extracting pigments from intact cells using a mixture of ionic liquids and organic solvents (e.g., ethyl acetate) keeping the microalgae cell walls intact [121], which could be followed by cell disruption and extracting the other intact components (e.g., fragile proteins), a

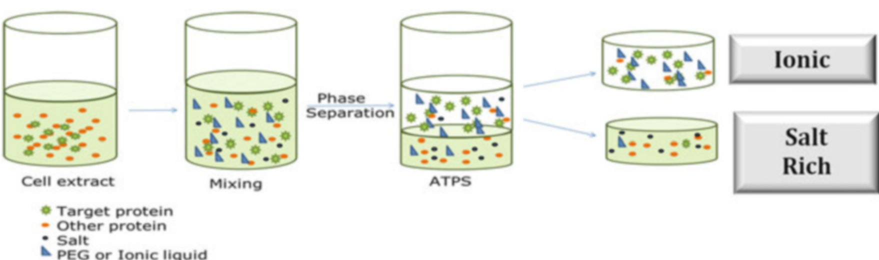

Fig. 5 Aqueous two-phase system (ATPS)

so-called pre-fractionation step which might open up new applications for extraction of high-value components from microalgae.

4.5 *Fractionation*

Fractionation is the prime technology for further isolation of individual components from extracts and mixtures of hydrophilic (e.g., proteins, carbohydrates) and hydrophobic (e.g., lipids, pigments) phases into purified components. For the hydrophilic phase, selective techniques such as (charged) membranes or beads [124, 125] together with alternatives such as protein precipitation can be applied [126]. However, selectivity tends to be low and only useful as an enrichment technique. This part of the biorefinery process is still an area where the developments are limited. Currently, fractionation technologies are mainly used for the purification/isolation of recombinant proteins from yeast, fungi, bacteria, and mammalian cells [125], and the reason is that these technologies are expensive and therefore only attractive for (high) value products in the food/cosmetics/health industry for isolation of specific proteins such as therapeutic proteins such as monoclonal antibodies. However, development of biorefinery processes can profit from the experience obtained in the biopharmaceutical field with regard to mild separation techniques. Other fractionation techniques such as ultrafiltration/diafiltration can be applied for concentrating, buffer exchange, or selective separation of proteins from carbohydrates [20, 70, 90, 127].

With respect to a further fractionation of the hydrophobic phase, such as specific lipids or pigments, techniques such as organic solvent extraction [20] or supercritical methods [108] are currently applied [20, 108].

Fractionation is the final step in the biorefinery process so the costs become higher and might only be a plausible solution for high-value products in the field of food/health/cosmetics.

4.5.1 Membranes

Proteins can be further separated from carbohydrates (long chain polysaccharides) and traces of pigments using membrane filtration techniques (dead end or tangential flow filtration). With tangential flow filtration, the different components can be fractionated by filter sizes ranging from 1 to 1,000 kDa [70, 90, 127–130] under mild conditions. Dead end filtration is the standard method for removal of solid particles from the solution. However, in the past few years, new developments opened up in the field of dead end filtration by coating the membrane with specific ligands (e.g., charged, hydrophobic, hydrophilic) for capturing specific protein components [131]. With these so-called membrane absorbers a further fractionation of proteins and/or carbohydrates is feasible by selective binding in aqueous buffer systems [132].

4.5.2 Resins

In the field of fractionation, chromatographic separation of different products (e.g., proteins) is a technology mainly used for high-value products in the food/health/cosmetics market such as soluble proteins [20, 133–135]. Preliminary work was carried out with *Tetraselmis species* for fractionating a protein mixture using ionic exchange chromatography [127, 129, 136]. More specifically, proteins such as phycoerythrin from *P. pruentum* can be purified with size exclusion chromatography or ionic exchange chromatography to achieve high purities [20, 135] and might be an interesting protein for the clinical research based on its pharmacological (e.g., immunomodulatory, anticancer, and antioxidant) properties.

4.5.3 Extraction

For the hydrophobic phase, after separation of the hydrophilic phase using aqueous two-phase extraction as described in Sect. 4.4.2, the pigments and lipids (TAGs, PUFAs, glyco-/phospholipids) can be further fractionated using solvent extraction or supercritical fluids [20, 108]. In particular, the last technique becomes more promising for scalable extraction of pigments and lipids as presented in recent papers [20, 108, 137]. Furthermore, modification with, for example, trans-esterification may yield fatty acid methyl esters and may be separated from the pigments (carotenoids and chlorophylls) using nanofiltration. Additionally, the methyl esters can be further separated by crystallization and microfiltration. Nice overviews are presented by Kumar and Taher [138, 139].

5 Future Perspectives Biorefinery

Finally, the goal would be to integrate the whole biorefinery chain (harvesting, cell disruption, extraction, and fractionation technologies) into a continuous concept coupled to the biomass production, as schematically presented in Fig. 6. Initially, hybrid concept (coupling of two unit operations) should be developed by integrating harvesting with cell disruption followed by integrating all other unit operations (extraction, purification) into a continuous biorefinery concept (Fig. 6). The complexity would be too high when integrating the different biorefinery unit operations directly into a complete continuous concept because current equipment is not suitable, so new equipment designs are needed. In that way a two-step approach would be preferred whereby single unit operations are first connected into hybrid concepts and in the next step additional unit operations are connected so that a continuous concept for high-value products (Fig. 6) is developed.

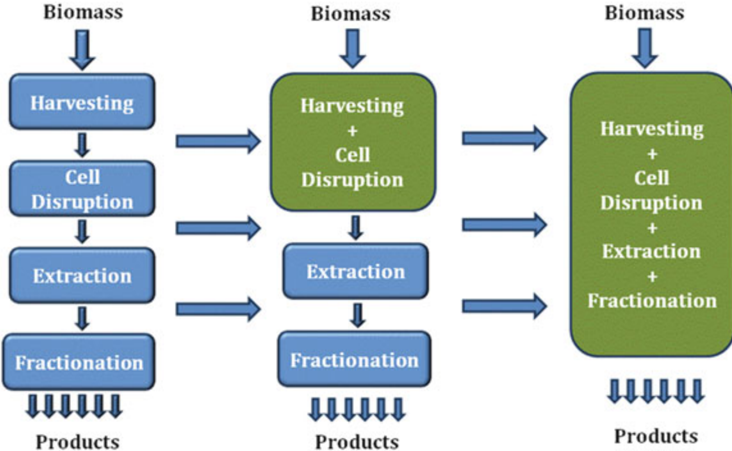

Fig. 6 From single unit toward a continuous biorefinery concept

5.1 *Hybrid and Integrated Technologies*

The first step would be to develop hybrid technologies with the intention of combining more biorefinery unit operations into one step such as biomass production/harvesting or harvesting/cell disruption or cell disruption/extraction or extraction/fractionation. Different possibilities are feasible, but it depends on the products, mildness, and biorefinery technologies used for the recovery of the products. Especially in the field of biofuel production, the combination of harvesting/cell disruption and cell disruption/extraction are being explored to recover the lipids for fuel production as presented in recent reviews [23, 140–142] and further improved by commercial companies (e.g., www.originoil.com). Although this biorefinery concept from Origin Oil is more straightforward, the focus is kept on biofuels/lipids, which can withstand harsh conditions that would damage other ingredient fractions (e.g., proteins) and these ingredients can then only be used as low-value feed or fertilizer. Next to the recovery of lipids, the fragile proteins and carbohydrates should keep their functional composition and therefore mild and new biorefinery techniques are needed. This is still in its infancy as, currently, focus is directed toward the development of single unit operations in a biorefinery concept.

5.2 *Continuous Concept*

Integration of all the unit operations into one process chain is the ultimate goal for a cost-effective process for obtaining all valuable (fragile) biomolecules (lipids, proteins, carbohydrates). In this concept, produced micro-algal biomass is fed as

a diluted or partly concentrated stream through the continuous flow cell disruption equipment under the influence of waves or pulses (e.g., steam, electricity, ultrasound, or light). A prerequisite is fine tuning of the cell breakage in such a way that the cells are gradually cracked and the components (lipids, carbohydrates, proteins) are released. Subsequently, the hydrophobic (lipids, pigments) components are separated from the hydrophilic (proteins, carbohydrates) components and, finally, fractionating the different components in the hydrophobic and hydrophilic phases yields different products in a mild sustainable continuous process concept and at low energy costs. If needed, the obtained fractions can be further fractioned toward different (non-)fuel products in the biorefinery plant by, for example, membrane filtration or extraction or chromatographic separation methods, or further processed at specialized industries (food, feed, chemicals, fuel, etc.). The industrial production process in this microalgal biomass/biorefinery plant should be sustainable, efficient, flexible, have low energy costs, and be applicable to a large variety of microalgae feedstock materials.

6 Future Perspectives Products

The demand for plant-based ingredients should most likely increase in the next decades, but functionality of such ingredients remains rather limited [143, 144]. In a search for sustainable ingredients with special functionalities, microalgae can play a significant role as one of the most promising foods for the future, particularly as a source of proteins, lipids, and phytochemicals [37, 144]. A dominant soluble protein fraction is RuBisCO and, once purified, was further tested for techno-functional properties for application as food additives [129, 130, 136] for replacement of egg-white or whey proteins. It was also shown that purified RuBisCO, albeit from spinach leaves, was able to form heat-set gels with a similar gel-strength as egg-white with only one-third of the of protein [145].

Algae with a complex cell wall matrix might also be a source of natural fibers. Currently, most research focuses on converting the carbohydrate fraction, either catalytically or fermentatively, to fuel-like components or bio-based building blocks [146]. By processing the polysaccharide fraction in a mild way, the fibers retain possible beneficial functionalities such as enhancing gut micro flora, water holding capacity, and laxation [42, 147, 148].

Recent advances in genomics and genetic engineering hold promise for targeted improvements of algal strains to adapt them specifically for enhanced growth, productivity, and high-value product accumulation (e.g., carotenoids, PUFAs) [37]. Biorefinery approaches for such high-value products and byproducts are essential for improving the economic balance of these production processes, and these new approaches need to be developed and tested for various components and strains.

7 Conclusion

Integrated, multiproduct biorefinery of microalgae for co-production of proteins, lipids, and carbohydrates has been discussed, showing that there are clear scientific opportunities to develop these processes further and to make them economically attractive in the coming years. To develop a sustainable and economically feasible process all (fragile) biomass components should be used and therefore an integrated continuous biorefinery technology concept for microalgae processing is needed. Evidently, various technological challenges need to be overcome, such as the integration of biomass production with cell disruption, extraction and fractionation technologies, and the simultaneous extraction and separation of hydrophilic and hydrophobic components, keeping all components in their fully-functional state, which require substantial technological developments. Biorefinery of microalgae is a promising area; however, major efforts have to be made to develop an economically relevant sector.

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Sugarcane-Biorefinery

Sílvio Vaz

Abstract Concepts such as biorefinery and green chemistry focus on the usage of biomass, as with the oil value chain. However, it can cause less negative impact on the environment. A biorefinery based on sugarcane (*Saccharum* spp.) as feedstock is an example, because it can integrate into the same physical space, of processes for obtaining biofuels (ethanol), chemicals (from sugars or ethanol), electricity, and heat.

The use of sugarcane as feedstock for biorefineries is dictated by its potential to supply sugars, ethanol, natural polymers or macromolecules, organic matter, and other compounds and materials. By means of conversion processes (chemical, biochemical, and thermochemical), sugarcane biomass can be transformed into high-value bioproducts to replace petrochemicals, as a bioeconomy model.

Keywords Bioeconomy, Biomass, Green chemistry, Sugarcane, Sustainable chemistry

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1 Introduction

The economic development of different biomass chains is part of the agenda for R&D&I in most developed and developing countries, such as Germany, France, USA, Brazil, and South Korea, among others, mobilizing large amounts of public and private effort and resources, and aiming at the optimized utilization of biomass. It is a trend that seeks to add value to supply chains and to reduce negative environmental impacts. Brazil is one of the major biomass producers for food and biofuels, and therefore these efforts are of great importance for the maintenance of a positive and less impactful economic scenario.

Concepts such as biorefinery and green chemistry focus on the usage of biomass, as with the oil value chain. However, it causes less impact on the environment, considering sustainable integrated systems (feedstock-processes-products-residues) according to technical parameters that take into account, among other things, mass and energy balances and lifecycle analysis.

It is possible to observe a great synergy between biorefineries and green chemistry, especially regarding the minimization of waste and negative environmental impacts, as well as promoting a "green economy." A biorefinery based on sugarcane as feedstock is an example, because it can integrate into the same physical space, of processes for obtaining biofuels (ethanol), chemicals (from sugars or ethanol), electricity, and heat.

We can consider on a scale of economic valuation [1] that fine chemicals and specialties developed from biomass are those with the greatest potential to add value on bioenergetic chains (as sugarcane). This is because of the strategic participation of the chemical industry in the supply of chemical commodities and final products to various economic sectors, such as petrochemical, pharmaceutical, automotive, construction, agroindustry, cosmetics, etc. Biofuels and materials are at a second level of valuation, followed by energy and chemical commodities such as fertilizers and pesticides.

In countries with a large production of sugarcane, such as Brazil, efforts have been made to promote the biomass economic potential, in accordance with the use of renewable sources to develop a sustainable chemistry as well as its use in biorefineries [2, 3]. It is important to mention that the use of residual biomass is crucial to enable the production of biofuels. Figure 1 shows the relationship between bioeconomy and renewable chemistry as a proposal for an innovative design to obtain good results for the usage of sugarcane in biorefineries.

In Brazil, sugarcane-biorefineries have a particularly great potential to share wealth based on a solid industrial leadership in this sector, when allied to technical and scientific development.

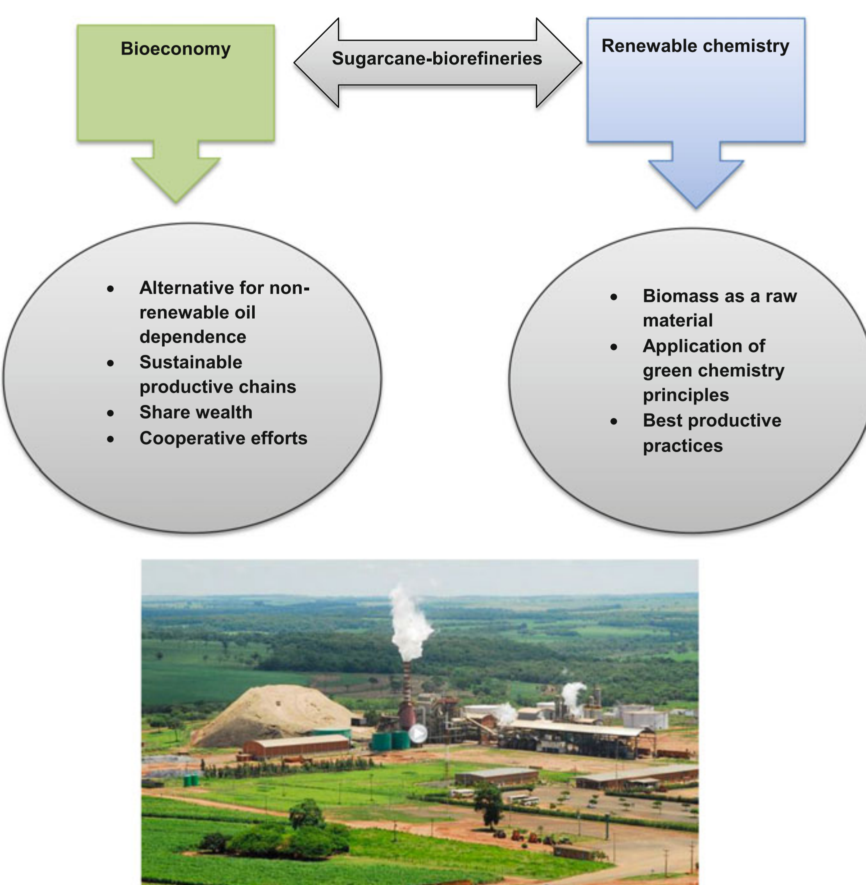

Fig. 1 Relationship between bioeconomy and renewable chemistry for the usage of sugarcane biomass. Sugarcane-biorefineries can establish a bridge between these two concepts. Sugarcane mill courtesy of the Brazilian Sugarcane Industry Association (UNICA)

Table 1 Worldwide sugarcane producers for the year 2013

Country	Production (tonnes)
Brazil	739,267,042
India	341,200,000
Mexico	61,182,077
Colombia	34,876,332
Indonesia	33,700,000
USA	27,905,943

Source: Food and Agriculture Organization of the United Nations [5]

2 Sugarcane as a Feedstock for Biorefineries

Table 1 describes the major worldwide sugarcane producers. As expected, the major producers are established in the tropical regions, excepted for the USA. We have a forecast of availability in these countries of 1.2 billion tonnes of sugarcane for biorefineries.

In Brazil, sugarcane is practically the only source of feedstock for sugar (sucrose) and its derivatives; in some countries, such as the USA, the major sugar source for ethanol is corn. Nowadays, the Brazilian production of ethanol (anhydrous and hydrated) is 27.5 million m^3 and the production of commercial sugar is 37.3 million tonnes [4].

The use of sugarcane (*Saccharum* spp.) as feedstock for biorefineries is desirable because of its potential to supply the following compounds and materials:

- Sugars: sucrose (Broth), glucose (derived from cellulose and sucrose inversion), and xylose (derived from hemicellulose)
- Ethanol: from sucrose fermentation
- Natural polymers or macromolecules: cellulose, hemicellulose, and lignin, which are present in bagasse and straw (lignocellulosic biomass); they can be converted into hexose, pentose, and phenol sources, respectively
- Stillage or wastewater rich in organic matter
- Other compounds, such as higher alcohols and carbon dioxide (CO_2) at a high purity content for food and chemical usages

The average mass composition of the sugarcane plant is presented in Table 2.

Bagasse, as the main lignocellulosic biomass, has the following average mass composition [6]:

- Cellulose: 41.7 wt%
- Hemicellulose: 34 wt%
- Lignin: 12.6 wt%

Sucrose, which is a disaccharide originating from fructose and glucose monosaccharides, is used for ethanol production by fermentation and for commercial sugar production through separation and crystallization. However, one could obtain other molecules of higher value from sucrose, glucose and xylose, constituents of cellulose and hemicellulose, respectively. Bagasse is frequently used for animal feeding and production of bioelectricity by cogeneration in Brazil; the sugarcane mills then are self-sufficient in electricity [7]. The use of bagasse and straw for second generation (2G) ethanol production is a topic that has a large amount of published literature. However, commercial production requires certain barriers to be overcome, such as reducing the cost of development of enzymes and yeasts that ferment the pentoses from hemicellulose [8]. Stillage has been used both in generating biogas and in soil fertigation. However, frequent monitoring of its application is required because of the high content of ions and organic matter,

Table 2 Mass composition of the sugarcane plant [6]

Component	Average mass (wt%)
Fiber	8–14
Fructose	0.2–1
Glucose	0.2–1
Sucrose	14–24
Various organic compounds	0.8–1.8
Various inorganic compounds	0.2–0.7
Water	75–82

Table 3 Sugarcane components and derivatives with relevance to a biorefinery

Raw material	Main composition	Usages
Sucrose	Glucose and fructose	Commercial sugar Ethanol Renewable chemicals
Bagasse	Lignin, cellulose, hemicellulose, inorganics, and water	Animal food Bioelectricity Renewable chemicals Ethanol 2G Fibers and polymers
Straw	Lignin, cellulose, hemicellulose, inorganics, and water	Ethanol 2G Soil recovery
Vinasse (aqueous effluent)	Solubilized organic matter, inorganic solids, solubilized inorganic salts, and water	Biogas Biofertilizer

which can alter the physicochemical properties of the soil, with subsequent leaching of ions (NO_3^-, PO_4^{3-}, K^+, etc.) and groundwater pollution [9].

Table 3 shows the main sugarcane components and derivatives with economic potential for biorefineries. Ethanol stands out as the main product because it can be obtained from three of the four sugarcane components (sucrose, bagasse, and straw). Furthermore, it can be used to produce other derivatives for renewable chemistry, such as “green” plastics. Figure 2 shows the product tree obtained from a sugarcane-biorefinery.

3 Perspectives for Sugarcane Usages in Biorefineries

A biorefinery incorporates the technologies and processes used for the conversion of biomass into five types of products: energy, chemical commodities or bulk chemicals, biofuels, materials, and fine chemicals. The technologies are compiled into processes, which are divided into biochemical (fermentation and enzymatic

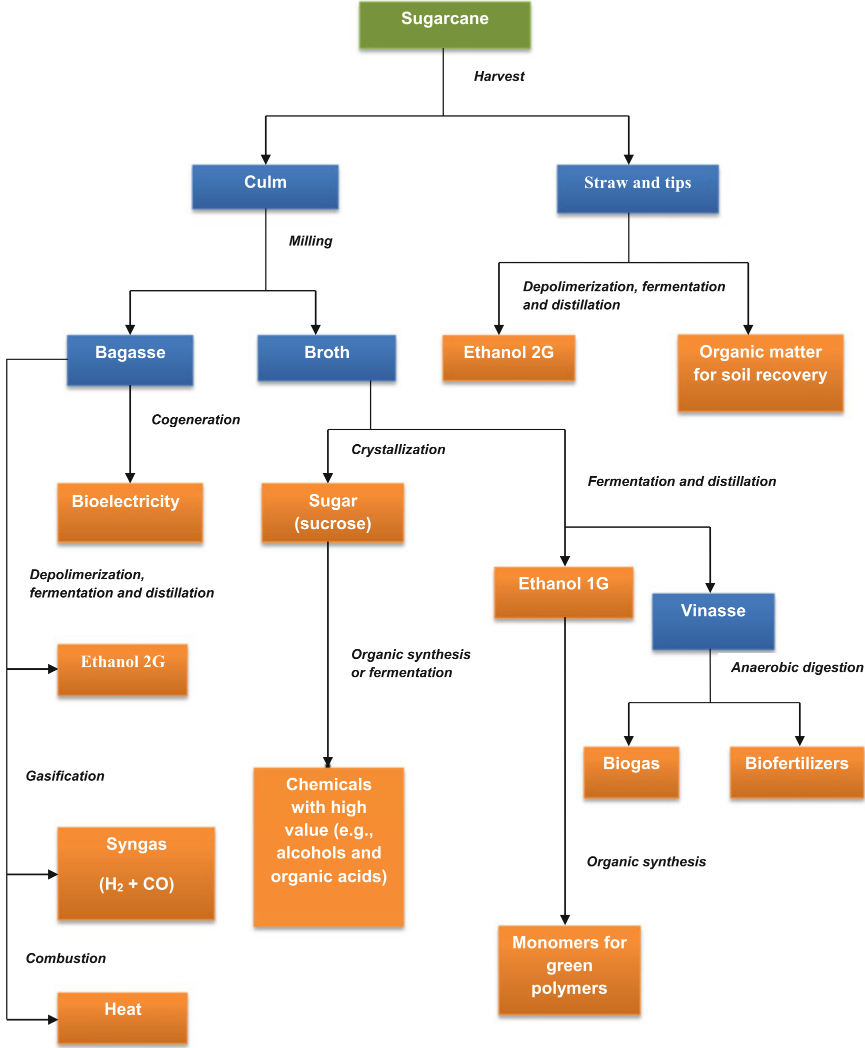

Fig. 2 Simplified flowchart of products (boxes in *orange*) for a sugarcane-biorefinery

catalysis), chemical (chemo-catalytic), and thermochemical (combustion, gasification, and pyrolysis).

Table 4 presents a description of target products with high added value that can be obtained from sugarcane by means of different processes. In this table, only building blocks and end-use products can be observed. They were defined according to the characteristics of Brazilian biomass, data from national and international scientific literature, and demand from the national chemical industry and related industries. Building blocks are molecules from which a number of other chemical products of economic interest originate. Synthesis intermediates are those

Table 4 New products from sugarcane identified as having high potential for biorefineries

Target compound	Precursor	Route	Status
2,5-Furanodicarboxylic acid	Glucose from cellulose	Organic synthesis Fermentation	In development: improvement of catalysts, biocatalysts, micro-organisms, yield, others
Succinic acid	Xylose from hemicellulose	Fermentation	In development: improvement of micro-organisms and yields
Antioxidants	Lignin	Catalytic cracking	In development: improvement of catalysts, yield, others
Derivatives from cellulose (acids, esters, nitrates, ethers, etc.)	Cellulose	Organic synthesis	Established industrial processes
2G ethanol	Glucose from cellulose Xylose from hemicellulose	Fermentation	In production but with improvement of enzymes, micro-organisms, yields, and cost reduction
Phenols	Lignin	Catalytic cracking	In development: improvement of catalysts, yield, others
Furfural	Xylose from hemicellulose	Organic synthesis	Established industrial processes: still needing to improve catalysts, yields, others
Gas of synthesis ($CO + H_2$)	Lignocellulosic biomass	Gasification	Established industrial processes based on petroleum: still needing to improve yields
5-Hydroxymethylfurfural	Cellulose	Organic synthesis	Established industrial processes: still needing to improve catalysts, yields, others
Sulfonated lignins	Lignin	Organic synthesis	Established industrial processes
Xylitol	Xylose from hemicellulose	Organic synthesis	In development: improvement of catalysts, yield, others

Source: adapted from [7]

compounds used in small quantities as fine chemical reagents and in the production of drugs, pesticide, and cosmetics, among others. Chemical specialties must be considered, which are restricted to certain specific purposes.

Even with the laudable efforts of institutions such as the DOE-NREL (US Department of Energy – National Renewable Energy Laboratory) in R&D, product survey, and potential routes, most "green" compounds (or bioproducts) have not reached the stage of industrial maturity. A strong exception is succinic acid: considered as one of the main opportunities for renewable chemicals because of its possible application as building blocks [3, 10, 11] industrial production has started [12]. However, it is necessary to evaluate each molecule according to the market scenario, and two major aspects that must be considered are (1) commercial balance for chemicals (deficit or surplus) and (2) the need for synthesis intermediates, mainly for drugs, which may become more interesting than the search for building blocks, usually seen on the international scenario.

The current technological scenario, taken from the information shown in Tables 2 and 3, suggests a production of a wide range of products – as covered in Fig. 2 – in a model similar to an oil refinery. Furthermore, the American Chemical Society published in 2014 a revision of compounds from DOE-NREL's forecasts considering building block molecules for biobased polymers [11], adding information to Table 4:

- 3-Hydroxypropionic acid from bacterial fermentation of glucose from sucrose – likely in 10 years for commercial step
- Sorbitol from hydrogenation of glucose from sucrose – likely in 10 years
- Levulinic acid from acid-catalyzed dehydration of sugars (hexose or pentose) – maybe likely in 10 years

The main processes to be applied in a sugarcane-biorefinery are described in the following.

3.1 Chemical Processes

Chemical processes are those based exclusively on chemical reactions (forming or breaking). In Fig. 2 it is possible to observe that organic synthesis is very relevant for biomass conversion. Usually a biomass component is extracted and purified and, subsequently, used as starting material in a synthetic route. Catalysts are often used to increase the yield of product of interest and to decrease the reaction time – the chemical processes are chemo-catalytic. This can be seen in several aspects of green chemistry, such as the use of renewable feedstock, catalysts, and the reduction of waste production [13].

In the case of utilization of cellulose and hemicellulose from lignocellulosic waste (bagasse and straw), first these polymers and their constituent sugars must be obtained, especially glucose (hexose) and xylose (pentose), respectively, to obtain products of industrial interest, such as ethanol 2G [14]. With lignin, the breaking of its molecular structure is initially attempted to release mainly phenolic compounds which may be tested, for example, as monomers for various routes of preparation. Obtaining building blocks and synthesis intermediates is the usual approach used by R&D projects to add greater value in bioenergetic chains in a biorefinery [3, 10, 15]. Building blocks, such as furfural and xylitol (derived from xylose constituent of hemicellulose) and 5-hydroxymethylfurfural (derived from glucose), among others, can add great value to carbohydrates, which can be extended to derivatives of lignin [1, 7, 10, 11, 14], as reported in Table 3.

It is worth commenting on the development and use of catalysts for these processes, given their importance for improving yields and selectivities (enantioselectivity, regioselectivity, and stereoselectivity). Zeolites have been used in the cracking of lignins [16]. The metals (soluble and insoluble salts and complexes) have been applied to heterogeneous catalysis (Ni, Pd/C, Ru/C, Co-Mo, Ni-Mo, Ru/Al_2O_3, etc.) to reduce lignins [10, 16] and metal complexes of V, Mn,

Co, Pd, Fe, Re, and Cu acting as homogeneous and heterogeneous catalysts for cellulose oxidation, among other reactions [17].

3.2 Biochemical Processes

The biochemical processes in Fig. 2 are fermentation for the production of first generation ethanol (1G) and other chemicals, such as alcohols and other organic acids, and anaerobic digestion for production of biogas and biofertilizer (mineralized fraction). Enzymatic catalysis contributes to increase the rate of metabolic reactions involved in biochemical conversion routes.

Biochemical processes have major operational similarity to chemical processes with respect to the stages of compositional analysis and characterization of the feedstock, pre-treatment (whenever necessary), structural identification, and study of industrial potential. However, the main feature of these processes relates to the use of microorganisms (fungi, bacteria and yeast), which have the biochemical mechanisms that allow the synthesis of organic chemicals, such as ethanol. For example, production of ethanol (1G and 2G) by the yeast *Saccharomyces cerevisiae* by means fermentation of sucrose also produces carbon dioxide (CO_2) [8], a carbon dioxide with a high purity degree and heat for industrial purposes.

Anaerobic fermentation of organic matter present in the vinasse mainly produces methane (biogas) and carbon dioxide, as well as mineral residue rich in inorganic salts of nitrogen, sulfur, and carbon (biofertilizer) [18]. In this case, bacterial consortia are used, formed by *Acinetobacter*, *Arthrobacter*, *Bacillus*, and *Brevbacterium*, among others [19].

The bacteria *Lactobacillus delbrueckii* can be used for the production of lactic acid via fermentation of glucose and *Anaerobiospirillum succiniciproducens* for the production of succinic acid through fermentation of pentoses and hexoses [10]. However, despite the high potential for production of a large amount of chemicals, the slow rate of reaction (kinetic of conversion) and the difficulty in separating final products (downstream step) can, in some cases, limit the use of bioprocesses in sugarcane-biorefineries for products other than ethanol.

On the other hand, enzymes, such as cellulase, β-glucosidase, and xylanase, are widely used in the hydrolysis of cellulose and hemicellulose to release glucose and xylose, respectively [8].

3.3 Thermochemical Processes

As for chemical and biochemical processes, the steps of compositional analysis and characterization of feedstocks and the structural identification and study of industrial potential are common. Nevertheless, the main characteristic of these processes is the use of thermal energy that leads to combustion, gasification, or pyrolysis.

Through the combustion, it generates thermal energy (heat) [20]; through the gasification it generates synthesis gas (syngas), which is mainly composed of carbon monoxide (CO) and hydrogen (H_2), to be used in organic synthesis of various molecules for the chemical industry [21] by Fisher–Tropsch reaction [22]. Fast pyrolysis produces bio-oil (rich in polar and non-polar organic compounds) and bio-char (a soil fertilizer). Cogeneration is a combined thermal process in which electrical energy is produced. Biomass combustion generates heat, which heats the water, generating steam, moving the turbines for the production of bioelectricity, which can either be used by the biorefinery itself or be sold to the electric grid.

As seen in Table 4, gasification is a process that can add value to the biomass waste. Syngas and fast pyrolysis can produce renewable chemicals to substitute for several petrochemicals.

4 Challenges Involved

As already mentioned, many of the target products shown in this chapter are subjects of extensive literature, mainly in the areas of organic chemistry, biotechnology, and chemical engineering. However, in some cases, there are no processes established for industrial production, which points scientific, technical, and economic challenges, separate or combined, which need to be overcome. It is important to consider, however, that the worldwide marketing of chemicals involves values around USD 100 billion per year, of which about 3% relates to bioproducts or biomass derivatives. It is estimated that the total market share could reach 25% by the year 2025 [23]. These figures give an idea of the possibilities and risks involved. For specialties and fine chemicals, the current participation of renewable – about 25% in both segments – could reach a market share of 50%, whereas for polymers, the current 10% could reach 20%, also in 2025 [24].

4.1 Technical Challenges

The technical challenges involve technological developments or improvements that allow the scale-up of processes developed in the laboratory such as separation methods, process optimization, energy efficiency, among others; a clear example of these observations is 2G ethanol.

Failure to overcome this kind of challenge can derail the production of certain bioproducts, as a value-added molecule, which can show great market potential in its R&D stage. Therefore, a well-planned R&D stage must have proper technological support to enable the laboratory scale to move on to an industrial scale.

4.2 Economic Challenges

One of the main economic challenges concerns attracting and allocating funds in R&D&I projects and, subsequently, in demonstration of technology projects. The rise and the possible decline of said "green" chemicals needs to be considered in the budget planning of development projects or for renewable compounds production, as previous international scenarios related to the chemical industry draw attention to external market factors [11, 24]. Nowadays, shale gas could be a threat for renewable chemicals based on its lower cost.

Nevertheless, the existing demand for ethanol 1G and the beginning of ethanol 2G production provides a favorable economic environment for the sugarcane-biorefinery.

5 Conclusion

This chapter attempts to show the great economic potential of sugarcane-biorefineries. Building blocks and synthesis intermediates are good examples of potential bioproducts in a medium and large horizon, integrated into ethanol, sugar, and bioenergy production. Energy and biofuel are very important in sugarcane industrial exploitation, in which the conceptual model is the petrochemical refinery.

When evaluating products mentioned as potential in other regions of the world, it should be noted that they do not always reflect local needs, justifying the use of own information obtained in studies conducted to adapt better for national scenarios, technical and scientific planning, and investment resources.

Chemical, biochemical, and thermochemical processes are fundamental to exploiting the full economic potential of sugarcane. However, strong actions are still required to overcome several challenges, comprising mainly pre-treatments, synthesis routes, catalysts, and microorganisms. However, is very important to establish an integrated sugarcane-biorefinery to achieve the real economic potential for the sugarcane biomass.

Scientific, technical, and economic challenges must be overcome by the government and private sector. It should allow sugarcane-biorefineries to turn into a viable alternative to the replacement of the productive chain based on oil – a non-renewable feedstock – leading to a positive environmental, social, and economic impact as a model of the bioeconomy based on renewable biomass.

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Starch Biorefinery Enzymes

Albrecht Läufer

Abstract Nature uses enzymes to build and convert biomass; mankind uses the same enzymes and produces them on a large scale to make optimum use of biomass in biorefineries. Bacterial α-amylases and fungal glucoamylases have been the workhorses of starch biorefineries for many decades. Pullulanases were introduced in the 1980s. Proteases, cellulases, hemicellulases, and phytases have been on the market for a few years as process aids, improving yields, performance, and costs. Detailed studies of the complex chemical structures of biomass and of the physicochemical limitations of industrial biorefineries have led enzyme developers to produce novel tailor-made solutions for improving yield and profitability in the industry. This chapter reviews the development of enzyme applications in the major starch biorefining processes.

Keywords Beer brewing, DDGS, Fuel ethanol, High fructose corn syrup, Industrial enzymes

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1 Enzymes in Starch Biorefinery: Status and Perspectives

The annual production of starch worldwide was 48.5 million metric tons (mt) in the year 2000 [1]. This grew to 62 million mt in 2008 [2]; this figure includes over 25 million mt of starch used for ethanol production in the US because of the growing support via RFS (Renewable Fuel Standard). Annual starch production is estimated to have grown to 85 million mt in 2015.

The revenue of the starch and starch derivatives industry was $51.2 billion in 2012 and is expected to reach $77.4 billion by 2018, at a compound annual growth rate (CAGR) of 7.1% between 2012 and 2018 [3].

Against this figure, the value of the starch enzymes market is comparatively small at $1.5 billion worldwide, the majority being in the USA [4] and there in the corn-to-ethanol and grain-to-glucose and high-fructose syrups industries. Starch enzymes are, however, of strategic importance to the starch industry; without enzymes, no refinery products. Major products are those enzymes which hydrolyze the 1-4 and 1-6 glucosidic bonds occurring in amylose and amylopectins, that is, α-amylases, pullulanases, and glucoamylases. A substantial amount of work has been devoted to the search and generation of new and better variants of these enzymes. Other enzyme types have been added to the portfolio. Proteases have been playing a role for some time, and cellulases and hemicellulases as well as phytases have been introduced more recently. These enzymes are employed to improve productivity of the biorefinery plant by improving process characteristics, for example, better flow, less pH adjustment, lower viscosity, less water usage, less nitrogen source added, less cleaning efforts in the downstream part of the factory. They improve the yield or quality or both of side products, for example, corn oil or DDGS (Distiller's Dried Grains with Solubles). Their development and usage is based on a deep understanding of the industrial biorefinery process and the physicochemical structure of the biomass from which the starch is retrieved, that is, corn, wheat, cassava, or other sources.

Innovation in starch enzymes is driven by the main market sectors, corn-to-ethanol and grain-to-sugar conversion. Within these industries the main drivers are increase of plant profitability and competitiveness between the major enzyme suppliers. The bioethanol industry is suffering from low crude oil prices and is looking for every means to improve the margins of their factories by reducing costs of process additives and increasing revenues from co-products. Large volumes of bulk enzymes from China are reaching the US and Europe at very competitive prices, and thus the innovators need to defend their portfolio and stay ahead with

ever novel unique selling points (USPs) of their products. A third driver for new enzyme applications is new feedstocks: improvements to existing crops, such as Syngenta's maize branded Enogen® containing α-amylase genes, and increased use of other feedstocks such as sorghum or cassava, give enzyme developers food for new thought and formulations.

2 Starch Biorefineries: Processes and Products

The industrial use of starch crops varies strongly from region to region. Number one product in the US is fuel ethanol and number two is high fructose corn syrup (HFCS). In other regions of the world the majority of starch goes into the food and beverage sectors as starch, sweetener, or potable alcohol, and, secondly, into the paper industry. Relatively small amounts are used for the production of organic acids and bioplastics [2]. In China the use of starch for non-food products such as fuel or chemicals is discouraged [5]. Despite this, fuel ethanol production in China is forecast for 2016 to reach 3.15 billion L (2.49 million mt), up 2.6% from 2015. China's ethanol production has caught up to become number three in the world after the US and Brazil [6].

In the food sector, enzymes are used to modify starch in baking or they are added in the brewery or distillery process. Modified and regular starches find a multitude of applications in cosmetics, pharmaceuticals, or adhesives.

The crop for producing starch varies by region. Although in the US 98% of starch comes from maize or corn [2], in other regions of the world wheat is the biggest source, followed by rice and potato. Cassava is increasingly used for fuel ethanol production in Asia and Africa [2]. The type of starch crop influences the choice of the enzyme formulation, as the molecular structures of the starch compounds and the structure of the complexes between starch and other constituents such as hemicellulose, cellulose, oil, and proteins differ from plant to plant.

Figure 1 shows in an abstract scheme the pathways from starch crops to products, highlighting in red those process steps where enzymes are being applied; these are explained further below.

All processes start with milling of the starch-containing crop followed by physical separation processes and addition of enzymes at various stages. *Wet milling* is primarily used for physical fractionation of native starch from fibers, proteins, and oil. Cellulases and xylanases are used in wet milling of wheat to improve the separation of starch and gluten proteins [7]. The resulting starch is either used directly in food, pharmaceutical, cosmetic, or technical applications or further fractionated; it can also be chemically or enzymatically modified or enzymatically converted to glucose, fructose, or cyclodextrins. One of the technical applications is use as a carbon source in fermentation processes.

Dry milling is technically less demanding than wet milling. It is the major process used in the corn-to-ethanol industry and is obviously the process of choice to prepare flour and fiber or protein co-products from grain.

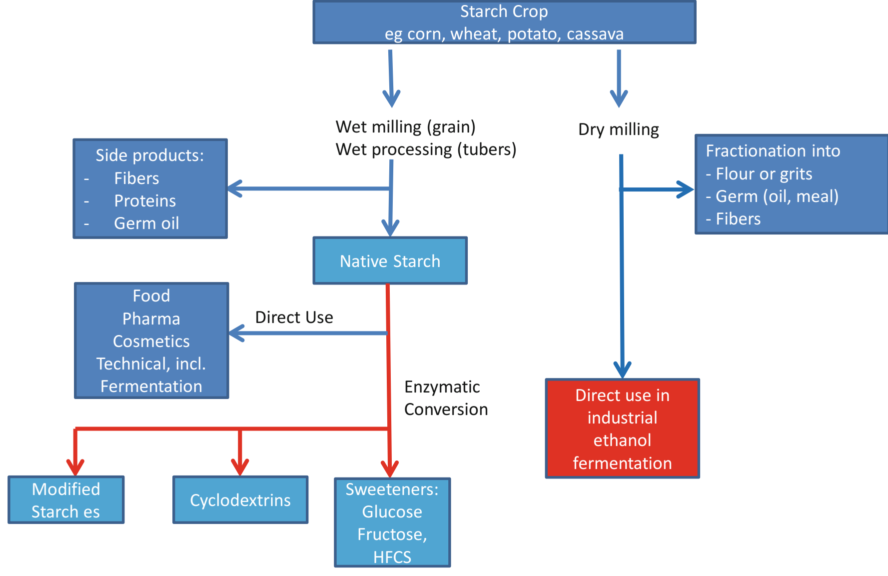

Fig. 1 Schematic outline of a starch biorefinery. Process steps where enzymes are applied are marked in *red*

Numerous reviews have been published on the starch refinery, for example, [8–10], and further process details can be found there. Those steps, however, where enzymes are relevant, are discussed below.

2.1 Glucose, Maltose, and HFCS

Glucose, maltose, and HFCS are by far the largest volume products from starch besides fuel ethanol. The value of the glucose syrup market was estimated at $24 billion in 2013 and is estimated to grow to around $33 billion by the end of 2018 [11]. The production volume worldwide is 18 million mt for glucose syrups, maltose syrups, and maltodextrins, and 14 million mt for HFCS [7].

Figure 2 provides a schematic view of the major enzymatic steps in producing various syrups from starch. To enable enzymes to act, starch needs to be gelatinized before the insoluble microgranules are prone to enzyme-catalyzed hydrolysis. Gelatinization is achieved in different ways: for HFCS and glucose syrups most plants use jet cookers and for maltose syrups temperatures around 60 °C are used. The first step in starch conversion is breaking down the two major polymers, amylose and amylopectin, into oligomers called dextrins, using α-amylases under starch-gelatinizing conditions. This process is called liquefaction, as the highly viscous slurry formed by solid starch granule polymers is transformed into a much

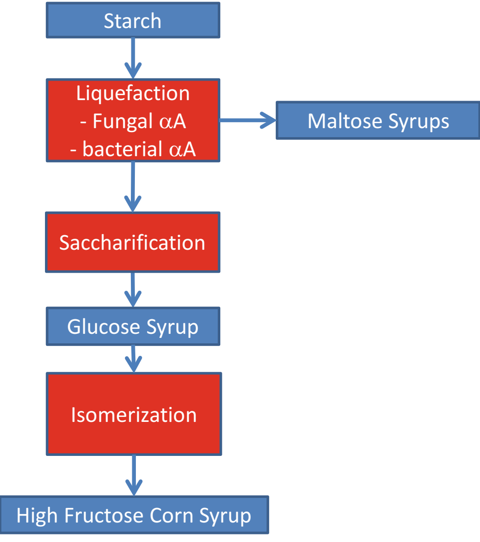

Fig. 2 Scheme of starch conversion to maltose, glucose, and HFCSs

less viscous syrup containing dissolved oligosaccharides or dextrins. The second step in sugar syrup generation is called saccharification. In this step the dextrins are converted to glucose by the action of glucoamylase. The chemistry and enzyme biochemistry of these conversion steps have been covered extensively elsewhere [12]. The application of industrial enzymes in processing starch to sugar syrups is reviewed in [7].

One enzymatic step is needed to produce maltose syrups, two for glucose syrup, and three for HFCS.

For production of the disaccharide maltose a fungal α-amylase from *Aspergillus oryzae* is used [7]. This enzyme breaks down gelatinized starch to maltotriose and maltose and is used to produce high maltose syrups. A combination of pullulanase and β-amylase from barley is used for very high maltose syrups. If glucose content should be higher, fungal glucoamylase is added. Maltose syrups are produced in wide varieties for specific applications in the brewing industry as well as for confectionary and baking.

Glucose syrups are produced in two steps: starch liquefaction followed by saccharification (see Fig. 2). Today, most plants operate a jet cooking process for starch gelatinization and liquefaction. This is made possible by development of thermostable α-amylases which are mixed into the starch slurry which is then pumped through a jet cooker where steam is injected and the temperature is raised to 105 °C for a short time. At such a temperature, full gelatinization of the starch occurs, followed by hydrolysis to dextrins; further hydrolysis to lower molecular weight dextrins occurs by holding the mash at 80–95 °C. Saccharification is then carried out at around 60 °C using glucoamylases and pullulanases. Pullulanase cleaves α-1,6-bonds in amylopectin and thus supports fungal glucoamylase which

bites away one glucose molecule at a time from the reducing end of the amylose molecule.

If glucose is converted partially (45–50%) to fructose, a syrup is obtained which is as sweet as sucrose syrup but cheaper in cost. Thus HFCS has become the preferred sweetener in the soft drink industry; about 14 million mt are produced annually, of which about 8 mt are produced in the US [7]. Fructose formation from glucose is catalyzed by glucose isomerase (GI). The enzyme is relatively expensive; moreover, the glucose syrup is a relatively pure feedstock stream. Therefore it is possible to use immobilized GI in cylindrical reactors, up to 1.5 m in width and up to 5 m in height. One such reactor can produce up to 50 mt of HCFS per day. The half-life of immobilized GIs can be as long as 200 days, that is, a reactor needs to be freshly loaded approximately every 600 days.

2.2 Fuel Ethanol

Ethanol is by far the largest volume product produced from starch. If produced from corn, as a co-product corn oil can be sold into the food and feed industries and into biodiesel production. Other alcohols are produced via fermentation from starch, such as *n*-butanol or iso-butanol, but these processes are not yet developed to the stage of fully integrated refineries as in the ethanol first or second generation industry. First generation ethanol covers the corn-to-ethanol plants mostly in the US and reviewed below; second generation ethanol means ethanol from lignocellulosic biomass, covered elsewhere in this volume.

Close to 15 billion gallons or 45 million mt of ethanol were produced in 2015 from corn in the USA [13]. RFS and the high octane value of ethanol – and thus its antiknocking action in gasoline – are responsible for this large volume. This amount is moderately stable for the time being, as crude oil prices are too low to allow good margins in the corn-to-ethanol industry and as the industry is encouraged to switch from first to second generation ethanol.

Corn-to-ethanol biorefineries mostly use the so-called dry-milling process (Fig. 3) for breaking down the corn kernels; only in a few cases is wet-milling used. These processes have been extensively reviewed [7–10] (Fig. 1).

In dry milling the corn is ground, in most plants by hammer mills, to a meal containing all constituents of the kernel, that is, mostly starch (amyloses and amylopectin) but also protein, oil, salts, and phytin. Then the gelatinization and liquefaction process starts and water is added together with lime and a thermostable α-amylase. The resulting meal slurry, 28–37 wt%, in some plants is passed through a jet cooker, where steam is injected, temperature is increased to 105 °C for about 5 min, and then the slurry is pumped further into a liquefaction tank and kept there for a further 1–2 h at around 90 °C. Other plants do not operate a jet cooker and the meal slurry enters directly into the liquefaction tank. The addition of lime is needed to provide Ca^{2+} ions to the active center of the α-amylase; in recent years one major focus of development has been the reduction of Ca^{2+} use. α-Amylases with higher

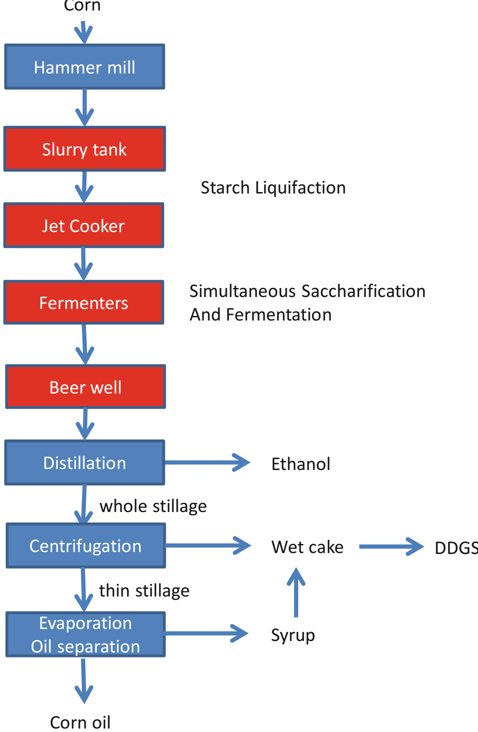

Fig. 3 Schematic view of the fuel ethanol process from corn, using dry milling. Process steps where enzymes are applied are marked in *red*

binding affinities for the Ca^{2+} ion at their active center have been generated by targeted modification and by screening of novel enzymes from other donor organisms.

In a novel variation of the corn-to-ethanol process, the feedstock itself has been used to provide part of the processing enzymes. Corn was genetically modified to contain a high level of α-amylase already as part of the enzyme portfolio in the gluten. This trait has been developed by the agrochemical giant Syngenta and branded Enogen®. The α-amylase released from the Enogen® corn kernel is said to reduce the viscosity of the meal slurry, energy cost, and water consumption. It may also reduce the amount of thermostable α-amylase needed for liquefaction. Although the idea of such a development is brilliant, and the practical advantages were obviously proven in quite a few plant trials, a practical problem was introduced for the farmers. This has to do with the reluctance of the European Union and some of its member countries to accept gene modification in food and feed, and it has been used politically by the Peoples Republic of China some years back. Enogen® should by no means be mixed with other corn which is targeted at the food or feed markets outside the US, otherwise the food or feed products containing traces of gene modified corn can be banned [14].

The mash coming from the liquefaction tank is pumped further into fermenters, huge stirred tanks of up to a million gallons in volume, that is, up to 4,000 m^3.

Fungal glucoamylase and yeast are added for simultaneous saccharification and fermentation at dry weight concentrations of meal ranging from 29wt% to 37wt%. The yeast is either delivered on site from a yeast factory in dry or liquid form, or it is produced in seed fermenters next to the ethanol fermenters. Other process enzymes may be added at the fermenter stage: acid proteases, phytases, and cellulases.

Proteases are added to hydrolyze maize proteins to amino acids and thus provide building blocks to the yeast for improved growth and reduce the need to feed a nitrogen source, mostly urea. Also attributed to the addition of proteases is an increase in corn oil recovery [15, 16]. Thermostable proteases have recently been developed for the purpose of increased corn oil recovery. These proteases are added at the downstream stages of the corn-to-ethanol process, after ethanol has been removed by distillation, to the whole stillage or thin stillage (see below) [17].

Phytase is added to hydrolyze phytin contained in the corn and generate phosphate. This phosphate in turn can be utilized by the yeast as a growth factor and thus less phosphate needs to be added to the fermenter, in turn less phosphate featuring on the bill for waste water treatment [18].

Finally, it has been observed recently that the addition of cellulases and hemicellulases into the fermenter improves the availability of starch and the release of corn oil from the microstructures still present after hammer milling and jet-cooking [16, 19].

After 48–60 h the fermenter broth is transferred into a holding tank, the so-called beer well. After the beer well, the process, which was continuous in the milling and jet-cooking stages, then becomes a batch process in liquefaction and fermentation becomes a continuous process again. Ethanol is removed by distillation and further purified by rectification and dewatered by molecular sieves. What is left after ethanol removal is called “whole stillage.” The whole stillage is pumped into a centrifuge, yielding “thin stillage” and “wet cake.” From 15% to 50% of the thin stillage is recycled as “back set” into the fermenter. The major part is concentrated into a syrup by passing through a series of evaporators. In the middle of the series of evaporators an oil centrifuge has been installed in recent years by almost all ethanol plants in the US: corn oil is separated from the syrup and sold into the biodiesel industry.

The final concentrated syrup is mixed into the wet cake and this is dried to DDGS. DDGS production is 45 million mt per year in the USA, and most of this is fed to cattle, both for milk and for meat production. The remainder is used for poultry or swine feeding.

Corn oil and DDGS were considered negligible side products 10 years ago. They are now co-products which each and every ethanol plant needs to examine closely to improve its margin which is under pressure from low crude oil prices. Thus it is of prime importance to the plant to increase corn oil recovery from syrup, and if possible to increase the feeding quality of the DDGS.

This trend makes the corn-to-ethanol plant a true biorefinery, converting starch-containing feedstock into a bundle of products: ethanol, corn oil, and DDGS.

An ethanol plant with an annual production capacity of 100 million gallons may typically produce 23–30 million pounds of corn oil, equivalent to 10,000–14,000 mt

per year. Corn oil recovery can be increased by 10–25% by adding proteases or cellulases and hemicellulases; see above [15–19]. Such increase means additional production of 1,000–1,400 mt per year.

The same ethanol plant, producing 100 million gallons of ethanol and 10,000–14,000 mt of corn oil, also produces about 160,000 mt of DDGS annually. DDGS has been discovered to be a valuable feedstock; in addition to the fibers coming from the corn kernel, it contains residual maize proteins, residual oil, and all the yeast biomass. DDGS is a good feedstuff for ruminants but for monogastrics such as poultry or swine there are limitations, mainly because of the structure of the fibers. Development work is underway to improve feeding value and inclusion rate by adding cellulase/xylanase enzyme blends to the beer well or the fermenter. Improvement of DDGS nutritional value has been shown to be effectuated by the addition of a blend of hemicellulases and cellulases to the beer well [20–22]. This enzyme blend reduces the amount of fibers in the DDGS which harm digestibility. The nutritional effects need to be studied more in detail.

Althgough corn oil and DDGS have become valuable co-products of the corn ethanol industry, enzymes are also being used to improve process efficiency and/or to reduce unwanted waste products. Protease for the purpose of supplying amino acid to yeast has been mentioned above. Phytase is another enzyme frequently added to the fermentation process [18]. Phytin or phytic acid is a phosphate storage compound in plants; the sugar inositol is sixfold phosphorylated. Phytin has a threefold effect: (1) in the waste water of ethanol plants, or secondary in the waste streams of poultry or swine who are on a DDGS diet but cannot metabolize phytin, a phosphate problem for the environment is generated; (2) phytic acid in the upstream part of the process acts as an inhibitor to at least some of the α-amylases commercially used, for example, the α-amylase from *Bacillus stearothermophilus*; (3) phytin in the downstream process of the ethanol plant enhances viscosity and fouling in the evaporators. Phytase, by hydrolyzing the phytin, solves all these problems; it provides better stability and activity to the α-amylase and thus better liquefaction, it decreases viscosity in the DSP, it provides free phosphate in the DDGS, and it enhances feeding quality. To do so, it must be added prior to the liquefaction process, a process scheme suggested by [18].

In summary, the processing of grain really is to be considered as a biorefinery: crude feedstock biomass is split up by physical and chemical means, part of the product stream (starch) is directly converted further to the final product ethanol, side product corn oil is recovered, and, as with pitch in the crude oil refinery, DDGS is a complex product which in the past was considered of low value but is now considered a valuable product stream.

2.3 Beer Brewing

Beer brewing may be considered the finest possible starch biorefinery, at least as far as taste of the end product is concerned. Looking at the complexity of the feedstock

it makes a lot of sense to support the brewing process by externally added enzymes. This is practiced worldwide on a large scale, except for Germany. Here the so-called Reinheitsgebot (= beer purity act) dating back to the year 1516 strictly interpreted forbids the addition of anything other than barley, hop and water to the beer-making process.

In the classical beer-brewing process the enzymes to liquefy and saccharify grain-based (mostly barley) starch are intrinsically provided by the mashing process from the grain's own enzyme constituents. Exogenous enzymes are added to supplement the endogenous enzymes and guarantee process outcome; additionally, new types of beer can be created such as low-carbohydrate "light beer", or the beer maturation time can be shortened. Moreover, overall cost can be reduced and raw material other than the relatively expensive malt can be used, for example, rice or sorghum. A complete overview of the possible use of commercial enzymes is provided in [23]. Table 1 has been extracted from this source.

Table 1 Overview of possible use of commercial enzymes in starch biorefineries

Operation	Enzymes	Enzyme action	Function
Decoction vessel (cereal cooker)	α-Amylase	Hydrolyse starch	Adjunct[a] liquefaction. Reduce viscosity
	β-Glucanase	Hydrolyse glucans	Aid the filtration
Mashing	α-Amylase	Hydrolyse starch	Malt improvement
	Amyloglucosidase	Increase glucose content	Increase % fermentable sugar in "light" beer
	Debranching enzyme	Hydrolyse α-1,6 branch points of starch	Secures maximum fermentability of the wort
	Proteases	Increase soluble protein, and free amino-nitrogen (FAN)	Malt improvement. Improved yeast growth
	β-Glucanase	Hydrolyse glucans	Improve wort separation
	Pentosanase/xylanase	Hydrolyse pentosans of malt, barley, wheat	Improve extraction and beer filtration
Fermentation	Fungal α-amylase	Increase maltose and glucose content	Increase % fermentable sugar in "light" beer
	β-Glucanase	Hydrolyze glucans	Reduce viscosity and aid filtration
	α-Acetolactate-decarboxylase (ALDC)	Converts α-acetolactate to acetoin directly	Decrease fermentation time by avoiding formation of diacetyl
Conditioning tank	Protease	Modify protein-polyphenolic compounds	Reduce the chill haze formed in beer

[a]Adjunct is starchy cereals such as maize, rice, wheat, sorghum, barley, or pure starch materials added to the mash

2.4 *Further Enzymatic Processes with Starch*

2.4.1 Potable Alcohol Distilling

As in beer brewing, starch from many sources can be converted to alcohol for human consumption. In principle, the enzymes applied correspond to those used in producing glucose syrups from starch; see above. The distillers industry is a much more fragmented industry than the starch refining industry. There are large manufacturers of potable alcohol such as Cargill [24], but numerous medium sized and small and specialist distillers exist. These are serviced by distributors who are selling specially confectionated blends and provide process application services to each plant. The enzymes used are basically the same as in the brewing industry.

2.4.2 Enzymes in Starch Modification

Starch can be modified by branching enzymes and amylomaltase; thereby the degree of branching is increased, the degree of digestibility in the digestive tract is reduced, and "low calorie starch" is generated. The increase in branching degree also causes better mouth feel and aroma release [7, 25, 26].

Starch-branching enzymes (SBEs) generate α-1,6-bonds in α-glucans by cleaving internal α-1,4 bonds and transferring the reducing ends released to C-6 hydroxyl groups. SBEs form one of the four major enzyme classes involved in starch biosynthesis in plants and algae.

2.4.3 Enzymes in Cyclodextrin Production

Cyclodextrins are cyclic oligosaccharides or dextrins. They are produced from starch by enzymatic conversion with cyclodextrin glycosyl transferase or CGTase. They are used in the food, pharmaceuticals, and chemical industries and in agriculture and environmental engineering. Typical cyclodextrins contain six to eight glucose monomers, α-cyclodextrin contains six, β-cyclodextrin contains seven, and γ-cyclodextrin eight glucose units. The cage formed by the cyclic molecules can reversibly bind small molecules such as flavor or fragrance molecules. Thereby, depot formulations can be generated, which are used in instant meals or in long-lasting cosmetic preparations. Delayed or controlled release forms of medicines are generated by encaging active pharmaceutical substances using cyclodextrins.

The annual production volume of cyclodextrins is one order of magnitude smaller compared to sugar syrup; it grew from 191,000 mt in 2009 to 353,160 mt in 2013 [27]. The products have a higher added value than sugar syrups, the global market value is reported as $649 million for 2013, and projected to grow to $904 million in 2018 [28].

3 Development and Manufacturing of Starch Enzymes

Where do the enzymes come from, these workhorse products used in hundreds of plants and in thousands of tons annually? From which species do the enzymes or the genes coding for these enzymes originate, and which host organisms are used to manufacture them on an industrial scale? Moreover, what are the major trends in enzyme development?

Before we start on these topics, a short look at whom this industry consists of.

3.1 Industrial Players in Starch Enzymes

The major industrial players in starch enzymes are market leader Novozymes followed by a strong number two, Dupont Enzymes. Dupont is in merger talks with Dow Chemicals and it could be interesting to see what will happen to Dupont's enzyme division, which started as Genencor in the early 1990s, grew by acquiring the enzyme divisions of Gist Brocades and Solvay, then became Danisco for a time until Danisco was acquired by Dupont in 2011. Novozymes and former Genencor have substantially contributed to the development of thermostable amylases or proteases, and improved enzyme blends, for example, glucoamylase + cellulase designed to increase efficiency and reduce process costs in the starch conversion and ethanol industry. They have also introduced the application of non-starch conversion enzymes such as proteases, cellulases, xylanases, and phytases as process enhancers. In the US quite a few Chinese suppliers have started to sell amylases into the market, such as CTE, Boli, and Sunson. Companies such as AB Enzymes and DSM are also active in starch conversion and are especially strong in enzymes for the food and beverage industries. BASF has shown interest in entering the enzyme industry by the acquisition of Verenium and technologies from other enzyme innovators. Japanese Amano is probably the world's largest manufacturer of fungal enzymes using surface culture in open trays.

3.2 Origins of Starch Enzymes

An extensive review of hundreds of enzymes active in starch chemistry, including reaction chemistry and kinetics, is available [12]. Those enzyme products commercially used are a small but powerful selection of this long list.

α-Amylases used in liquefaction originally came either from *Bacillus licheniformis* or from *Bacillus stearothermophilus*. α-Amylases hydrolyze internal α-1,4-glycosidic bonds of amylose or amylopectin in gelatinized starch and thus break down the polymers into smaller oligomers of a chain length of typically 8–12 dextrose equivalents. The wildtype enzymes have been genetically modified mostly

(1) to increase the activity and stability at higher temperature, (2) to eliminate the need of calcium addition, and (3) to act at lower pH. Instead of genetic modification, enzymes acting and stable at harsher conditions can be found from nature in thermophilic or extremophilic microorganisms [29, 30].

The first efforts to improve the commercial properties of α-amylases were directed at increasing activity at higher temperature, to allow starch hydrolysis at 80 °C, or even, for a short time, at 105 °C under jet cooking conditions. Enzymes active and stable at boiling water conditions have been isolated and genetically modified, as described and patented by [31–33]. The patent literature is extensive, proving the high commercial importance.

Calcium ions are needed in the active center of the α-amylase and lime was previously added to the slurry before liquefaction in order to have an active α-amylase. Later in the process, calcium ions had to be removed again by ion exchange because they inhibit GI in HFCS production. α-Amylase variants have been created which bind the calcium ion tightly to the active center, and the calcium coming with the corn slurry is sufficient for enzyme activity.

The pH optimum of α-amylase activity is normally between 6.0 and 7.0. The pH optimum of the saccharification and fermentation step is below 5.0. In the last 10–15 years, α-amylases have been engineered which can be used at pH 5.0, thus avoiding pH adjustment, and ion exchange which used to be necessary to remove ion load which disturbs glucose isomerization in HFCS production [34].

β-Amylase is an enzyme that hydrolyzes starch from the nonreducing end producing maltose-units in the β-form. Such enzymes were only known from plants, barley, soy, or wheat for many years. Today β-amylase is also industrially produced by extraction from barley. In Japan Amano was able to identify microbial β-amylases and also to produce one of these from a Bacillus species industrially [35]. The optimum reaction temperature and stability is about 10 °C higher than that of the barley enzyme, thus allowing a higher reaction rate. Besides generating maltose units, the enzyme inhibits retrogradation of starch in cakes, thereby allowing baked products to remain fresh and soft for a longer period.

Glucoamylase is used for the saccharification step; it hydrolyzes starch from the nonreducing end, removing glucose units one by one. Thus for glucose syrup production, liquefaction by α-amylases is needed to prepare shorter chain length oligomers which are then submitted to glucoamylase action. Industrially used glucoamylases normally originate from fungal origin, the main products being GA from *Aspergillus niger* and *Aspergillus oryzae*.

Pullulanase catalyzes hydrolysis of α-1,6-glycosidic bonds which are present in amylopectin. Thus pullulanase is also called a “de-branching enzyme.” Although glucoamylase is able to hydrolyze α-1,6-glycosidic bonds in amylopectin, it does so only slowly. Normally pullulanase is therefore added to the process to speed up reaction time. The enzyme can be found in plants and bacteria, less in fungi. One broadly used type originates from *Bacillus acidopullulyticus*.

3.3 Expression Host Strains

As described above, starch processing is a multimillion ton business; the amount of enzyme needed is substantial, the cost pressure as well. The enzyme industry uses a number of expression platforms to produce enzymes as efficiently as possible. The most frequently used bacterial hosts are *Bacillus subtilis* and *B. licheniformis*. The most used fungal systems are *Aspergillus oryzae*, *A. niger*, and *Trichoderma resei*. More recently, a platform with very high versatility and productivity was introduced by Dyadic [36] and licensed by BASF. Dyadic is now also part of Dupont's enzyme business. This platform is based on a fungus *Chrysosporium lucknowense* isolated from alkaline soil.

In searching and defining a good recombinant production host strain for commercial application, the following criteria have to be fulfilled [37]:

- Versatility, that is, a broad variety of genes must be expressed
- Safety, that is, the organism has to be nonpathogenic toward humans, animals, and plants; ideally, the strain would be registered as GRAS, Generally Registered as Safe
- Good protein secretion, that is, the enzymes produced should be secreted by the cells into the fermentation media, thus avoiding costly cell disruption and purification steps

The host strain should be fermentable in an industrial fermentation process, that is, in large submerged, stirred, and aerated fermentation systems. Only this technology allows a commercially reasonable time and space yield.

Besides working on suitable host organisms, it is important to have appropriate and, if possible, proprietary expression vectors and transformation procedures. Today most companies are working with stable integration of the genes of interest into the host chromosome in one or more copies.

4 Trends in Development of Enzymes for the Starch Conversion Industry

The major drivers of enzyme development are (1) cost, (2) competitiveness, and (3) new substrates and products. Obviously these topics are interlinked.

Cost reduction can be achieved by reducing the number of process steps or the use of additives. Thus development continues to look at fine tuning pH and temperature profiles, in respect of both reaction activity and stability. Activity and stability at higher temperatures has been and still is a "hot topic," running from the 1990s until today; new patent applications are filed and there is fierce debate on intellectual property rights between the two major companies of this industry [38].

Another way to reduce costs and improve efficiency is the addition of acid protease to reduce the addition of urea as a nitrogen source to the ethanol fermentation process. Proteases have been used for some time already; they are added at the beginning of the fermentation process to hydrolyze the proteins in the corn mash coming from the corn kernel, thereby providing amino acids to the yeast. This reduces the amount of urea which normally needs to be added as a nitrogen source to the fermentation, and thus saves on the cost side. Recently, a new, thermostable acid protease has been introduced which can be added prior to the liquefaction step [17]. It is claimed that not only the supply of nitrogen source to the fermentation is increased but also the release of corn oil, thus providing both cost saving and increased revenue to the ethanol factory. The theory behind this is that corn oil is stored in the form of oleosomes, and oleosomes in turn are stabilized by proteins, so-called oleosines [39], which are hydrolyzed by the protease.

The above considerations apply not only to the corn-to-ethanol process but also to all other starch-converting processes: an innovative enzyme supplier has a dedicated application laboratory to study and thus understand the target process as deeply as possible, better than his customer. Better understanding leads to discovery of special enzymes, which in turn provide a competitive edge to the supplier.

Cost reduction obviously means profitability increase. Profitability can also be increased by enzymatically helping to produce value-added co-products, for example, in the case of the corn-to-ethanol industry, the production of corn oil or value-enhanced DDGS, or enhancing the ethanol yield. Enhanced use of feedstocks such as sorghum or cassava for ethanol production also triggers development of specially adapted enzymes.

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Organosolv Processes

Nicolas Brosse, Mohd Hazwan Hussin, and Afidah Abdul Rahim

Abstract Biofuels and chemicals can be produced from lignocellulosic feedstocks using biotechnological processes. The effective utilization of carbohydrates from biomass for the production of biofuels necessitates the development of pretreatment technologies to enhance their enzymatic digestibility. Among all the various pretreatment methods currently studied and developed, the organosolv processes, in which organic solvents or aqueous organic solvent mixtures are used as the pretreatment medium, appear to be specially promising in the context of the biorefinery because (1) they produce cellulosic pulp with a good enzymatic digestibility for monomeric glucose production and (2) they allow a clean fractionation of the major biomass components (cellulose, lignin, and hemicelluloses) into three process streams. In this chapter we give an updated overview of organosolv methods using conventional solvents and ionic liquids which have recently gained considerable interest as solvents for lignocellulosic biomass and pretreatment.

Keywords Ionic liquid, Lignocellulose, Pretreatment

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1 Introduction

The most crucial issue faced by the world today is the sustainability of consumption of energy and natural resources. As fossil fuel creates problematic issues (because of global warming, increase in price, and running out), the use of renewable resources to shift the oil-based economy into a bio-based economy is one of the alternative choices. With a goal of reducing net greenhouse gas emissions, this marks an important turning point in the effort to promote the use of renewable energy to fulfill the commitments of the Kyoto Protocol and the COP 21 [1, 2]. Biomass has been considered as one of the potential sources of renewable energy throughout the world. Several agencies worldwide have recently reported an increase in the gross domestic energy and chemical production from renewable energy, especially biomass. It was reported that the European Union (EU) utilized about 66.1% of its renewable energy (including heat generations) from biomass which surpassed the contribution of other energy sources such as hydro, wind, geothermal, and solar power [3]. The development of (bio)technologies and (bio) processes for biomass valorization is focusing not only on the production of energy but also on the production of biofuels and biomaterials.

Dry matter of plants can be referred as lignocellulosic biomass. It has been acknowledged as the most abundant source of renewable energy (approximately 200×10^9 tons/year) obtained from crops, wood, and agricultural waste [4, 5]. Lignocellulosic biomass is best suited for energy and chemical applications because of its sufficient availability, and it is inexpensive and environmentally safe. It is composed of cellulose, hemicellulose, and lignin with small amounts of proteins, lipids, and ash that later form the complex structure of the plant cell wall. The composition of these compounds essentially depends on the origin of the plant as listed in Table 1.

The lignocellulosic materials (cellulose, hemicellulose, and lignin) are interconnected with each other through covalent crosslinks. Recent work in this area has mainly focused on the delignification of lignocellulosic biomass separating lignin, cellulose, and hemicelluloses to be used in both physical and (bio)chemical applications. The cellulose and hemicellulose can be hydrolyzed to monomeric sugars and often converted to value added products such as ethanol, additives,

Table 1 Representation of different fractions of lignocellulosic materials[a]

Lignocellulosic materials	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Hardwood stems	40–55	24–40	18–25
Softwood stems	45–50	25–35	25–35
Nut shells	25–30	25–30	30–40
Corn cobs	45	35	15
Grasses	25–40	35–50	10–30
Wheat straw	30	50	15
Cotton seed hairs	80–95	5–20	0
Coastal Bermuda grass	25	35.7	6.4
Typha capensis	34.2	11.6	26.4
Miscanthus x giganteus	38.2	24.3	25
Poplar aspen	42.3	31	16.2
Empty fruit bunch	59.7	22.1	18.1
Switch grass	45	31.4	12
Oil palm fronds	35.7	28.4	24.6

[a]Source: [6–11]

organic acid, and others by chemical and biochemical processes [12]. All of these lead to the utilization of lignocellulosics not only for second generation energy, chemical and material production but also for synthesizing food additives and feed supplements [13].

Organosolv pulping is the process of extracting lignin from lignocellulosic feedstocks with organic solvents or their aqueous solutions. Since the 1980s, organosolv pulping using low boiling point solvent (e.g., ethanol) has been an alternative to Kraft and sulfite pulping, which have some serious shortcomings such as air and water pollution. It was originally developed as Alcell® pulping process for hardwood. With the recent emerging need to develop alternative sustainable transportation fuel, the organosolv process for the production of ethanol is among the pretreatment strategies currently being studied and developed on an industrial scale for the conversion of lignocellulosic feedstock to biofuels and biomaterials [14, 15]. Moreover, organosolv pretreatment processes allow a clean fractionation of lignocellulosic feedstocks and the recovery of high-quality lignins, which are of great interest and are currently a focus of attention [16]. The availability of such lignin fractions in large quantities should stimulate development in new lignin utilizations [17]. In the last few years, a new type of solvent, referred to as an ionic liquid (IL), has been extensively described for the pretreatment of biomass and the extraction of lignin and holds great promise. Although the recovery yield is low, the primary advantages of the ILs for pretreatment technologies are the very low emissions of volatile organic compounds and their excellent solvent properties allowing them to dissolve the lignocellulosic matrix [18, 19].

In this chapter we focus on the organosolv process as a method for fractionation of biomass and some discussion on organosolv pulping and pretreatment, together

with the chemistry of organosolv delignification. The current applications of organosolv lignin and the role of ILs in the organosolv process are also discussed.

2 Conventional Organosolv Pretreatment

2.1 Organosolv Pulping

Generally, organosolv pretreatment has been shown to be a very effective process in fractionating lignocellulosic feedstocks into rich cellulose pulp, a water-soluble hemicellulose stream, and a solid organosolv lignin fraction [15, 20]. Since the 1970s it has attracted much interest for addressing the environmental concerns with regards to the conventional Kraft and sulfite processes, which cause serious issues such as water and air pollution [14]. It is important to note that organosolv pretreatment is similar to organosolv pulping except for the degree of delignification which is not as high as that of pulping. Some advantages of organosolv pretreatment are as follows: (1) it allows easy recovery of organic solvents via distillation and (2) it allows isolation of lignin and carbohydrates that show promise as chemical feedstocks [21–23], thus making it more feasible for biorefinery of lignocellulosic feedstocks. The organosolv process utilizes ethanol or methanol as solvent and mineral acids as catalyst. Previous studies have shown that yields of organosolv softwood pulps are higher than those of conventional Kraft and sulfite pulps at equivalent Kappa number [22]. Generally, Kappa number is a measure of the amount of lignin remaining in pulp. The higher the Kappa number value, the greater the amount of bleaching chemicals required to brighten the pulp. At the industrial scale, organosolv pulp mills can be operated on a smaller volume (around 300 tons pulp/day) to that of Kraft pulp mills (1,000 tons pulp/day) [24], which can easily be bleached (chlorine-less) and are environmentally safe.

2.2 Overview of Ethanol Organosolv Pretreatment

It is important to note that different cooking conditions of lignocellulosic feedstocks on organosolv pretreatment/pulping are very crucial to preserve high quality fiber. Recycling the solvent used in the organosolv process is essential to lower the total costs. Organosolv pretreatment uses a large number of organic or aqueous-organic solvent systems with or without catalysts but the ethanol organosolv pretreatment is more described and is the subject of industrial developments (e.g., lignol process). The general ranges are as follows: cooking temperature of 100–250 °C, cooking time of 30–90 min, ethanol concentration of 35–70 wt%, and solid to liquid ratio from 1:4 to 1:10 (w/w) [15, 25]. Lignin can be recovered as a precipitate by flashing the black liquor to atmospheric pressure followed by

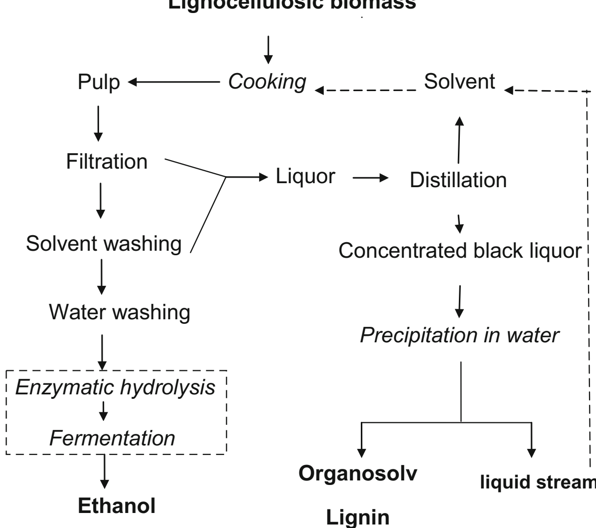

Fig. 1 Process flowchart of low boiling point alcohol pretreatment

dilution with water. The process of ethanol (and more generally of low boiling point solvent) organosolv pretreatment is illustrated in Fig. 1. Neilson et al. [26] have revealed that ethanol organosolv pretreatment was said to be an effective method for enzymatic hydrolysis of cottonwood. It gave about 1.8–2.5 times the sugar when the pulp was enzymatically hydrolyzed with *Trichoderma reesei* cellulase to that of untreated cottonwood. Ethanol organosolv pretreatment can be performed in the presence or absence of catalyst with auto-catalyzed pretreatment performed at higher temperatures (185–210 °C) [14, 15]. In such conditions, the severity of the organosolv pretreatment can be calculated as the combined severity factor, CS, with specific pH, pretreatment time (t in minutes), and pretreatment temperature (T in degrees Celsius; note that T_{ref} is 100 °C) [27]:

$$\mathrm{CS} = \log\left[t \exp\left(\frac{T - T_{\mathrm{ref}}}{14.7}\right)\right] - \mathrm{pH}$$

Additionally, co-products such as hemicellulose sugars and furfural can be recovered from the water stream. During ethanol organosolv pretreatments there are four main reactions or processes that occur: (1) hydrolysis of lignin hemicelluloses linkages via cleavage of 4-*O*-methylglucuronic ester bonds to α-carbons of lignin and cleavage of α- and β-*O*-aryl ether linkages, respectively; (2) glycosidic bonds cleavage in hemicelluloses; (3) acid-catalyzed degradation of monosaccharides to furfural, hydroxymethylfurfural (HMF), and other degradation products; and (4) lignin condensation reactions [15]. More information on the mechanism during organosolv pretreatment is discussed in the next section.

If the pretreatment is performed at high temperature, acid addition is not necessary as it is believed that organic acids released from the feedstock act as

catalyst for the degradation of the lignin–carbohydrate complex [20]. Nevertheless, if the acid catalysts are added, this increases the delignification rate that results in higher yield of xylose. The use of mineral acids (such as hydrochloric acid, sulfuric acid, and phosphoric acid) and organic acids (oxalic, formic, salicylic, and acetylsalicylic acid) as catalysts could be useful to accelerate delignification and degradation of xylan [28]. Sulfuric acid is the most frequently used mineral acid catalyst for ethanol organosolv pretreatment, which has been applied to various types of lignocellulosic feedstocks such as pine, hybrid poplar, miscanthus, switchgrass, and oil palm residues [11, 29–32]. The usual concentration of sulfuric acid based on dry weight of biomass is usually in the range of 0.5–1.75%. Higher concentrations of acid lead to greater delignification and greater hemicellulose degradation.

The cellulose-rich pulp produced during acid catalyzed ethanol organosolv pretreatment is suspected to exhibit high glucose yields after enzymatic hydrolysis than base catalyzed pretreatment. In a previous study, Del Rio et al. [33] have shown that NaOH-catalyzed pretreatment of lodgepole pine gave high lignin removal but low cellulose to glucose conversion. Compiled results of enzymatic digestibility of cellulose after ethanol organosolv pretreatment from different biomass are summarized in Table 2. Some characteristics of ethanol organosolv pretreated pulp are (1) low hemicellulose and lignin content with decreased cellulose chain length and (2) low molecular weight and high pore volume that could improve their digestibility [31, 33]. The resulting pulp can be subjected to any fermentation process for the production of ethanol. There are two most suitable fermentation processes for the production of ethanol: simultaneous saccharification and fermentation (SSF) and separate hydrolysis and fermentation (SHF). For the industrial scale, SSF is more preferable as it leads to lower overall process time, less enzymatic inhibition by hydrolysis products, and thus lower capital and operational costs [34].

2.3 *Other Solvents for Organosolv Pretreatment*

The use of solvent for organosolv pretreatment is not only limited to methanol or ethanol. High boiling point alcohols (e.g., ethylene glycol, glycerol, and tetrahydrofurfuryl alcohol) and other organic compounds (e.g., dimethylsulfoxide, ethers, ketones, and phenols) could also be used as solvents for organosolv pretreatment. Table 3 shows some advantages and disadvantages of these solvents.

2.4 *Combinative Pretreatment Process*

Recently, combinative pretreatment process of lignocellulosic biomass has become an important process because it promises higher efficiency, higher delignification

Table 2 Fractionation of biomass by ethanol organosolv pretreatment as percentage dry weight of untreated feedstocks [15]

Biomass	Cellulose			Hemicellulose			Lignin			
	Untreated	Liquid fraction	Solid fraction	Untreated	Liquid fraction	Solid fraction	Untreated	EOL[a]	Liquid fraction	Solid fraction
Loblolly pine[b]	42.0	3.0	33.3 (55)	21.6	0.5	15.3	29.9	5.4	12.4	11.6
Lodgepole pine[c]	50.5	4.2	37.6 (100)	23.9	1.2	11.1	25.1	19.6	4.8	4.2
Hybrid poplar[d]	48.9	0.6	43.2 (98)	22.4	4.9	11.2	23.3	15.5	5.2	6.2
Miscanthus[e]	37.7	1.7	35.5 (98)	37.3	3.3	25.1	26.3	18.1	0.2	7.8
B. davidii[f]	38.9	1.4	32.3 (98)	26.1	5.7	11.0	30.2	8.9	3.4	19.0

[a]EOL = ethanol organosolv lignin
[b]65% aqueous EtOH, 1.1% H_2SO_4, 170 °C, 60 min [35]
[c]65% aqueous EtOH, 1.1% H_2SO_4, 170 °C, 60 min [36]
[d]60% aqueous EtOH, 1.3% H_2SO_4, 180 °C, 60 min [29]
[e]80% aqueous EtOH, 0.5% H_2SO_4, 170 °C, 60 min [31]
[f]50% aqueous EtOH, 1.8% H_2SO_4, 180 °C, 40 min [32]

Table 3 Advantages and disadvantages of other solvents for organosolv pretreatment

Solvent	Advantages/disadvantages	Reference
Butanol	– Excellent delignification agent because of its hydrophobicity – Limited miscibility of aqueous butanol leads to higher pretreatment severity, which leads to the formation of pulp with lower hemicellulose content, lower degree of polymerization (DP_w) and increased in pore size	[33]
Ethylene glycol and glycerol	– Pretreatment can be conducted at atmospheric pressure, which reduces energy costs – Effective for the delignification of wood chips – High costs of solvent recovery	[9, 37]
Organic acids and peracids	– Good lignin solvents – Limited because of their corrosive properties – Xylose did not undergo significant conversion to furfural	[38, 39]
Acetone and ketones	– Excellent solvent of lignin for both auto-catalyzed and catalyzed acetone organosolv pretreatments – Almost all hemicellulose solubilized and about 47% lignin can be recovered as organosolv lignin – 50% of acetone-water mixture gives highest lignin recovery (~60%)	[40, 41]
Phosphoric acid	– Pretreatment can be conducted at atmospheric pressure at relatively low temperature (around 50 °C) – High cellulose enzymatic hydrolyzability – Problem of corrosion	[42]

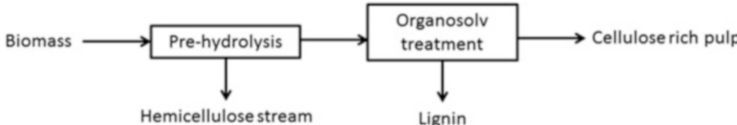

Fig. 2 Schematic of a combinative organosolv pretreatment

rate, less severity of cellulose pulp, and less concentration of fermentation inhibitors [43–45]. The pre-extraction of hemicelluloses could also improve the process economics; the hemicelluloses extracted could be used in high-value-added products such as barrier films, coatings, hydrogels, or paper additives [46]. In this approach, a presoaking or prehydrolysis (as the first step) of biomass is involved to hydrolyze the hemicelluloses and is followed by the organosolv delignification process (as the second step) where the solid residue from the first step is retreated (Fig. 2).

The main objective of the two-step processes is to reduce the degradation of carbohydrate fragments into furfural and hydroxymethylfurfural [31, 45]. It was believed that the lignin deconstruction during the prehydrolysis treatment increases the extractability of organosolv lignin through the breaking of lignin–carbohydrate bonds, resulting in smaller lignin fragments. Nevertheless, the deconstruction of lignin is often associated with repolymerization reactions through the formation of

new C–C bondings (β-β, β-1, and β-5) which sometimes still affect the delignification rate and lignin structure. Some common pretreatment/prehydrolysis for combinative pretreatment processes are listed below.

2.4.1 Acid Pretreatment

Acid pretreatment is the most common method that utilizes mineral acids such as hydrochloric acid (HCl) and sulfuric acid (H_2SO_4) for the treatment of biomass. According to Sun and Cheng [28], acidic treatment of lignocellulosic biomass improves the enzymatic hydrolysis of pulp and at the same time gives higher recovery of fermentable sugars. However, the pretreatment with concentrated acids is not suitable in economic and environment perspectives because it is toxic, hazardous, and corrosive to the reactor. Thus researchers have mainly focused on the utilization of dilute acids for the biomass pretreatment. To date, various kinds of dilute mineral acids such as sulfuric, hydrochloric, nitric, phosphoric, and peracetic acids have been experimented with for this pretreatment [47]. Among these acids, sulfuric acid is of interest because of its low cost and efficiency [48]. It was also reported that the dilute sulfuric acid can be used as an alternative for the production of furfural from biomass hemicellulose [49].

2.4.2 Autohydrolysis

Autohydrolysis is a biomass pretreatment with water at very severe conditions (elevated temperatures and pressures). In this process, the lignocellulosic biomass is heated at high temperature and pressure which results in the solubilization of acid components, de-esterification of ester groups, and formation of organic acids in the hemicellulose structure. The resulting organic acid (such as acetic acid) causes a hydrolytic breakdown of hemicellulose which is spontaneously repeated [50]. Because of the mechanistic action of hydronium ions on lignocellulosic biomass, it was believed that the autohydrolysis process selectively dissolves most of the hemicellulose portion which can be recovered in the residual solution [51]. The hemicellulose-rich liquid portion can be used potentially for the synthesis of furfural derivatives or other green chemicals [48].

The removal of hemicellulose from the lignocellulosic biomass would enhance the hydrolyzability of cellulose, leaving the solid pulp rich in cellulose and insoluble lignin residue [52]. Optimization of the operating conditions during autohydrolysis is considered important to ensure the effectiveness of the delignification ability of treated biomass. Brosse et al. [53] have reported that the operation conditions of *M. x giganteus* during autohydrolysis have affected the removal of hemicellulose, delignification yield and the efficiency of cellulose conversion to glucose during enzymatic hydrolysis.

2.4.3 Addition of Organic Scavengers in Combinative Pretreatment

Previous studies have shown that hydrothermal treatments such as autohydrolysis could lead to the change in lignin structures and overall delignification yield because of the repolymerization of lignin [54–59]. The repolymerization of lignin through the formation of carbonium ion intermediates produces new linkages of β-β, β-1, and β-5 bonds [60], where the carbonium ion is formed from the lignin phenylpropane units during acidic conditions of autohydrolysis. The resulting lignin after repolymerization is highly condensed and insoluble with high molecular weight which impairs the delignification of any pulping process [48].

It was also demonstrated that the presence of carbonium ion scavengers could substantially improve lignin extractability. These aromatic organic compounds compete with the aromatic rings of lignin during the incorporation of the carbonium ion. Therefore, it scavenges the carbonium ion intermediate from the self-condensation process during autohydrolysis. In a previous study, Wayman and Lora [50] have tested on 40 different types of aromatic compounds in combination with the autohydrolysis pretreatment. They have revealed that 2-naphthol gave lower lignin content in the residual pulp. The utilization of 2-naphthol as lignin–lignin recondensation inhibitor in different feedstocks has been further studied by other researchers [55, 57, 59]. More recently, some authors have studied the effect of adding different carbonium ion scavengers (*o*-cresol, *p*-cresol, hydroquinone, and dihydroxyanthraquinone) [58] on the delignification of *M. x giganteus* and oil palm fronds and it was reported that all organic scavengers used gave different organosolv lignin yields and properties.

2.4.4 Enzymatic Pre-hydrolysis

Enzymatic pre-hydrolysis using the industrial enzymatic cocktail Cellulyve® (Sigma Aldrich) was assessed as a first step in a pretreatment process of *Miscanteus* biomass involving an aqueous-ethanol organosolv treatment. It was demonstrated that, despite a very low impact on the fiber structure and composition (in terms of sugars and polyphenolics content), the enzymatic pretreatment disrupted the lignocellulosic matrix to a considerable extent. This weakening enhanced the removal of lignin during the organosolv pulping and the hydrolyzability of the residual cellulosic pulp for the production of monomeric glucose [44].

2.5 Organosolv Lignin and Delignification

Lignin is a natural aromatic amorphous macromolecule, a binder that holds together the lignocellulosic fibers to ensure rigidity of all vascular plants. The polyphenolic structure of lignin is known for its role to provide resistance of both chemical and

biological degradations in woody biomass. Perhaps this is because of the hydrophobic and insolubility nature of lignin in aqueous system that prevents the full access of chemicals and organisms. Generally, it is built up of three major C6–C3 (phenylpropanoid) units: *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol, which form a randomized structure in a 3D network inside the cell wall [61–63].

2.5.1 Mechanisms of Organosolv Delignification

It has been acknowledged that the cleavage of aryl ether bonds is responsible for lignin breakdown during organosolv pretreatment. The α-*O*-aryl ether bonds are more easily cleaved (also known as the rate-controlling step in organosolv delignification) than β-*O*-aryl ether bonds, which are normally broken under more severe conditions, especially at elevated acid concentrations [64, 65]. In the organosolv delignification process, several mechanistic pathways have been proposed: (1) solvolytic splitting of α-*O*-aryl ether linkages through quinone methide intermediate, (2) solvolytic cleavage via nucleophilic substitution benzylic position by S_N2 mechanism, and (3) formation of a benzyl carbocation under acidic conditions (Fig. 3). The β-*O*-aryl ether bonds (Fig. 4) can be homolytically cleaved with the loss of γ-methylol groups (as formaldehyde), which later give rise to the formation of stilbenes [32]. Additionally, the cleavage of β-*O*-aryl ether bonds can also form Hibbert's ketone functional groups [66].

Fig. 3 Solvolytic cleavage of α-*O*-aryl ether bonds in lignin [66]

R = OH, OEt or OAc
R = CH_3 or CH=CH-Ar(OH)

Fig. 4 Solvolytic cleavage of β- and γ-*O*-aryl ether bonds in lignin [66]

Lignin condensation can occur under severe conditions (normally under acidic condition), which are counter-productive to organosolv delignification [15]. In such a situation the lignin condensation occurs when the benzylic carbocation intermediate interacts with the electron-rich carbon atom of a neighboring lignin unit (Fig. 3). Condensation of lignin leads to the formation of high molecular weight (HMW) lignin fractions that are not soluble in organosolv pretreatment solvent, thus making the lignin recovery difficult. It has been reported that lignin condensation reactions can be prevented by blocking the reactive benzyl position (via electrophilic aromatic substitution) with phenolic solvents [15, 64]. In addition, the use of organic scavengers during combinative pretreatments can impede lignin condensation reactions (as mentioned in the previous section).

2.5.2 Current Applications of Organosolv Lignin

Much research work is ongoing toward the use of lignin for new green approaches [16]. This includes the use of lignin as dispersant in cement [67, 68], emulsifier [69], chelating agent for heavy metals removal from industrial waste effluents [70], absorbent [71], and phenol formaldehyde adhesives [72, 73] and tannins-based adhesives [74]. Organosolv lignin contains diverse functional groups of phenolic and aliphatic –OH, carbonyls, and carboxyls, which can also act as neutralizer or inhibitor in oxidation processes, via stabilizing reactions induced by oxygen radicals and their respective species. Thus, recent studies have been done on the applicability of lignins from different sources as potential antioxidants [57, 58, 75–77]. Hussin et al. [76] have revealed that organosolv lignin has a good antioxidant tendency with an inhibitive value of 60%. The antioxidant properties exhibited by lignin can lead to broader applications as anti-microbial, anti-aging agents and corrosion inhibitors. Recent findings have agreed that organosolv lignin and its derivatives possess inhibitive properties toward the corrosion of metals in corrosive media [76, 77]. Potential high-value products could also be produced from isolated lignin; these include low cost carbon fiber, engineering plastics and

thermoplastic elastomers, polymeric foams and membranes, and a variety of fuels and chemicals all currently sourced from petroleum [17]. However, the complexity and the multifunctional nature of lignin have yielded multiple extraction product streams, which require extensive separation and purification procedures. Proper modification/alteration of the organosolv lignin structures is still required to increase its applicability.

3 IL Pretreatments

Lignocellulose pretreatment for the production of bioethanol using ILs as solvent is an organosolv technology receiving growing interest from the biofuels community. ILs have been developed as a potentially 'greener' alternative to organic solvents. ILs are salts that are in liquid state at ambient temperatures. ILs present a high thermal stability (~200–250 °C) and a low vapor pressure. ILs have often been called green solvents mainly because of their negligible vapor pressure, which mitigates any emissions of volatile organic compounds (VOCs). The possible number of combinations cations/anions is huge although only around 100 ILs are described in the literature to this day. It is thus possible to modulate their physico-chemical properties (melting point, viscosity, density), except for vapor pressure which always remains extremely low [78].

The capacity of certain ILs to solubilize the (ligno) cellulose and to increase its enzymatic digestibility [79] has also been highlighted. The advantages are that ILs do not inhibit cellulose hydrolysis enzymes or accelerate enzymatic hydrolysis. The ILs can be supplemented with an antisolvent such as water, ethanol, or acetone for better cellulose regeneration, and can be recovered and recycled [80].

3.1 IL in Biomass Dissolution

Biomass dissolution is dependent on variables such as type and size of biomass, IL/biomass ratio, temperature and time of dissolution, and water content in the solution mixture. The dissolution of cellulose and lignocellulosic biomass has been extensively reviewed in recent years [19, 81–85]. ILs have been found to influence the biomass dissolution via several mechanisms. The lignocellulose dissolution is induced by the formation of electron donor–electron acceptor interactions (1) between cellulose oxygens and the cations of ILs and (2) between cellulose hydrogens and the anions of ILs [83]. It was shown that the dissolution is promoted by the presence of a high chloride concentration and activity of [BMIM][Cl] which is responsible for breaking the hydrogen bonding network of cellulose [79]. The imidazolium cation has also been proposed to have hydrophobic interactions with the hydrophobic side of the cellulose [86, 87] (Fig. 5).

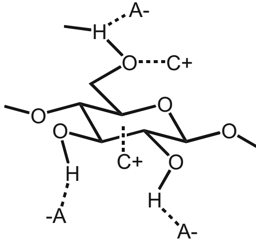

Fig. 5 Proposed dissolution mechanisms for cellulose in ILs

Table 4 Application of ILs for dissolution of lignocellulosic biomass

IL	Biomass	Loading (w/w)	Particle size (mm)	Conditions	References
[C2MIM][OAc]	Pine	5%	<0.125	175 °C, 30 mn	[93]
	Oak	5%	0.25–0.50	185 °C, 7 mn	[94]
	Spruce	5%	0.1–0.5	115 °C, 72 h	[95]
	Miscanthus	4%	4	130 °C, 8 h	[96]
[BMIM][OAc]	Corn stover	10%	<4	140 °C, 3 h	[97]
	Spruce	5%	0.3–0.8	120 °C, 1 h	[98]
	Rice hulls	10%	1.5	110 °C, 8 h	[99]
[BMIM][Cl]	Pine	5%	0.25–0.5	110 °C, 16 h	[94]
[C2MIM][Cl]	Beech	3%		120 °C, 24 h	[100]

These interactions lead to the disruption of the intra/inter-chain H bond network of cellulose and thus to its dissolution. These mechanisms of dissolution are enhanced by effective stirring and microwave and ultrasound irradiation [88–90]. Dissolution improvements have also been associated with hydrogen bond basicity of the IL anion such as [CH_3COO^-] wherein strong hydrogen basicity are effective in weakening the hydrogen bonding network of the polymer chains [91, 92]. However, increased hydrogen bond basicity could lead to the incorporation of water molecules in the IL structure, thus reducing the dissolution of biomass. Another important parameter to be considered is the viscosity of ILs that has an impact on the mixing and mass transfer of lignocellulose and the IL itself. Low viscosity ILs are able to extract higher amounts of carbohydrates from bran [92]. Table 4 summarizes recent examples of lignocellulosic dissolution in ILs.

3.2 *ILs in Pretreatment of Biomass*

In view of some of the drawbacks of the classical methods of biomass pretreatments (recycling issues, toxicity, hazardousness, etc.), application of ILs is an alternative solution. However, the complexity of the biomass matrix and ILs themselves makes

Fig. 6 The most used ILs for lignocellulosic pretreatment

the process very challenging. Among the advantages of the pretreatment with ILs are (1) the physicochemical properties of the biomass macromolecular components could be altered, (2) the specific property of the IL enables specific macromolecular components to be extracted, and (3) different fractionation approaches after biomass dissolution in IL could be performed. The success of the pretreatment is dependent on the IL, lignocellulosic biomass (type, moisture, size, and load), temperature, time of pretreatment, and the precipitating solvent. By far, [EMIM][CH_3COO^-], [AMIM][Cl^-], [BMIM][Cl^-], and dialkylimidazolium diphosphonates have been considered as the most suitable ILs for the pretreatment of lignocellulosic biomass because of their good solvent power (Fig. 6).

The use of [EMIM][CH_3COO^-] for various types of biomass utilizing pretreatment processes of various conditions has been reviewed extensively by da Costa Lopes et al. [101]. With the use of ILs in these pretreatments, cellulose, hemicellulose, and lignin fractions were obtained with higher purity that with conventional methods. Following the pretreatment step, different fractionation approaches for selective fraction separation can be applied using the specific properties of ILs. The entire process was found to be more economical than other conventional pretreatments such as alkali and acid pretreatments with respect to the energy consumed.

Recent approach to biomass pretreatment involves the addition of water to the dissolution process. This new approach allows the usage of small amounts of IL, easier processing operations because of reduced viscosity, and recycling of the aqueous IL mixtures is facilitated. In one of the earlier works to pretreat wood flour, a blend of 5% and 10% of water and [EMIM][CH_3COO^-] yielded a decrease in glucose and xylose that is attributed to a moderate inter-crystalline swelling effect with the wood fibers still intact and the crystallinity and lignin content being greater than in pure IL [102].

3.3 Enzymatic Saccharification of IL Pretreated of Biomass

For biofuel production, the polysaccharide fraction must be depolymerized into monomers before a fermentation step. The saccharification yield (e.g., monomeric glucose yield) is an important measure of the success of any lignocellulose pretreatment and remains as one of the critical bottlenecks. The development of

Table 5 Example of recent studies using ILs and glucose yields after enzymatic hydrolysis

Biomass	IL	Conditions	Glu%	References
Corn cob	Choline chloride and imidazole	15 h, 150 °C	94	[104]
Corn stover	[EMIM][OAc]	3 h, 110 °C	88	[105]
Softwood	[BMIM][Cl]	15 h, 130 °C	78	[106]
Miscanthus	DMIMMPh	6 h, 100 °C	72	[107]
	[BMIM][Cl]		68	
	[EtOHMIM][Cl]		66	
	[EMIM][SCN]		44	
	[DMIM][MPh]		72	
Spruce	[DMIM][MPh]	40 min, 110 °C	73	[108]
Oak	[DMIM][MPh]		79	
Douglas	[C2MIM][Ac]	3 h, 160 °C	92	[109]
Maple	[BMIM][OAc]	24 h, 90 °C	74	[102]
Willow	[BMIM][HSO_4] + 20% H_2O	22 h, 120 °C	81	[110]
Miscanthus	[BMIM][OAc]	6 h, 110 °C	91	[111]
Typha			82	

new and efficient IL-tolerant lignocellulosic enzymes to attain simultaneous pretreatment and saccharification is attracting vigorous scientific attempts [18, 103]. To date, the saccharification experiments have generally been performed after IL pretreatment (through separation of the cellulosic pulp from the IL liquor) and then adding the enzymes. Recent examples of glucose yields after enzymatic saccharification of IL treated and washed biomass are given in Table 5. Pretreatments with ILs permit the removal of a large part of the lignin and the hemicelluloses, increasing the porosity of the biomass and the accessibility of the cellulose fraction to hydrolytic enzymes.

Combinative pretreatments have also been proposed with ILs: a prehydrolysis step using aqueous ammonia, sodium hydroxide, or dilute sulfuric acid followed by treatment with ILs ([EMIM][OAc] and [BMIM][OAc] were reported to improve cellulose recovery and enzymatic glucose conversion) [111, 112].

3.4 IL Recycling

Because ILs are more expensive than conventional pretreatment agents, such as sulfuric acid, reusability of recycled ILs is a crucial factor to be considered economically. Generally, after the regeneration process, a mixture of antisolvent (antisolvent is a solvent in which the compound is less soluble), dissolved IL, and soluble biomass compounds are present. These compounds can be recovered with proper fractionation steps which allow limitation of the deactivation of enzymes because of high concentrations in residual IL in the medium [80, 113]. In fact it was demonstrated that low concentrations of ILs, such as [EMIM][OAc] or [BMIM]

[Cl], provoked an important decrease of the cellulase activity [114, 115]. Audu et al. [111] studied the recyclability of [BMIM][OAc] during the pretreatment of typha grass. The IL was recovered after precipitation of the pulp with an antisolvent (water or ethanol), dried under reduced pressure, and reused for up to 20 cycles. Treatment of the recycled IL at the 10th and 15th cycles (by washing with an acetone/water solution) enabled recovery of about 93% of the IL-soluble lignin released into the liquid stream and improved the effectiveness of the process [111]. To date, three types of IL recovery processes have been developed, the easiest way being evaporation of the antisolvent after the regeneration process with 94–98% recovery for legume straw. The drawback of this method is that some impurities may still be present in the IL [116]. Alternatively, IL recovery can be achieved by their ability to form a biphasic liquid–liquid system with the addition of an aqueous solution of a kosmotropic anion such as phosphate or sulfate whereby the salting-out effect leads to precipitation of a solid-phase rich in cellulose. The main advantage of this process is the decreased amount of water in the IL to be evaporated, thereby reducing energy costs [117]. For the third process, as proposed by Dibble et al. [97] when using [EMIM][CH_3COO^-], an antisolvent mixture of ethanol and acetone creates a quaternary solution of IL–water–ketone–alcohol that would be separated accordingly. This process requires minimal addition of reagents and minimal IL degradation and resulted in 89% of the initial IL.

As presented above (Sect. 2.5.2), the organosolv lignin fraction is potentially valuable for new applications. In the case of IL pretreatment, a two-stage process appears promising for lignin recovery. After the precipitation of the cellulosic pulp using a protic solvent, the lignin fraction is precipitated from the solution through acidification, which lowers the lignin solubility [94].

3.5 *Techno-Economic Analysis of IL Pretreatment*

IL pretreatments present several advantages over other pretreatment technologies, including low solvent toxicity, high monomeric sugar yields, short saccharification times, and efficient delignification. Nevertheless, there are several issues to overcome before commercialization and this new technology is facing great challenges with respect to its economic viability. These include (1) the high cost of ILs, (2) a lack of knowledge regarding the industrial processes to be developed for a biorefinery based on these solvents, and (3) the ability of the co-products of the pretreatment (lignin and hemicelluloses) to make significant impact as a substitute for fossil resources [118]. To address these challenges, the development of suitable IL regeneration technologies, including the removal of impurities and the understanding of their mechanism of formation, is essential. The development of new low-cost ILs and the reduction of IL loading are also important. Klein-Marcuschamer et al. [119] demonstrated that reducing IL cost could be the most important challenge which could positively impact the competitiveness of the whole process.

4 Conclusions and Recommendations

Development of technology for generating biofuels and platform molecules from lignocellulosic biomass is arousing a growing interest. Organosolv pretreatments are especially promising in this context because (1) they are capable of fractionating lignocellulosic biomass into separate streams rich in lignin, hemicellulose, and cellulose, (2) they produce a cellulosic pulp which is very amenable to enzymatic deconstruction and subsequent fermentation, and (3) they produce large amounts of lignin fractions which are relatively pure, unaltered, sulfur-free, and less condensed than other pretreatment lignins. All around the world, pilot plants based on the organosolv biorefinery concept are being progressively established. There are tremendous opportunities for future research and industrial developments in this area, including (1) development of efficient solvent and co-product recovery systems, (2) pre-extraction of hemicelluloses and extractive compounds and their contribution to improving process economics, and (3) development of ILs-based processes. Thus, the future development of organosolv pretreatment should be focused on the integrated utilization of biomass components and decrease of the pretreatment costs.

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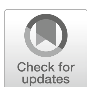
Lignocellulose-Biorefinery: Ethanol-Focused



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Abstract The development prospects of the world markets for petroleum and other liquid fuels are diverse and partly contradictory. However, comprehensive changes for the energy supply of the future are essential. Notwithstanding the fact that there are still very large deposits of energy resources from a geological point of view, the finite nature of conventional oil reserves is indisputable. To reduce our dependence on oil, the EU, the USA, and other major economic zones rely on energy diversification. For this purpose, alternative materials and technologies are being sought, and is most obvious in the transport sector. The objective is to progressively replace fossil fuels with renewable and more sustainable fuels. In this respect, biofuels have a pre-eminent position in terms of their capability of blending with fossil fuels and being usable in existing cars without substantial modification. Ethanol can be considered as the primary renewable liquid fuel. In this chapter enzymes, microorganisms, and processes for ethanol production based on renewable resources are described.

Keywords Bioethanol, Biorefinery, Lignocellulose feedstook

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1 Current Process Technologies

The development prospects of the world markets for petroleum and other liquid fuels are diverse and partly contradictory [1]. Scenarios reflecting optimistic and pessimistic assumptions alternate. In any case, comprehensive changes for the energy supply of the future are essential. Among the conventional sources of energy, crude oil is regarded as the most important energy source in the world with a share of around 33% of primary energy consumption. In 2013, oil production increased by 1.6% to a record high of nearly 4.2 billion tons, and oil consumption grew by 2.5% over the same period [2]. Notwithstanding the fact that there are still very large deposits of energy resources from a geological point of view, the finite nature of conventional oil reserves is indisputable. To reduce our dependence on oil, the European Union, the U.S., and other major economic zones rely on energy diversification [3–5]. For this purpose, alternative materials and technologies are being sought, which is most obvious in the transport sector. The objective is to replace fossil fuels progressively with renewable and more sustainable fuels. In this respect, biofuels have a preeminent position in terms of their capability of blending with fossil fuels and being usable in existing cars without substantial modification. Ethanol can be considered as the primary renewable liquid fuel. Blended with gasoline it can be used in over 80% of automobile and other light duty transportation vehicles [6].

It should be noted that the use of ethanol in cars is not a new topic. In 1908, Henry Ford designed the famous Ford Model T, the “Tin Lizzy,” based on the use of ethanol, gasoline, or a combination of both. By 1918 more than half the cars in America were Model Ts and Ford already envisioned a bio-based economy with the integration of agriculture and the sustainable development of biofuel production. “There is fuel in every bit of vegetable matter that can be fermented,” commented the automobile pioneer prophetically to The New York Times in 1925 [7]. However, during prohibition and under pressure from the rapidly growing oil industry, gasoline became the main fuel.

Nowadays, along with the goal of energy diversification, and therefore energy security, overall environmental benefits, such as the reduction of CO_2 emissions, are envisaged. Although first generation biomass, such as maize, wheat, and sugarcane, can be converted relatively easily into ethanol, their use is seen as inappropriate because of the “food vs fuel” dilemma, including problems

concerning direct and indirect effects of land use [8–10]. In this context, the sustainable production of bioethanol from lignocellulosic biomass has become one of the most conceivable alternatives. Apart from the use of bioethanol as renewable fuel, current fields of applications include [11]:

- Octane enhancer in unleaded gasoline to replace methyl-*tert*-butylether (MTBE)
- Oxygenated compound for clean combustion of gasoline, thus reducing the tailpipe emissions and improving the ambient air quality
- Conversion to H_2
- Conversion into various organic chemicals with either a higher number of carbon atoms in their molecules, or/and with an added-value (e.g., ethylacetal, butanone, butanol, pentenes)

For biofuel production, the conceptual model of a biorefinery provides the foundation for the development toward a sustainable bio-based economy: "A biorefinery is a facility that integrates biomass conversion processes and equipment to produce fuels, power, and chemicals from biomass" [12]. This concept is closely associated with the petroleum refinery, in which crude oil is processed and refined into many different marketable products. In a biorefinery, the biorenewable lignocellulosic feedstocks can analogically be converted into biofuels and value-added chemicals.

The improvement of biorefineries is generally considered as a priority, both at European and international levels, because the biorefinery supports several main drivers such as energy diversity, supply security, climate change, and rural development. Besides Germany, other EU member states, such as the Netherlands, Denmark, Finland, and Sweden, are developing their own specific bio-economy strategies [13, 14]. At the international level, aside from Brazil, the U.S. in particular has been very active in this field [15]. Numerous government programs and subsidies enable a substantial and fast-growing biofuel production industry. Under the 2007 Energy Independence & Security Act, the U.S. congress created the Renewable Fuel Standard (RFS) and mandated biofuels use. For 2016, the blending requirement, determined by the Environmental Protection Agency (EPA), is 230 million gallons. In 2014, the U.S. ethanol production reached 15 billion gallon per year from corn. The Renewable Fuels Association (RFA) lists at the present time 213 biorefineries for biofuel production at its website [16]. Moreover, the U.S. Department of Energy (DOE) and the U.S. Department of Agriculture (USDA) support the development of ethanol production from cellulosic feedstocks. The DOE supports at least 29 biorefineries and several more are in operation, under construction, or at the design stage [17–19].

Although bioethanol is currently produced predominantly from sugarcane and corn, governments are increasingly pressured to move away from food crops [10, 19]. Lignocellulosic ethanol production is increasing considerably on the basis of straw, corn stover, and sugarcane bagasse. More generally speaking, ethanol production increases on the basis of agricultural and municipal solid waste as they represent abundant, inexpensive, and readily available sources that are not directly interfering with food production [20]. Table 1 lists selected bioethanol pilot, demonstration, and commercial plants in Europe and the U.S.

Table 1 Selected bioethanol pilot, demonstration, and commercial plants in Europe and the U.S.

Substrate	Operator	Location	Ethanol capacity per year	Scale	Status
Wheat straw	*Abengoa Bioenergy*	York (Nebraska) U.S.	20,000 gallons	Pilot	Start of operation 2007
Wheat and barley straw		Salamanca, Spain	4000 tons/ 1.3 million gallons	Demonstration	Start of operation 2009
Agriculture residues, dedicated energy crops, prairie grasses		Hugoton (Kansas), U.S.	25 million gallons	Commercial	Start of operation 2014
Mixed Hardwood (Green Power+ Technology)	*American Process*	Alpena (Michigan), U.S.	700,000 gallons	Demonstration	Start of operation 2012
Wood chips and agricultural residues (Avap Technology)		Thomaston (Georgia), U.S.	300,000 gallons	Demonstration	Start of operation 2013
Non-food agricultural materials	*Beta Renewables*	Rivalta, Italy	40,000 tons	Demonstration	Start of operation 2009
Agriculture residues (PROESA™ Technology)		Crescentino, Italy	60,000 tons/ 20 million gallons	Commercial	Start of operation 2012
Softwood, hardwood, sugarcane bagasse BALI™ Technology)	*Borregaard Industries*	Sarpsborg, Norway	15,800 tons	Demonstration	Start of operation 2013
Agriculture residues (wheat, barley and rice straw, corn stover, sugarcane bagasse, miscanthus,) (Sunliquid® Technology)	*Clariant*	Munich, Germany	2 tons	Pilot	Start of operation 2009
		Straubing, Germany	1,000 tons/ 330,000 gallons	Demonstration	Start of operation 2012
Wood chips	*Fraunhofer CBP*	Leuna, Germany	Not specified	Pilot	Start of operation 2012
Corn stover, switchgrass	*DuPont Danisco*	Nevada (Iowa), U.S.	30 million gallons	Demonstration	Start of operation 2015

(continued)

Table 1 (continued)

Substrate	Operator	Location	Ethanol capacity per year	Scale	Status
Wheat straw	*Inbicon, DONG Energy*	Kalundborg, Denmark	1.5 million gallons	Demonstration	Start of operation 2009
		Maabjerg, Denmark	21 million gallons	Commercial	Planned start-up 2016
Multiple feed-stock (wood chips, switch-grass, corn sto-ver, sugarcane bagasse)	*Mascoma*	Rome (New York), U.S.	200,000 gallons	Demonstration	Start of operation 2009
Wood chips, sug-arcane bagasse, wheat, corn sto-ver, energy grass (CelluApp® Technology)	*SEKAB/ EPAB*	Örnsköldsvik, Sweden	100 tons	Pilot	Start of operation 2004
Wheat straw, poplar, miscanthus	*Futurol Procethol 2G*	Pomacle, France	47,550 gallons	Pilot	Start of operation 2011

In the field of lignocellulosic ethanol production, the technical development status until 2009 was based on laboratory experiments and pilot plants [21, 22]. Nowadays, the state-of-the-art has continually expanded through further demonstration and commercial plants.

There are basically two conversion technology paths that form the foundations of current research and development work – the biochemical approach and the thermochemical approach [6, 23–26]:

- In the biochemical route, the lignocellulosic base material has to be pretreated/ fractionated, then saccharified through hydrolysis, which can be achieved either chemically by acid hydrolysis or enzymatically, and finally the sugars can be fermented into ethanol
- The thermochemical route is based on gasification of the raw material to produce syngas (CO, CO_2, H_2) which is then converted into ethanol by a chemical reaction utilizing chemical catalysis or by fermentation

Currently, the biochemical route is the most commonly used process and is illustrated in detail in the following.

Comparable to petrochemical refineries, in lignocellulosic biorefineries the raw material is first separated into its main components. On this foundation, the individual product lines are developed with different processing technologies. However, unlike crude oil, which consists mainly of a mixture of hydrocarbon

compounds, lignocellulosic biomass has a smaller percentage of carbon and hydrogen, and the share of oxygen is higher. It is mainly composed of carbohydrate polymers (cellulose, hemicellulose) and an aromatic polymer (lignin). The proportions of these components vary depending on the source and seasonal changes (Table 2). Although the basic separation process at the petrochemical refinery is the crude oil distillation, a myriad of process technologies are applied for the separation of the polymeric constituents, depending on the feedstock or which products are made [25].

To give the reader an impression of the aforementioned diversity and to provide an overview of existing process technologies, we introduce several biorefineries in the following.

Regarding the world's largest commercial cellulosic biorefinery in Hugoton (*Abengoa Bioenergy*) with an ethanol capacity to produce up to 25 million gallons per year, acid catalyzed steam explosion is the prior processing method that is currently used. After pretreatment, the sugars from the hemicellulose (liquid fraction) are separated from the technical cellulose (solid fraction). The conversion technology of the cellulose is Separate Enzymatic Hydrolysis and Fermentation (SHF). Both the hexoses and the pentoses are fermented separately into ethanol. The separation of ethanol from the stream coming from the fermenters is done by means of distillation. The state-of-the-art facility also features electricity cogeneration using residual lignin component, allowing it to operate as a self-sufficient

Table 2 Difference in composition of lignocellulosic feedstocks and petroleum

Parameter	Hardwood (%)	Softwood (%)	Agricultural residue (%)	Herbaceous energy crops (%)	Petroleum (%)
Cellulose	41.1	41.7	32.6	31.0	
Hemicellulose	17.0	20.5	22.6	24.4	
Lignin	24.3	25.9	16.9	17.6	
Acids	1.4	2.5	2.2	1.2	
Extractives	6.5	2.7	13.0	17.0	
Ash	1.6	0.3	10.2	5.8	
Paraffins					30
Naphthenes					49
Aromatics					15
Asphaltics					6
C	51.7	50.3	43.9	47.3	83–87
H	4.5	6.0	5.3	5.3	10–14
O	35.1	42.1	38.8	41.6	0.1–1.5
N	0.20	0.03	0.63	0.51	0.1–2
S	0.03	0.01	0.16	0.10	0.05–6.0

Sources: Hardwood (Biomass sample type: Hybrid Poplar Caudina, DN-34 whole tree w/o leaves or needles #13), Softwood (Biomass sample type: Monterey pine (*Pinus radiata*) Debarked wood #153), Agricultural residue (Biomass sample type: Wheat straw (*Triticum aestivum*) Thunderbird whole plant #154), Herbaceous energy crops (Biomass sample type: Switchgrass Alamo whole plant #74) [27], Petroleum [28, 29]

renewable energy producer. According to *Abengoa Bioenergy*, the biorefinery concept is currently being assessed. In this connection, the company is developing proprietary technologies suited for the manufacturing of coproducts from the ethanol production process, such as coproducts from lignin (asphalts, bioplastics, biopolymers, resins) and coproducts from sugars [30–32].

Various kinds of pretreatments are applied by *American Process* [30]. The GreenPower+™ process is used to extract the hemicellulose from woody biomass by using steam or hot water which is then hydrolyzed into fermentable sugars. The extracted biomass is returned to the biomass boiler for the production of electricity and the sugars are converted to bioethanol. Through reverse osmosis, potassium acetate is also obtained [33]. The AVAP (American Value Added Pulping) technology co-produces cellulosic sugars, ethanol, and cellulose. The biomass is converted to sugars using a two-step process. In the first step, biomass is fractionated by a mixture of SO_2 and ethanol into its three major components – cellulose, hemicelluloses, and lignin. In the second step, hemicellulose and cellulose are hydrolyzed to sugars using heat and enzymes, respectively. The pentose sugars from the hemicellulose and the glucose from the cellulose are fermented to ethanol. Lignin is removed and burned to produce the energy required to run the process [34].

Beta Renewables, a joint venture between *Chemtex* and the investment company *TPG* (Texas Pacific Group), applies the PROESA™ (PROduzione di Etanolo da biomasSA) technology. The biomass is pressure-cooked with steam and hot water followed by a fast decompression to separate lignin. With Simultaneous Saccharification and Co-Fermentation (SSCF), the cellulose and the hemicellulose are transformed into ethanol. The plant uses enzymes from *Novozymes*, a Denmark-based biotech company, which acquired a 10% share in Beta Renewables. The yeast comes from Leaf Technologies (France). Lignin is isolated by a filtration process and used as energy source or it is sold on the market [30, 35, 36]. In October 2014, the company announced that it had signed a definitive agreement with *Energochemica SE* for the construction of a second generation ethanol plant in Strazske (Slovak Republic), which should deliver 55,000 metric tons per year of cost-competitive cellulosic ethanol with the PROESA™ technology, using non-food biomass as its feedstock. The start-up of the plant is expected in the first half of 2017 [37].

Borregaard Industries uses the BALI™ (Borregaard Advanced LIgnin) technology. Pulp for the paper mill is produced by cooking spruce chips with acidic calcium bisulfite cooking liquor and the hemicellulose is hydrolyzed to various sugars during this process. The sulfite spent liquor is then concentrated and the sugars are fermented. Ethanol is distilled off in several steps. Cellulose, lignin, vanillin, and ethanol are obtained [38, 39].

The pretreatment in the Sunliquid® technology from *Clariant* is carried out using hot water vapor. Ethanol is produced by an SHF process in which C6- and C5-sugars are converted simultaneously in a one-pot reaction. The enzyme production facilities are integrated in the process Sunliquid® plant. The lignin is separated and burned [30, 40].

The pilot plant of the German Project Lignocellulose Feedstock Biorefinery in Leuna is part of the *Fraunhofer Center for Chemical-Biotechnological Processes* (CBP). Pretreatment and component separation into cellulose, hemicelluloses, and lignin is carried out on the basis of the Organosolv pulping process. Cellulose and hemicelluloses are enzymatically converted to sugars and fermented to ethanol, lactic acid, acetic acid, or succinic acid. Lignin is precipitated from the pulping liquor via water dilution or thermal precipitation to receive Organosolv lignin. Unlike other pretreatment processes, the resulting lignin is very pure and contaminated neither with sulfur nor with inorganic salts. Thus, applications for a wide range of materials are possible either under structure-preserving of different thermoplastic and thermoset polymers or by breaking down the structure in terms of depolymerization to aromatics [41, 42].

DuPont's facility in Nevada uses the dilute ammonia-based pretreatment. Using Separate Enzymatic Hydrolysis and Fermentation (SHF), the facility employs Accellerase® Biomass Enzymes and an engineered bacterium strain of *Zymomonas mobilis*, which can convert both the C6- and C5-sugars from plant biomass into ethanol [43, 44].

Based on the "IBUS" (Integrated Biomass Utilization System) concept and under the EU 7th Framework Programme "KACELLE" (KAlundborg CELLulosic Ethanol project), *Inbicon*, a subsidiary of the Danish *DONG Energy*, uses a hydrothermal pretreatment and high-gravity enzymatic hydrolysis. The enzymes used in the Kalundborg Demonstration Plant are supplied by *Novozymes*, *DuPont Genencor* and *DSM* [45]. They focus on two fermentation concepts. The first concept comprises the fermentation of C6-sugars to ethanol and the use of the C5-sugars for livestock feed or biogas production. The second concept describes a C5- and C6-mixed sugar fermentation. Lignin pellets are sold to *DONG Energy* and are used as a high-quality solid biofuel in power plants. In Maabjerg, it is envisaged to produce – besides ethanol, heat, and electricity – biogas, and to use the remaining nutrients as fertilizer [22, 30, 39, 46, 47].

With the Consolidated Bioprocessing (CBP) concept, the *Mascoma Corporation* has chosen a different approach that features cellulase production, cellulose hydrolysis, and fermentation in one step [30, 39, 48].

In Sweden, the CelluApp® Technology of *SEKAB* is based on dilute acid pretreatment followed either by Separate Enzymatic Hydrolysis and Fermentation (SHF) or Simultaneous Saccharification and Fermentation (SSF) to produce ethanol. Lignin is removed and burned to produce the energy required to run the process [49].

The French project Futurol (*Procethol 2G*) is developing cellulose extraction techniques, selecting enzymes and yeasts, and developing the hydrolysis and fermentation processes best suited to each raw material configuration. Further details are not yet known [39, 50, 51].

Summarizing the above, it can be said that:

- Hydrothermal or acidic/sulfuric pretreatment are the main applied methods to fractionate the lignocellulosic biomass into cellulose, hemicellulose, and lignin

- Other pretreatments, for instance dilute ammonia- or Organosolv-based, are not yet established in industry
- Generally, biological conversion to ethanol is performed either through SHF, SSF, or SSCF (see Sect. 4.1)
- Current cellulolytic enzymes are derived from fungi
- Mainly yeast strains were used to transform the sugars into ethanol
- In most cases, lignin is only used for producing process energy
- Ethanol is removed from the fermentation broth by distillation

From the literature, we observe that there have been tremendous research efforts to develop advanced technologies for ethanol production in the context of a lignocellulosic biorefinery. However, a lot of challenges for the commercial applications remain. Besides continuing debate over water, land, and ecological impacts, in particular, the conversion technologies for cellulosic biofuels from non-food lignocellulosic plants are not yet optimized. Despite government programs and subsidies, federal loans, and other financial aids, the cost of commercial production of bioethanol is still very high. Tangible approaches to overcome these challenges reside in appropriate and cost effective pretreatment, improved enzymes, new yeast or bacterial strains, advanced process strategies concerning fermentation, and ethanol separation. Furthermore, technological innovations focus on utilizing every fraction of the biomass feedstock and producing more-valuable coproducts. The potential of lignin in particular is far from exhausted.

2 Efficient Enzymes for Lignocellulose Degradation

In the development and implementation of biorefinery processes, the hydrolytic conversion of the lignocellulosic substrate and its goal of providing monomeric sugars for microbial ethanol fermentation play a central role. A significant challenge is overcoming the recalcitrance of the complex lignocellulosic biomass. The plant cells have thick cell walls that primarily consist of polysaccharides and the aromatic polymer lignin. In numbers, the cell wall consists of 40–50% cellulose, 25–30% hemicellulose, and 15–20% lignin and other extractable components. The cellulose microfibrils, which are composed of linear chains of β-1,4-linked D-glucose units, are tightly packed and embedded in an amorphous matrix of lignin and hemicellulose. Intra- and intermolecular hydrogen bonds between hydroxyl groups as well as van der Waals forces lead to crystalline cellulose structures (Iα and Iβ polymorphs) comprised with amorphous regions. Cellulose is coated by hemicellulose, a heterogeneous, branched polysaccharide. The building blocks of hemicelluloses include xyloglycans, xylans, mannans, and glucomannans. The backbone of the polymer is made up by mostly one type of saccharide, the sidechains of other sugars such as L-arabinose, D-glucuronic acid, or D-galacturonic acid. Via chemical ester or ether bonds, hemicellulose is linked to lignin. This heterogeneous aromatic polymer is comprised of the three phenyl-propanoid

monomers *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol [52–55]. Because of the ordered tertiary structure, lignocellulose is insoluble in conventional solvents such as water, and is very difficult to depolymerize.

The catalytic conversion of cellulose and hemicellulose into fermentable hexoses and pentoses is being conducted either chemically or enzymatically. Possible chemical reactions are acid-catalyzed hydrolysis, bond scission in alkaline medium, and the application of ionic liquids [56, 57]. In particular, acid hydrolysis using sulfuric or hydrochloric acid, for example, applied in the Scholler, Madison, Noguchi, or Bergius process, is widely used, as acid hydrolysis was industrialized in the early part of the twentieth century [58]. However, these methods have various disadvantages. They are energy inefficient because high temperatures are always required, the equipment suffers from corrosion, there are difficulties in the separation and recycling of acids, there is the demand of neutralization, and the processes produce large amounts of waste. A more promising approach involves the use of solid acid catalysts, sulfonic group-functionalized or metal-based solid acid-catalyzed hydrolysis (e.g., MC-SO_3H, Amberlyst 15DRY or Pt/γ-Al_2O_3, Ru/CMK-3). The solid catalysts can be easily separated and recycled, are environmentally friendly, and have high hydrothermal stability. However, research is largely confined to model systems such as pure cellulose. Furthermore, there are significant drawbacks concerning high catalyst:substrate mass ratio, as well as concerning the transfer resistance between catalysts and insoluble or partially soluble cellulose that restricts the catalytic activity [56, 57, 59]. A further field of research is ionic liquids (ILs). An increasing number of research articles have been published in recent years [60]. Organic salts with melting points around or below the ambient temperature were used as solvents for the dissolution of cellulose, and also for hydrolysis with and without chemical catalysts. Even the enzymatic hydrolysis of lignocellulosic polysaccharides in the presence of ionic liquids has been investigated [61]. High thermal stability, negligible vapor pressure, wide liquid temperature range, and tunable solubility are named as the main advantages. Regardless of the practical advantages, tremendous challenges remain for the application of ILs in terms of biocompatibility and the need to develop effective recovery and recycling methods.

In the final analysis with respect to the implementation in a biorefinery process, enzymatic hydrolysis represents at the time the best option for an efficient disruption and hydrolytic cleavage of the hydrogen-bond network. Some of the most decisive advantages for enzymes in bioethanol production are [62, 63]:

- High selectivity of the enzymatic reaction
- Mild reaction conditions, thereby reducing the amount of energy and expensive equipment
- Low formation of inhibitory by-products
- Possibility to combine the hydrolysis with the subsequent fermentation step
- Advanced technology concerning environmental safety

However, the physicochemical factors make the native lignocellulosic biomass recalcitrant to degradation, and the access of the enzymes to the polysaccharides in the lignocellulose remains a major issue. Therefore pretreatment is required.

Generally, the methods are divided into physical, physicochemical, chemical, and biological pretreatment categories. In turn, each category can again be divided into subcategories. Focusing on chemical pretreatments, these subcategories can further be classified into acidic, alkaline, and neutral pretreatments. These methods have been addressed in a number of reviews and the specific modifications that make the polysaccharides more accessible to enzymes are determined by the pretreatment method used [64–69]. Targeting increased digestibility but avoiding the formation of sugar degradation products and fermentation inhibitors, pretreatment assessment depends on various properties. For instance, the sugars released in the liquid and the carbohydrate content of the solids after pretreatment are important factors. In addition, the enzymatic hydrolysis and the fermentation of either the washed or the unwashed substrate to target potential inhibitors and the assessment of additional biotechnological potential of the fractions are of interest. Yields of enzymatic hydrolysis higher than 90% achieved within reasonable time periods (up to 1 day) that work in combination with high sugar concentration provide the basis for efficient, enzymatic lignocellulose degradation [70].

2.1 Synergistic Effects of Carbohydrate-Active Enzymes

The kingdom of life covers many organisms which naturally developed the necessary capabilities to convert lignocellulose biomass to soluble species that are then used as a source for food and energy. Facing the recalcitrance of lignocellulose, these biomass-degrading organisms require an array of enzymes with synergistic function to break down polysaccharides and lignin. Table 3 gives an overview of the potential sources and their characteristics.

Among all organisms, fungi play a pivotal role in industrial biomass depolymerization [55]. The sources for commercialized cellulase production are mainly fungi such as *Trichoderma reesei* or *Aspergillus niger*. These fungi produce extracellular cellulases in significant amounts and are therefore of particular interest. In comparison with each other, the cellulases from *Aspergillus* usually have higher β-glucosidase activity but lower endoglucanase levels, whereas those from *Trichoderma* have high endo- and exoglucanase components but lower β-glucosidase levels. Unlike soft rot or white rot fungi, brown rot fungi involve low molecular weight degradation agents such as hydrogen peroxide. These low molecular weight oxidants penetrate through the cell wall of the lignocellulosic material and react with endogenous iron or other transition metals to produce hydroxyl radicals via the Fenton reaction [71]. However, bacteria such as *Bacilli*, *Paenibacilli*, or *Clostrida* are also valuable resources for enzymes [72]. Apart from these usual sources, significant recent activities and progress were made in the field of termites. Various recombinant expression platforms can be used, with bacteria and yeast being most notable, to produce the recombinant digestive enzymes [73].

Two primary nanocatalytic degradation paradigms have to be distinguished: the “free” enzyme paradigm and the “cellulosomal” paradigm [55, 76]. Regarding the

Table 3 Selected sources and characteristics of lignocellulosic degradation organisms [71–75]

Natural biomass utilization system		Characteristics	Example
Fungi	Soft rot fungi/ white rot fungi	• Degradation of cellulose and hemicellulose, some can selectively degrade lignin • Single-cell microorganism system, enriched with extracellular lignases and GHs • Are known to produce multiple cellulases (e.g., CBHs, EGs, BGLs) • Binding module seems to belong invariably to the CBM family	*Trichoderma reseei* *Aspergillus niger* *Trametes versicolor*
	Brown rot fungi	• Degradation of cellulose and hemicellulose, some can selectively degrade lignin • Single-cell microorganism system, enriched with extracellular GHs • Lack of both CBMs and processive cellulases • Involvement of non-enzymatic low molecular weight oxidants through the production of reactive oxygen species (OH-, peroxide-, or superoxide radicals, Fenton reaction system)	*Postia placenta* *Gloeophyllum trabeum* *Fomitopsis pinicola*
Bacteria	Aerobic bacteria	• Secrete various amounts of free cellulases that act synergistically to degrade cellulose and contain CDs, linker peptides, and CBMs	*Bacillus halodurans* *Paenibacillus mucilaginosus*
	Anaerobic bacteria	• Anaerobic bacteria have cellulose-degrading and hemicellulolytic enzymes • Enzymes assembled as cellulosome	*Clostridium acetobutylicum* *Clostridium thermocellum*
Termites		• Degradation of cellulose, hemicellulose, lignin • Complicated multi-organism systems with synergies between host and symbionts to lignocellulose degradation	*Reticulitermes flavipes*

CBH cellobiohydrolase, *EG* endoglucanase, *BGL* β-glucosidase, *GH* glycoside hydrolase, *CBM* carbohydrate binding modules, *CD* catalytic domain

“free” enzyme paradigm, cell wall-active enzymes are secreted as a mixture of individual enzymes. They diffuse as single catalytic units with different substrate specificities. Some of them are complemented with binding modules, which are covalently attached together via linker domains. Concerning the “cellulosomal” paradigm, the enzymes are tethered to large scaffolds. Through the noncovalent interaction of a cohesin module on the scaffoldin and a dockerin module on each enzymatic subunit, up to hundreds of enzymes form a large catalytic multi-enzyme complex in a self-assembling manner.

All components of the cellulose complex act synergistically. At least three major types of glycoside hydrolases (GH, EC 3.2.1.x) are involved in the degradation of cellulose [77]:

1. *Endoglucanases* (EGs; 1,4-β-D-glucan 4-glucanohydrolases; EC 3.2.1.4)
2. *Exoglucanases*, including cellodextrinases (1,4-β-D-glucan glucanohydrolases; EC 3.2.1.74) and cellobiohydrolases (CBHs; 1,4-β-D-glucan cellobiohydrolases; EC 3.2.1.91)
3. *β-Glucosidases* (BGL; also termed cellobiases; β-glucoside glucohydrolases; EC 3.2.1.21)

Endoglucanases mainly act on amorphous parts of cellulose and hydrolyze accessible intramolecular β-1,4-glucosidic bonds of cellulose chains randomly. Exoglucanases release soluble cellobiose or glucose through cleaving the cellulose chains at the end. β-Glucosidases act on the soluble substrate cellobiose and produce glucose. Both, endo- and exoglucanases are highly dependent on β-glucosidases because product inhibition through cellobiose limits efficient hydrolysis. For profitable biomass conversion, product inhibition is one of the main obstacles and additional β-glucosidases contribute to economic feasibility by achieving high sugar concentrations that come along with high yields [78, 79].

Beside the hydrolysis of the cellulose, the conversion of the hemicellulose is crucial for increasing the overall yield of bioethanol, as hemicellulose represents 20–35% of lignocellulosic biomass. Grass biomass contains a considerably high proportion of hemicellulose. The ethanol fermentation of the hemicellulose fraction should be achieved by co-saccharification and co-fermentation with the cellulosic fraction to establish an economically feasible process. Moreover, an effective sugar release is achieved under low enzyme loadings. Reduction of enzyme loading is made possible by an optimized enzyme combination that allows realistic prospects for the success of an economic biorefinery. By using the synergetic effect of cellulase, xylanase, and cellobiase activity, it is possible to hydrolyze at low enzyme loadings with high yields [80–83]. Enzymes that degrade hemicellulose can be arranged into two groups. The first group is responsible for the hydrolysis of the β-1,4 bonds in the backbone. The second group performs the de-branching and they are also known as ancillary and/or auxiliary enzymes. The core enzymes include endo β-1,4-xylanases (EC 3.2.1.8), xylan 1,4-β-xylosidases, (EC 3.2.1.37), endo-1,4-β-mannanases (EC 3.2.1.78), and β-1,4 mannosidases (EC 3.2.1.25). Auxiliary enzymes are, for instance, α-L-arabinofuranosidase (EC 3.2.1.55), β-glucuronidase (EC 3.2.1.139), acetylxylan esterase (EC 3.1.1.72), ferulic acid esterase (EC 3.1.1.73), and *p*-coumaric acid esterase (EC 3.1.1.1) [72]. Special emphasis should be put on the application of esterases, which are involved in the hydrolysis of ester bonds. Feruloyl esterases, for example, participate in the breakup of linkages connecting hemicellulose and lignin. This leads to an increase of free saccharides and supports their hydrolysis in combination with carbohydrate-degrading enzymes [84].

Many of these hydrolases are multimodular. They consist of a single or multiple catalytic modules connected by linker peptides of varying length and structure to one or more carbohydrate binding modules (CBMs) [55, 70]. The conformation of the catalytic module and the CBMs in the cellulosome differ from those of the free enzymes. Hence, the enzymatic activity and specificity differ substantially. For example, cellulosomes have an advantage in the digestion of pure cellulose,

whereas free fungal enzymes are more active on thermochemically treated biomass than cellulosomes [76]. In general, cellulosomes have the synergistic advantages of multi-enzyme complexes but the large size of cellulosomes may limit their diffusion on lignocellulosic substrates. A model approach that uses the advantages of cellulosomes but reduces its disadvantages is achieved by using free enzymes through the application of cross-linked enzyme aggregates (CLEAs) [72]. These combi-CLEAs, which are composed of different immobilized enzymes through precipitation and cross-linking, involve the physical aggregation of a thermostable endoglucanase and β-glucosidase from fungi along with highly stable hemicellulase from thermophilic bacterial strains. Magnetic-CLEAs further improve the process control and the operation stability [85].

The catalogue of cellulolytic enzymes has consisted, until now, primarily of hydrolytic enzymes. In addition, there are further enzymes with functional benefits available.

A more recently discovered class of enzymes known as lytic polysaccharide monooxygenases (LPMOs, formerly GH61 or CBM33) has turned out to be important for the complete saccharification of the lignocellulosic biomass. LPMOs are selective, oxidative enzymes. They lack measurable hydrolytic activity, but are capable of cleaving polysaccharide chains in crystalline regions by oxidative reactions. By doing so, they increase the accessibility for hydrolytic enzymes [55, 86]. As already mentioned, endoglucanases primarily act in more accessible, amorphous regions of cellulose. The ability to act on crystalline regions thus offers evident synergy of LPMOs in combination with EGs [87].

Ligninolytic enzymes represent another efficient consortium of enzymes with high redox potential that can be used in the lignocellulose biorefineries, in both the pretreatment and the hydrolysis stages [88–90]. The access of the cellulolytic enzymes to the polysaccharides in the lignocellulose is hampered by the cross-links in the plant cell walls with the hydrophobic network of lignin. Thermochemical pretreatment is placed upstream to the saccharification and fermentation step to dissolve or delocalize lignin. However, this process requires large inputs of energy, causes pollution, and may also generate inhibitors and reduce the overall yield of fermentable sugars. Therefore, environmentally friendly technologies are required and biological treatment with ligninolytic enzymes or the combination of conventional and enzyme-based treatment are promising approaches. Moreover, most ethanol biorefineries focus on cellulose and hemicellulose valorization, whereas lignin, because of its inherent heterogeneity and recalcitrance, is usually burned for process heat [91–93]. The depolymerization of lignin through these enzymes can help to valorize lignin selectively. A variety of white rot and brown rot fungi, as well as bacteria, have been reported to degrade lignin, by means of different enzymes and catabolic pathways [94, 95]. Lignin peroxidase (LiP, EC 1.11.1.14), manganese peroxidases (MnP, EC 1.11.1.13), versatile peroxidases (VP, EC 1.11.1.16), and laccase (EC 1.10.3.2) are the major lignin-degrading enzyme systems of white rot fungi [88]. The enzymology of bacterial lignin breakdown is currently not well-understood, but extracellular peroxidase and laccase enzymes appear to be involved. Actinomycetes, α-proteobacteria, and γ-proteobacteria are

specified in the corresponding literature. Many bacteria were isolated from guts of termites and wood-boring beetles [95–97].

2.2 Protein Engineering and Production

Enzyme production improvements can be achieved by a multitude of approaches, including the use of cheaper carbon sources as substrates for enzyme production and notably through bioengineering the microorganisms.

Protein engineering is nowadays a well-established technology and enzyme properties can be tailored to obtain improved abilities. The two basic approaches are rational protein design or directed evolution. The prerequisite for the protein design method is the availability of a protein 3D structure or a reasonable homology model. This enables the prediction of the type and position of amino acid mutations. By site-directed mutagenesis, these mutations are introduced into the protein-encoding gene and produced by recombinant expression. In the case of directed evolution, mutations are introduced randomly or homologous genes are recombined manually. This method mimics natural evolution. Such an approach demands a suitable high-throughput screening method to identify the desired variants [21, 70].

Goals in this context are enhanced activity [98–100], a different pH-optimum [89, 101–103], or improved thermostability. Particularly improving thermoactivity (>50°C) is an important target, as it would allow hydrolysis at higher solids concentration because of lower viscosity at elevated temperatures. This results in reduced contamination by microorganisms and increased hydrolytic activity. Many small cumulative changes in the hydrophobic and electrostatic interactions, as well as in hydrogen bonds, presumably enable increased thermostability. Enhanced thermostability and activity has been achieved with β-glucosidase of *Trichoderma reesei* by mutation of specific amino acids in the outer channel of the active site [104]. The engineered *Talaromyces emersonii* Cel7A cellobiohydrolase has three extra disulfide bonds and a T_m value of 84°C [105]. Gene shuffling of the genes encoding β-glucosidases from *Thermobifida fusca* and *Paebibacillus polymxyxa* resulted in a mutant with increased thermostability compared to both parental enzymes. The mutant showed a 144-fold increase in half-life of inactivation and an increased catalytic turnover frequency (94%, k_{cat}) toward cellobiose [106].

An entirely different approach for reducing the cost of lignocellulosic bioconversion to ethanol should be mentioned. The use of non-enzymatic proteins such as expansin, swollenin, and loosinin can enhance cellulase activity, presumably through their ability to disrupt hydrogen bonds. This disruption reduces cellulose crystallinity and increases cellulase accessibility [80]. The swollenin gene from *Trichoderma reseei* has been heterologously expressed in *Saccharomyces cerevisiae*, *Kluyveromyces lactis*, and *Aspergillus niger*, and also co-expressed with cellulase in *S. cerevisiae* [93, 107]. More work is needed to determine the mechanism by which these molecules increase lignocellulose conversion. Protein engineering allows industrial exploitation of this class of protein.

A large part of the costs in a lignocellulosic biorefinery arises with enzyme production [108]. To make enzyme production part of the processes within the biorefinery would help to reduce costs. Therefore, research looks at producing enzymes on-site with cheap carbon sources. For instance, cellulase production on pretreated corn stover by two filamentous fungi (*Trichoderma reseei* RUT-C30 and *Aspergillus saccharolyticus*) was investigated [109]. Different pretreated sugarcane bagasses were evaluated as carbon source for *Penicillium echinulatum* enzyme production [110]. The filter cake left after hydrolysis and fermentation from wet oxidized wheat straw was chosen as substrate for enzyme production. Sørensen et al. screened fungal isolates and *Aspergillus niger* IBT25747 and *Aspergillus saccharolyticus* CBS127449 were found to be promising candidates for on-site enzyme production on the selected substrate [111]. The liquid fraction from steam-exploded wheat straw was used as a carbon source for cellulase production by *Trichoderma reseei* RUT-C30 [112].

3 Microorganisms for Ethanol Fermentation

Fermentation constitutes the core process in the ethanol-focused lignocellulosic biorefinery. The selection of the sugar fermenting microorganism is an important success factor, as influences of the previous process steps become apparent in the fermentation step. Depending on the raw material and the pretreatment conditions, specific chemical compounds are contained in the substrate or can be generated. These compounds reduce the yield or productivity of ethanol, lower the viability of the microorganisms, and, in the worst case, they cause a complete standstill of the fermentation. Issues related to high sugar concentrations, or the concentration of the fermentation product itself, can additionally reduce the microorganism's ability of ethanol production. The most frequently used microorganisms for converting C6 sugars in the bioethanol fermentation are the facultative anaerobic yeast *S. cerevisiae* and the Gram-negative bacterium *Z. mobilis* (laboratory scale). Both microorganisms are adapted to ethanol fermentation and have turned out to be very robust, but neither of them is able to ferment C5 sugars, which is another main requirement for a complete conversion of all the lignocellulosic polysaccharides [11, 113, 114].

However, the above-mentioned aspects are only some of several key attributes that are desirable or even required for an industrially suitable microorganism. The following list gives an overview [114, 115]:

- High ethanol tolerance (>40 g/L)
- High ethanol productivity (>1 g/L/h)
- High resistance to inhibitors
- Tolerance of high solid and substrate loadings
- Robust and simple growth on inexpensive medium
- Culture growth at acidic pH or higher temperatures

- Minimal by-product formation
- Co-fermentation of hexose and pentose sugars
- GRAS (Generally Recognized As Safe)-Status

3.1 Overcoming Inhibitors

As with many microorganisms, *S. cerevisiae* metabolizes glucose via the Embden–Meyerhof–Parnas (EMP) pathway of glycolysis. Many bacteria, such as *Z. mobilis*, take the Entner–Doudoroff (ED) pathway together with the pyruvate decarboxylase (PDC) and the alcohol dehydrogenase (ADH) for ethanol production [116–118]. The theoretical maximum yield of ethanol from glucose during fermentation is 2 moles/mole (or 0.511 g/g) [119]:

$$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2$$

Glucose uptake into the cell of *S. cerevisiae* is achieved through facilitated diffusion and requires a concentration gradient across the plasma membrane. The EMP pathway oxidizes one molecule of glucose to two molecules of pyruvate, resulting in the net formation of two ATPs per glucose. Under anaerobic conditions, pyruvate is decarboxylated with the release of CO_2 to acetaldehyde via the pyruvate decarboxylase. Acetaldehyde is further reduced to ethanol by alcohol dehydrogenase. Together with ethanol and CO_2, 1.0% (w/v) of glycerol is produced for most ethanol fermentations, as a response, for instance, to osmotic stress [116]. All wild-type *S. cerevisiae* strains can also ferment mannose, fructose, and galactose, which are isomers of glucose [117].

In the ED pathway of *Z. mobilis*, only one molecule of ATP per glucose molecule is produced. As a result, *Z. mobilis* produces less biomass than *S. cerevisiae* and maintains a higher glucose metabolic flux and a higher ethanol productivity [116, 120]. More recently it was observed that the ED pathway requires significantly less enzymatic protein than the EMP pathway to metabolize the same amount of glucose per second [121, 122].

Despite these advantages, *S. cerevisiae* is preferred over *Z. mobilis*. *S. cerevisiae* is widely employed for the commercial production of bioethanol from sugars by the industry. *Z. mobilis* is less robust in terms of tolerance to inhibitors, salts, and low pH conditions [120]. In general, toxicity of substances differs between organisms, and thus the biochemical processes in fermentation pathways affects the choice of the microorganism.

A number of inhibitors are present in the different lignocellulosic substrates used for industrial processes. Moreover, pretreatment of the raw materials usually involves physical factors (such as temperature and pressure) or chemicals (acids, bases, and organic solvents) and this results in the formation of many by-products such as furan derivatives, weak acids, and phenolic compounds [65, 123, 124]. In the following, these inhibitors are discussed in more detail.

The formation of furans is observed at high temperatures and low pH in the presence of monomeric sugars. Sugar monomers with five carbon atoms such as xylose are dehydrated under these conditions and form furfural. Furfural and 5-hydroxymethylfurfural are potent inhibitors of bacteria and yeasts at concentrations as low as 1–2 g/L [65, 125]. Furfural is known as one of the main fermentation inhibitors of many microorganisms. Various mechanisms lead to lower performances of microorganisms [126].

Furfural inhibits several intercellular enzymes involved in the growth, fermentation, and energy metabolism [127–129]. Dehydrogenases appear to be the most susceptible enzymes. Furfural was found to decrease the activities of triosephosphate dehydrogenase, hexokinase, pyruvate dehydrogenase (PDH), and alcohol dehydrogenase (ADH) [129, 130]. Triosephosphate dehydrogenase catalyzes the conversion of glyceraldehyde 3-phosphate to D-glycerate 1,3-bisphosphate. This is the first energy preserving step of two in the glycolytic breakdown of glucose to produce ATP. Additionally, hexokinase is inhibited by furfural. This enzyme phosphorylates six-carbon sugars. Glucose is the most important substrate for the hexokinase and the phosphorylation of the glucose ring is the first step of glycolysis and ensures glucose is trapped within the cell. PDH is responsible for the transformation of pyruvate into acetyl-CoA, an intermediate, which links the glycolysis to the citric acid cycle, and thus contributes to releasing energy via NADH. ADH, on the other hand, is responsible for the transformation of acetaldehyde to ethanol. Therefore, furfural can inhibit the cellular respiration and consequently the growth of the microorganisms and also the formation of ethanol by inhibiting ADH [131]. Besides the effects on enzymes, furfural induces the accumulation of reactive oxygen species (ROS). In addition, furfural was shown to cause cellular damage, which includes damage to mitochondria and vacuole membranes, the actin cytoskeleton, and nuclear chromatin [132]. In a similar way to furfural, 5-hydroxymethylfurfural (HMF) is a product of dehydration, but results from sugar monomers with six carbon atoms such as glucose. HMF and furfural are chemically related compounds as both contain a furan ring and an aldehyde group. The decrease in fermentation rate and the effect on the growth rate is lower in the case of HMF, so it is a less severe inhibitor for microorganisms than furfural [133, 134].

Carboxylic acids, namely acetic acid, formic acid, and levulinic acid, have also been identified as inhibitory substances at concentrations of 10 g/L [65]. Acetic acid is formed by deacetylation of the hemicellulosic part of lignocellulose. Levulinic and formic acid are produced under acidic and high temperature conditions. Levulinic acid is a degradation product of HMF and formic acid is a product of the two furans, furfural and HMF. The inhibitory effects of carboxylic acids are related to the accumulation of their intracellular anions [135–137]. Undissociated weak acids are liposoluble and can diffuse across the plasma membrane. Thus, the toxicity of the carboxylic acids is pH dependent. The pH of the medium is typically 4.5–5.5 for yeasts. After entry in the cytosol, dissociation of the acid occurs because of the neutral intracellular pH of 7. Consequently, the cytosolic pH decreases and

the cells try to pump out the protons across the cell membrane. This pumping needs energy in the form of ATP, which inhibits cell proliferation and viability.

Finally, phenolic compounds are another group of inhibitors that can cause significant inhibition at concentrations below 1 g/L [65, 138]. The molecular weights, the polarity, and the side groups of these compounds differ and depend on the lignin structure in the raw material and on the pretreatment. Phenolic compounds such as syringaldehyde, 4-hydroxybenzaldehyde, vanillin, cinnamic acid, coniferyl aldehyde, and syringic acid are reported as lignin degradation products. The mechanisms of inhibition are manifold. They cause, for instance, the loss of cell membrane integrity and damage to the cytoskeleton, they decrease cellular pH and ATP, and inhibit translation or induce apoptosis [126, 139–141].

Pretreatment conditions play a crucial role in producing the previously discussed inhibitors and one should thoroughly evaluate the resulting effects on the used microorganisms. However, pretreatment is necessary in lignocellulosic biorefinery and it seems nearly impossible not to generate potential inhibitors. One way to solve the problem is process technology solutions with active removal of these substances, which is, nevertheless, associated with additional investment and operating costs. Another solution for overcoming inhibitory effects is the choice of a suitable fermenting strain. The same applies to the other inhibitory effects such as high ethanol and sugar concentrations.

Ethanol, the fermentation product, can also be a toxic compound for microorganisms at high concentrations [142, 143]. This is the case with very high gravity fermentation processes. The inhibition mechanism is not fully understood, but it is reported that ethanol induces chaotrope-stress, can damage cellular membranes and DNA, and inhibits enzymes [125, 126].

High concentrations of sugars can decrease ethanol yield and productivity [142, 144]. The sugar inhibition depends on the strain, can reduce the activity of the enzymes in the fermentative pathway, and typically starts at a concentration of 150 g/L glucose [135].

Screening of ethanol-fermenting microorganisms to get tolerant strains is one possible method for overcoming inhibitors. Field et al. screened a collection of 71 environmental and industrial isolates of *S. cerevisiae* and its closest relative *Saccharomyces paradoxus* in terms of their tolerance for furfural and HMF [145]. *S. cerevisiae* (NCYC) 3451 displayed the greatest furfural resistance and was able to grow in the presence of up to 3.0 mg/mL furfural. Pereir et al. screened *S. cerevisiae* and *Kluyveromyces marxianus* yeast strains that were isolated from industrial environments. Fermentation results showed the superior ability of industrial *S. cerevisiae* strains isolated from industrial distilleries, compared to an industrial *S. cerevisiae* beer strain, to industrial *K. marxianus* isolates and compared to laboratory background strains [146].

The screening approach can also serve to understand and manipulate inhibitor resistance, and thus helps to develop tolerant yeast and bacteria strains for their use in bioethanol production.

Adaptation of microorganisms to toxic compounds is suggested as another advantageous procedure to increase the fermentation rate and yield of ethanol

production from an inhibitory media. *S. cerevisiae* is able to detoxify furfural to furan-3-methanol and to detoxify HMF to 2,5-furan dimethanol in situ. This process is taking place as a response mechanism in the presence of these inhibitors and several improvements have been reported in the literature [138, 142, 147–150].

Understanding the adaptation responses can also be fundamental in the design of metabolic engineering strategies for the generation of robust fermentation strains. The identified stress response mechanisms could be used by metabolic engineering to solve inhibition problems. The overexpression of genes that confer resistance to inhibitors, such as cloning of the laccase genes and altering cofactor balances, is among the studied experimental approaches [151, 152]. For instance, Lu et al. improved the robustness under heat, acetic acid, and furfural stresses for *S. cerevisiae* using genome shuffling. After 3 h of fermentation at 40°C with 0.5 vol% acetic acid, 194.4 ± 1.2 g/L glucose in the medium was utilized by recombinant strain R32 to produce 84.2 ± 4.6 g/L of ethanol. The amount of glucose utilization and ethanol concentration of recombinant strain R32 was 6.3 and 7.9 times those of the original strain CE25 [153]. Furthermore, genetic engineering is predominantly employed in altering the capacity of fermenting hexose and pentose sugars simultaneously.

Another possibility is the integration of other microorganisms that are able to detoxify some inhibitors in situ. The Gram-negative bacterium *Cupriavidus basilensis* was shown to grow on HMF as a sole carbon source. It was demonstrated that the degradation of HMF proceeds via 2,5-furandicarboxylic acid. This compound is decarboxylated to furoic acid, which is further metabolized by the furfural degradation route. Furthermore, other bacteria with the ability to utilize furfural or HMF were identified [154]. The edible, ligninolytic white rot filamentous fungus *Pleurotus ostreatus* has the capability of metabolizing HMF to 2,5-dihydroxymethylfuran and 2,5-furandicarboxylic acid [155]. This active removal of inhibitory compounds can also be performed as pre-fermentation. It is, however, accompanied with high costs for manufacturing.

3.2 Fermentation of Pentoses

The conversion of the first generation substrates to bioethanol is a proven technology. The first generation feedstock is mainly sugarcane and corn, and the monomeric sugars are hexoses that can be easily fermented by *S. cerevisiae* and *Z. mobilis*. The production of second generation bioethanol is characterized by using the lignocellulosic feedstock, which has a valuable pool of several hexose and pentose sugars [156, 157]. A complete utilization of all types of monomeric sugars is required to make lignocellulosic ethanol processes economically viable. Therefore, microorganisms that efficiently perform this conversion under industrial conditions are in demand.

One approach is the use of pentose-fermenting microorganisms which leads to arrangements involving separate fermentation processes of pentoses and hexoses.

Natural xylose-fermenting yeasts are *Pichia stipitis*, *Candida shehatae*, and *Candida parapsilosis*. They can metabolize xylose via the action of xylose reductase (XR) to convert xylose to xylitol, and of xylitol dehydrogenase (XDH) to convert xylitol to xylulose [115].

Other yeasts and bacteria are under investigation for fermenting both hexoses and pentoses into ethanol.

A natural yeast that is able to use both pentose and hexose is *Pachysolen tannophilus* [158]. Thermophilic bacteria show a good potential as well, as they degrade a much wider range of carbohydrates and have many other properties that make them well-suited for second generation ethanol production. Mass transfer rates are higher at increased temperatures and the contamination risk is lower. Additionally, direct ethanol recovery from the fermentation broth is possible by in situ vacuum distillation. They are especially usable in SSF processes because of their ability to perform the fermentation under elevated temperatures that are closer to the optimum for hydrolytic enzymes. Worth mentioning are typical members of the genera *Bacillus*, *Clostridium*, *Caldanaerobacter*, *Geobacillus*, *Kluyveromyces*, *Paenibacillus*, *Thermoanaerobacter*, and *Thermoanaerobacterium* [11, 114, 159].

However, the major drawback for natural organisms that have the ability to convert both hexose and pentose sugars is their low tolerance to ethanol [11]. Genetically modified microorganisms are, therefore, research subjects to alter the microorganism's capacity to ferment glucose and pentose sugars simultaneously [152, 160–164]. Genetic engineering has, for instance, succeeded in altering sugar utilization for *Z. mobilis* [165]. In addition to glucose, various engineering strains are able to use xylose and arabinose. This becomes, for example, possible through integration of relevant catabolic genes. Zhang et al. integrated the xylose catabolic genes xylA, xylB, tktA, and talB from *E. coli* into *Z. mobilis* for the construction of the xylose-utilizing metabolic pathway [166].

3.3 Microorganisms for Consolidated Bioprocessing (CBP)

A novel development combines different process steps in the lignocellulosic biorefinery to a single process step with a single type of microorganism. These steps are the production of hydrolytic enzymes, the hydrolysis of the polysaccharides present in the pretreated lignocellulose, and the fermentation of hexose and pentose sugars. This so-called consolidated bioprocessing (CBP) is considered to be the most advantageous biomass-to-bioethanol conversion technology. It offers a number of benefits such as having neither capital, substrate, nor operating costs for enzyme production, but having higher hydrolysis rates, a reduced reactor volume, and a reduced capital investment [11, 119].

Z. mobilis is regarded as an important CBP platform organism, as this ethanol-fermenting bacterium has been successfully engineered to ferment the hexoses and pentoses. In the development of the CBP, achieving high-level expression of hydrolytic enzymes is a central challenge to overcome. Furthermore, these enzymes have to be translocated to the extracellular medium to contact the lignocellulosic

substrate directly. Linger et al. demonstrated the exogenous expression and the extracellular secretion of two endo-1,4-β-glucanases (E1 and GH12) from *Acidothermus cellulolyticus* in *Z. mobilis*. They further showed that *Z. mobilis* is capable of translocating both E1 and GH12 through the periplasmic space into the extracellular medium [167]. The authors pointed out that more research has to be done on increasing the translocation through the inner membrane to the periplasm, the translocation through the outer membrane to the extracellular space, and exploring the use of alternate secretion pathways [167].

Zymobacter palmae, another ethanol-fermenting bacterium, is capable of utilizing a broad range of sugar substrates, but it cannot utilize cellulose. Kojima et al. thus introduced and co-expressed six genes encoding the cellulolytic enzymes (CenA, CenB, CenD, CbhA, CbhB, and Cex) from *Cellulomonas fimi* and the *cenA* gene and β-glucosidase gene (*bgl*) from *Ruminococcus albus* in *Z. palmae*. The fermentation of water-soluble cellulosic polysaccharides to ethanol was shown [168].

The heterologous production of cellulases has also been pursued with several thermotolerant yeast strains as hosts [169]. The XYN2 gene, encoding endoxylanase of the fungus *Trichoderma reseei,* and the xlnD gene, coding for β-xylosidase of the fungus *Aspergillus niger*, were expressed in *Hansenula polymorpha*. Resulting transformants were capable of growing and fermenting on a minimal medium supplemented with birchwood xylan as sole carbon source [170]. *Paecillomyces variotii* (ATHUM8891) was used with an initial concentration of 20 g/L on corn cob and brewers spent grain. Corn cob was the poorest substrate in terms of ethanol production and productivity, because a minimum enzyme production was observed in this substrate. For the spent grain, an ethanol yield of 60 mg ethanol per gram spent grain was reached [171].

In conclusion, it can be stated that, although considerable progress has been made by genetic engineering of yeasts and bacteria, most studies have a proof-of-concept character with low ethanol titers achieved. Nevertheless, considerable effort should lead to further developments in this promising technology.

4 Advances and Developments in Fermentation Strategies

There have been vast research efforts in developing cost effective second generation or advanced technologies for ethanol production. However, there are major obstacles for the commercial application of these advanced technologies. Opportunities for reducing costs to achieve economically efficient conditions are categorized into different technical approaches. In the first instance, the advantages and disadvantages of the different technological configurations of the fermentation processes have to be identified. Another perspective is evaluating high-gravity technologies as, on the one hand, this strategy improves the water economy of the process and results in lower distillation costs. On the other hand, it has negative influence on the physiology and metabolism of fermenting microorganisms, the action of enzymes,

and other process-related factors. Further technological innovations focus on advanced ethanol separation strategies as the common distillation step is the most energy-demanding process. The following sections give an overview of the mentioned technical approaches, starting with a comparison of different fermentation processes.

4.1 *Comparison of Different Fermentation Processes*

Regardless of the several technological configurations that have to be distinguished, basically, the biochemical route for converting lignocellulose in a biorefinery to ethanol comprises three main steps: pretreatment, enzymatic hydrolysis, and the fermentation of the derived sugars. Figure 1 provides an overview of the operating options.

The pretreatment step is executed first, separately to the hydrolysis and fermentation. A number of pretreatments have been developed and applied to make cellulose more accessible for the enzymatic hydrolysis. These pretreatment methods are divided into physical, physicochemical, chemical, and biological pretreatment categories. Each pretreatment leads to specific modifications of the raw lignocellulosic material and has been addressed in a number of reviews [64–69]. All these methods aim at ensuring increased digestibility avoiding the formation of sugar degradation products and fermentation inhibitors. The specific changes are determined by the pretreatment method used and include:

- Modifications in the degree of polymerization and the crystallinity index
- Increased porosity and specific surface area of the substrate
- Disruptions in lignin–carbohydrate linkages
- Hemicellulose and lignin removal
- Hemicellulose and lignin degradation
- Lignin transformation

The hydrolysis and fermentation steps can be carried out consecutively, partly simultaneously, or fully simultaneously. Each process configuration has different advantages and disadvantages. Several additional aspects must be considered for determining an appropriate configuration concept for an efficient production of ethanol.

The following technological configurations are described in more detail:

- Separate Hydrolysis and Fermentation (SHF)
- Simultaneous Saccharification and Fermentation (SSF)
- Simultaneous Saccharification and Fermentation with delayed inoculation (dSSF)
- Simultaneous Saccharification and Co-Fermentation (SSCF)
- Consolidated BioProcessing (CBP)

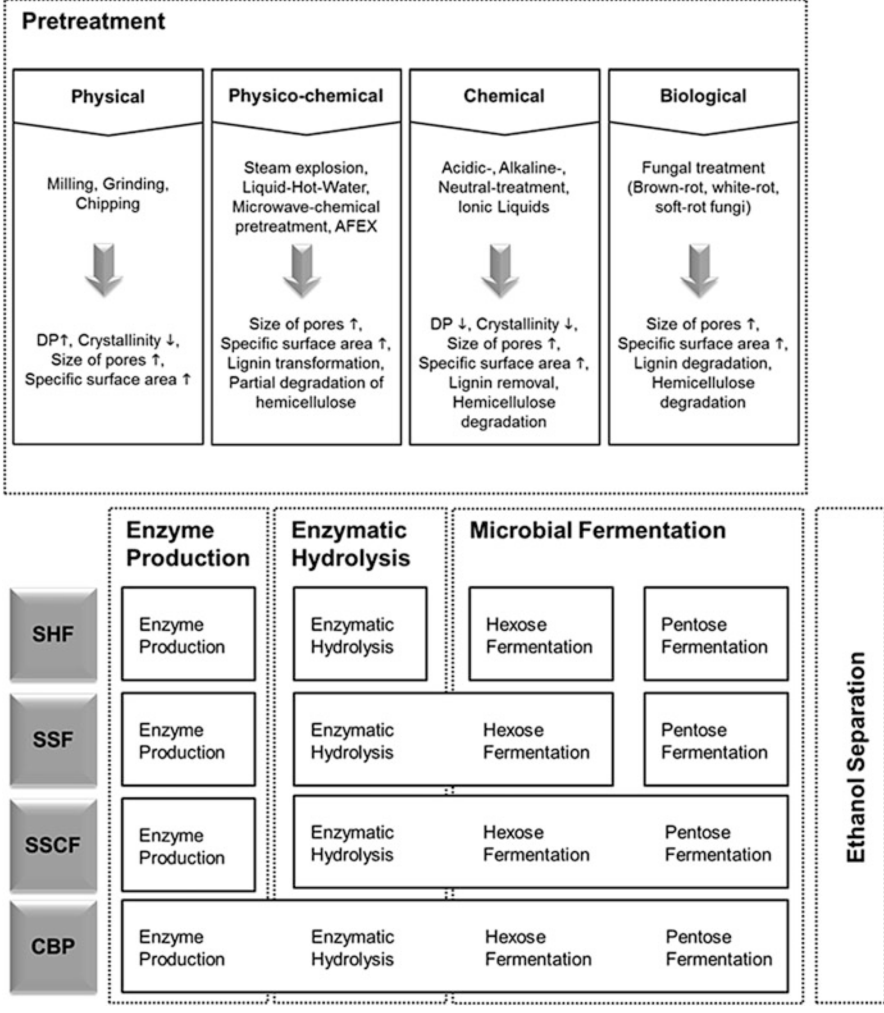

Fig. 1 Technological configurations of the lignocellulosic biorefinery

4.1.1 Separate Hydrolysis and Fermentation (SHF)

The Separate Hydrolysis and Fermentation (SHF) process is one of the most conventional technological configurations. In the process of lignocellulosic ethanol production, two consecutive operations follow after the pretreatment. The first saccharification step consists of the enzymatic hydrolysis of the cellulose. In the second step, the released sugar monomers are converted to ethanol by fermentation. In the majority of cases the hemicellulose is already separated and hydrolyzed in the pretreatment step (e.g., chemical acidic pretreatment) and, in turn, the produced

pentoses are also fermented to ethanol. The fermentation step can be achieved either by separate fermentation of the individual cellulose and hemicellulose hydrolysates or fermentation of mixed hydrolysate using co-culture.

The major advantage of the SHF concept is that both yeasts and enzymes can work at their optimal temperature. With respect to temperature, the cellulolytic enzymes typically have their optimum around 50°C, whereas yeasts typically grow best at 30–35°C [113]. Additionally, the activity of cellulolytic enzymes is not influenced by the presence of ethanol, as seen for simultaneous process options [172]. Furthermore, the viscosity is reduced prior to fermentation. This is mainly advantageous for high gravity processes, because of the positive effects on microbial strain viability, efficient mixing, and nutrient and heat transfers. Finally, SHF offers the possibility of cell recycling, whereas SSF offers no possibility to separate cells and solid raw material particles [157, 173].

However, the accumulation of end-products and therefore end-product inhibition of the enzymes has traditionally been regarded as the major drawbacks of SHF [21]. Another disadvantage is the risk of microbial contamination caused by the relatively long residence time (1–3 days) for the enzymatic hydrolysis process [174]. Economic drawbacks can arise from increased investment costs from the use of multiple vessels [173].

4.1.2 Simultaneous Saccharification and Fermentation (SSF)

In the Simultaneous Saccharification and Fermentation (SHF) process, the enzymatic hydrolysis and the fermentation step become intertwined. This allows both processes to benefit from each other. Through the continuous fermentation of the sugars to ethanol by the selected microbial strain, end-product inhibition of the cellulolytic enzyme can be avoided. In addition, high initial concentrations of sugars can decrease ethanol yield and productivity [142, 144]. A further advantage is the reduced risk of microbial contamination caused by the presence of ethanol. In comparison to the SHF process, the SSF needs to run just one vessel offering lower investment costs [113]. The hemicelluloses have been hydrolyzed in the pretreatment and converted separately to ethanol.

Nonetheless, the enzymes can be substantially affected by the released ethanol. At high gravity processes, mixing problems and mass transfer limitations may occur, caused by the high viscosity of the medium. Moreover, the SSF process requires a temperature compromise between the optimum temperature of the enzymatic hydrolysis and that of the fermentation [11]. Given that the temperature tolerance is rather strain-dependent, some moderate thermophilic bacteria strains have attracted increased interest because of their ability to ferment in a temperature range of 50–64°C [114].

4.1.3 Simultaneous Saccharification and Fermentation with Delayed Inoculation (dSSF)

Simultaneous Saccharification and Fermentation with delayed inoculation (dSSF) [175] is known in the literature by different names [113] – for instance, Prehydrolysis and Simultaneous Saccharification and Fermentation (PSSF) [176] or Semi-Simultaneous Saccharification and Fermentation (SSSF) [177]. The difference to the conventional SSF process is a prehydrolysis step with enzymes at the optimum temperature. The reaction is then cooled to the temperature at the SSF condition and inoculated with the microbial preculture. This procedure can significantly improve the mixing properties of the substrate slurry through the pre-liquefaction stage and therefore represents an enhanced SSF process [175].

4.1.4 Simultaneous Saccharification and Co-Fermentation (SSCF)

Simultaneous Saccharification and Co-Fermentation (SSCF) exhibits several advantages over the conventional SHF and SSF processes. Advantages include lower enzyme requirement, shorter process time, and cost reduction [157]. However, the major drawback is the fact that natural organisms with the ability to convert both hexose and pentose sugars do not tolerate high ethanol concentrations. Therefore, the use of genetically modified microorganisms is proposed, but further research effort is needed to overcome this problem (see Sect. 3.3). The use of mixed cultures of yeasts was also suggested. However, hexose-utilizing microorganisms grow faster than pentose-utilizing microorganisms and the conversion of hexoses to ethanol is consequently more elevated, which can lead to problems [178].

4.1.5 Consolidated Bioprocessing (CBP)

Consolidated Bioprocessing (CBP) constitutes the most favorable process. This technological configuration combines the production of hydrolytic enzymes, enzymatic hydrolysis, and ethanol fermentation of the polysaccharides present in the pretreated lignocellulose.

No additional capital, substrate, or operating costs arise from enzyme production. Higher hydrolysis rates, a reduced reactor volume, and a reduced capital investment are regarded as advantages [11, 119]. As shown in Sect. 3.3, considerable progress has been made by genetic engineering of yeasts and bacteria, but extensive research is still needed to establish feasibility.

In all these process strategies, the residual lignin is mostly burned to generate energy for the overall process. Nonetheless, the potential of the aromatic polymer is high. The kind of potential uses range from low cost carbon fibers, engineering plastics and thermoplastic elastomers, polymeric foams and membranes to a variety of fuels and chemicals that are currently produced from petroleum [179]. Most

recently published, for instance, Linger et al. used *Pseudomonas putida* KT2440, an aromatic-catabolizing bacterium, to produce medium chain-length (C6–C14) polyhydroxyalkanoates (mclPHAs) in an integrated process. Mcl-PHAs are high-value polymers that can serve as plastics or adhesives, or can be depolymerized and converted to chemical precursors or methyl ester-based fuels [92]. Vardon et al. produced adipic acid from lignin. They used also *P. putida* KT2440, engineered the bacterium to funnel lignin-derived aromatics to *cis*,*cis*-muconate acid, and produced adipic acid through hydrogenation with Pd/C [180]. Li et al. demonstrated the production of lignin-based Ts-SME (Thermally stimulated Shape Memory Effect) dual shape memory copolymers using a variety of industrial lignins. This polymer could be used for a broad range of applications, for instance as heat shrinkable tubes for electronics, or self-deployable sun sails in spacecraft [181]. Comprehensive reviews provide a good overview and have already been published [182, 183].

4.2 High-Gravity Technology

The scientific and technical research of ethanol-focused lignocellulosic biorefineries focuses on the development of efficient and sustainable processes. Therefore, high gravity operations (>10 wt%) are currently under development [67, 175, 184, 185]. Considerable advantages are obtained by increasing the solids loading [11, 186]. The process improves the water economy, decreases reactor volumes, and leads to increased ethanol concentrations that again result in a reduced need for energy during the downstream processing of the fermentation broth. However, there are still a few obstacles to overcome:

- High concentrations of inhibitory substances from the pretreatment
- Inhibition by sugars
- Inhibition by ethanol
- High osmotic pressure
- Mixing and mass transfer limitations

The relatively high concentrations of inhibitory components, sugars, and ethanol, as well as high osmotic pressure can affect both the enzymes and the microorganisms used. Different strategies to circumvent these problems are discussed. Starting at the pretreatment, avoiding the use of high temperatures, and optimizing the application of chemicals and the retention time have been studied in order to produce lower concentrations of inhibitors [67]. Research has been undertaken to overcome these inhibitions through synergistic effects of enzymes and protein engineering (see Sect. 2). Equally, the choice of a robust microbial strain is a major factor contributing to the successful implementation of high-gravity technology (see Sect. 3.1). Moreover, the high viscosity problem needs to be solved. Efficient mixing is required for the convective heat transfer and the consistent distribution of chemical components. Finally, the rheological properties are also

essential for an appropriate contact between the substrate and the enzymes. Various approaches are available to overcome the mass transfer limitations. Feeding strategies [187–189] and liquefying pre-hydrolysis [175–177] have been shown to surpass limitations. Freefall mixing in horizontal rotating reactors provide homogenous mixtures and the system requires relatively low power for the viscous slurries as they rotate at low speed [185, 190–192].

4.3 Ethanol Separation

The fermentation broth typically contains 8–14 vol% of ethanol. The separation method by means of traditional distillation and rectification yields an azeotropic solution of 95.5% alcohol and 4.5% water. The remaining wastewater from the distillation column is known as stillage, or vinasse. The stillage is mainly used for production of Distillers Dried Grains with Solubles (DDGS) or as fertilizer, although negative impacts on the soil structure and water resources in cases of excessive dosages are being discussed [193, 194]. Therefore, research approaches focus on anaerobic digestion of the organic material in stillage to convert compounds into biogas [195, 196] and methane [197].

The ethanol–water azeotropic mixture is then dehydrated to obtain an anhydrous ethanol containing up to 99.6% alcohol and 0.4% water. The major techniques available to produce anhydrous ethanol are heterogeneous azeotropic distillation, extractive distillation, and adsorption on molecular sieves [198].

The overall challenge of ethanol production from lignocellulosic biomass is to improve the process economics. Protein engineering and the new robust yeast or bacterial strains lead to higher productivity. Energy-efficient technologies, such as high-gravity strategies in combination with the selected fermentation processes, and technological innovations for the simultaneous production of valuable coproducts, for instance on the basis of lignin, contribute to an economically feasible, commercial implementation. A multitude of ethanol production plants have started their operation within the past few years. Implementation of new technologies can provide valuable experiences. In this context, biorefinery concepts are still in the early stages of development, but hold great potential for producing high value components, transportation fuels, and energy.

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Synthesis Gas Biorefinery

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Abstract Synthesis gas or syngas is an intermediate, which can be produced by gasification from a variety of carbonaceous feedstocks including biomass. Carbon monoxide and hydrogen, the main constituents of syngas, can be subjected to a broad range of chemical and microbial synthesis processes, leading to gaseous and liquid hydrocarbon fuels as well as to platform and fine chemicals. Gasification of solid biomass differs from coal gasification by chemical composition, heating value, ash behavior, and other technical and biomass related issues. By thermo-chemical pre-treatment of lignocellulose as the most abundant form of biomass, for example, by torrefaction or fast pyrolysis, energy dense fuels for gasification can be obtained, which can be used in the different types of gasifiers available today. A number of pilot and demonstration plants exist, giving evidence of the broad technology portfolio developed so far. Therefore, a syngas biorefinery is highly flexible in regard to feedstock and product options. However, the technology is complex and does not result in competitive production costs today. Added value can be generated by suitable integration of thermochemical, biochemical, and chemical processes.

Keywords Gasification, Syngas, Synthetic fuels

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1 Introduction

A biorefinery usually consists of successive primary and secondary conversion steps [1]. First, intermediates are produced by biomass pre-treatment processes, which then are further converted to a variety of products (Fig. 1). In a synthesis gas or syngas biorefinery, biomass is usually pre-treated mechanically (e.g., by grinding) or thermally. Then it is sequentially degraded thermally by gasification to the smallest building blocks of synthetic chemistry: carbon monoxide (CO) and hydrogen (H_2). From these molecules, fuels and chemicals can be produced by chemical synthesis.

High temperature processes are not sensitive to the feedstock so much. From syngas a large variety of bulk and fine chemicals as well as all important types of transportation fuels in use today can be produced. The specific advantage of such a thermochemical biorefinery with intermediate syngas production is its feedstock and product flexibility. Heat is an inevitable by-product in syngas refineries, which can be converted into steam and electricity sufficient to cover a substantial share of the process energy demand. Therefore, biomass-derived products from a syngas

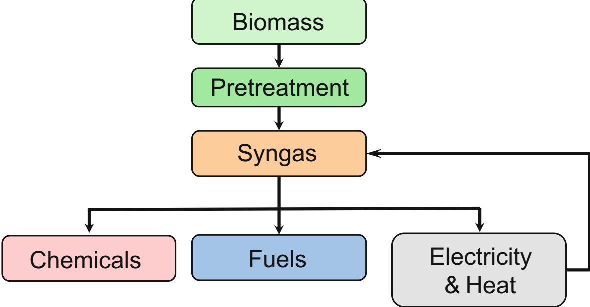

Fig. 1 Principle of a synthesis gas biorefinery according to [1]

refinery usually show considerable carbon dioxide (CO_2) reduction potentials, which in the case of fuel production is one of the essential key performance indicators.

The so-called BtL (Biomass to liquids) technologies allow for the use of almost every type of dry biomass, organic residue, or waste. Principally, the technologies are similar to the already established Coal to Liquid (CtL) or Gas to Liquid (GtL) processes also proceeding via syngas by gasification of coal and reforming of natural gas, respectively. In CtL plants, high pressure fixed bed gasifiers (FBDB™ gasifiers) have already been in commercial use for several decades in South Africa (Secunda, 170,000 bbd (barrels per day) Fischer–Tropsch products) and entrained flow gasifier units up to 500 MW thermal fuel capacity (Ningxia, China, coal-to-polypropylene plant, 5 × 500 MW) are state-of-the-art today [2]. Examples for GtL processes are the Fischer–Tropsch synthesis plants in Qatar (260,000 bbd) and in Malaysia (Bintulu, 14,700 bbd).

First experiments on the hydration of CO with H_2 were carried out by Paul Sabatier in 1902, mainly yielding methane. While BASF in the 1910s favored methanol production, Franz Fischer and Hans Tropsch invented “their” hydrocarbon synthesis in 1925 after attempts to produce fuels directly from hydrogenation of coal failed. The first trade name, Kogasin, nicely describes the product sequence from char (German: *Ko*ks) converted to syn*gas* and further to gasoline (German: Benz*in*).

This is still the principle route today as shown in Fig. 2, but with different feed materials and a much broader product range. Syngas can be reacted to produce an amazing variety of chemicals and fuels such as methane, hydrogen, methanol, ethanol, dimethyl ether (DME), olefins, gasoline, kerosene, and diesel. Several catalytic processes are involved, such as the direct synthesis of methanol or DME, the methanol-based MtO (Methanol to Olefins), and MtG (Methanol to Gasoline) processes, the production of fuels by oligomerization of olefins (Conversion of Olefins to Distillate, COD), and the synthesis of a wide range of hydrocarbons by Fischer–Tropsch (FT) processes. However, the main use of syngas today, produced mainly from natural gas, coal, or petroleum fractions, is for hydrogen generation for ammonia synthesis mainly, methanol production, hydroformylation, and FT synthesis.

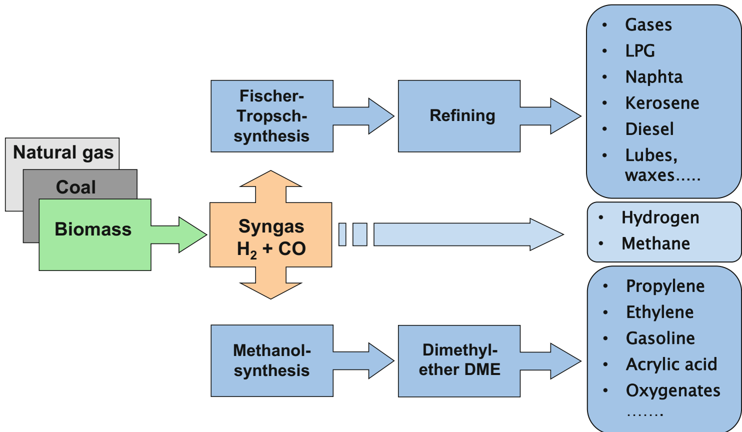

Fig. 2 Products available from syngas conversion

2 Gasification Principles

Gasification is a process that converts organic carbonaceous materials of biogenic or fossil origin mainly into carbon monoxide (CO), hydrogen (H_2), methane (CH_4), carbon dioxide (CO_2), and traces of water (H_2O). This is achieved by reacting the solid or liquid feedstock at high temperatures (>700 °C) with a controlled amount of gasification agents. When subjected to high temperatures, the usually small or fine fuel particles are first dried (Fig. 3). Then volatile components are released, leaving a solid char residue. Both gas and char are transformed in homogenous and heterogeneous reactions with the gasification agent to raw syngas. The gasification agents that can be used are water (steam), oxygen or air (leading to lower heating value of the product gas because of dilution by N_2), but also CO_2 if CO is the main product.

The resulting gas mixture is called producer gas when subjected to combustion for process heat production and electrical power generation or synthesis gas (syngas) when subjected to chemical syntheses, where high syngas purity is required. Gasification is an endothermic process, thus requiring an energy supply. In autothermal gasification, the heat required for gasification is produced by partial combustion under addition of oxygen or air as oxidizing agent to the fuel feed in the gasification reactor. For allothermal gasification, externally generated heat is supplied by a heat exchanger or a circulated heat carrier. Autothermal or allothermal gasification is possible with potential plant capacities of up to several hundred megawatts fuel input capacity (MW_{th}).

In Table 1, the equilibrium gasification reactions are given for the chemical species C, CO, CO_2, H_2, O_2, H_2O, and CH_4, which are sufficient to describe the gasification process at temperatures above 1,000 °C. Reactions R_1–R_4 are four heterogeneous key reactions where solid carbon reacts with different gaseous species. Reactions R_5–R_8 are other important gasification reactions, formed by linear combinations of R_1–R_4. Based on this, the syngas composition of high

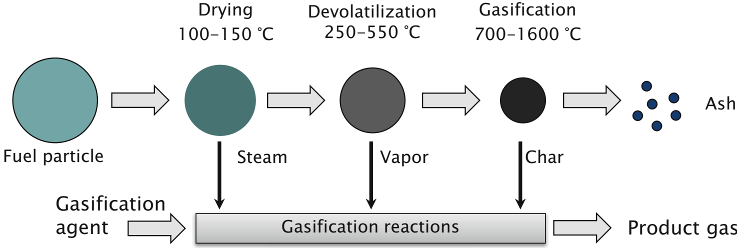

Fig. 3 Principle of gasification

Table 1 Fundamental gasification equations

Reaction		Reaction equation	$\Delta_r H$/kJ/mol
Key reactions			
R_1	Combustion	$C + O_2 \rightleftarrows CO_2$	−394
R_2	Hydrogenation	$C + 2\ H_2 \rightleftarrows CH_4$	−75
R_3	H_2O gasification	$C + H_2O \rightleftarrows CO_2 + H_2$	+119
R_4	CO_2 gasification	$C + CO_2 \rightleftarrows 2\ CO$	+173
Linear combination of key reactions			
$R_5 = R_4 – R_3$	Water gas shift reaction	$CO + H_2O \rightleftarrows CO_2 + H_2$	±54
$R_6 = R_1 + R_4$	Partial oxidation	$2\ C + O_2 \rightleftarrows 2\ CO$	−221
$R_7 = R_1 – R_4$	CO oxidation	$2\ CO + O_2 \rightleftarrows 2\ CO$	−567
$R_8 = R_1 + R_4 – 2\ R_3$	H_2 combustion	$2\ H_2 + O_2 \rightleftarrows 2\ H_2O$	−459

temperature gasification can ususally be described in good approximation by thermodynamic equilibrium calculations.

The syngas composition of gasification depends significantly on reaction temperature. In Fig. 4 the product gas yields are shown for gasification of lignocellulosic biomass with sum formula $C_3H_4O_2$ using oxygen for autothermal and water for allothermal gasification, respectively. In all cases with increasing temperature the hydrogen and carbon monoxide yields increase at the cost of methane and carbon dioxide formation. At lower gasification temperatures the formation of methane is thermodynamically favored. With increasing gasification pressure, higher methane yields can be obtained. For downstream chemical synthesis, the $CO{:}H_2$ ratio in the syngas is of importance (see Table 6). It is usually adjusted by the water gas shift reaction (WGS, R_5), in which carbon monoxide is converted into additional hydrogen and carbon dioxide.

3 Biomass Feedstock

In the context of biomass gasification, lignocellulose is usually considered as feedstock, covering a broad range of materials. Lignocellulose consists mainly of three natural polymers contained in plant material; its simplified average composition consists of about 40–55 wt% cellulose, 15–35 wt% hemicellulose, and 20–40 wt%

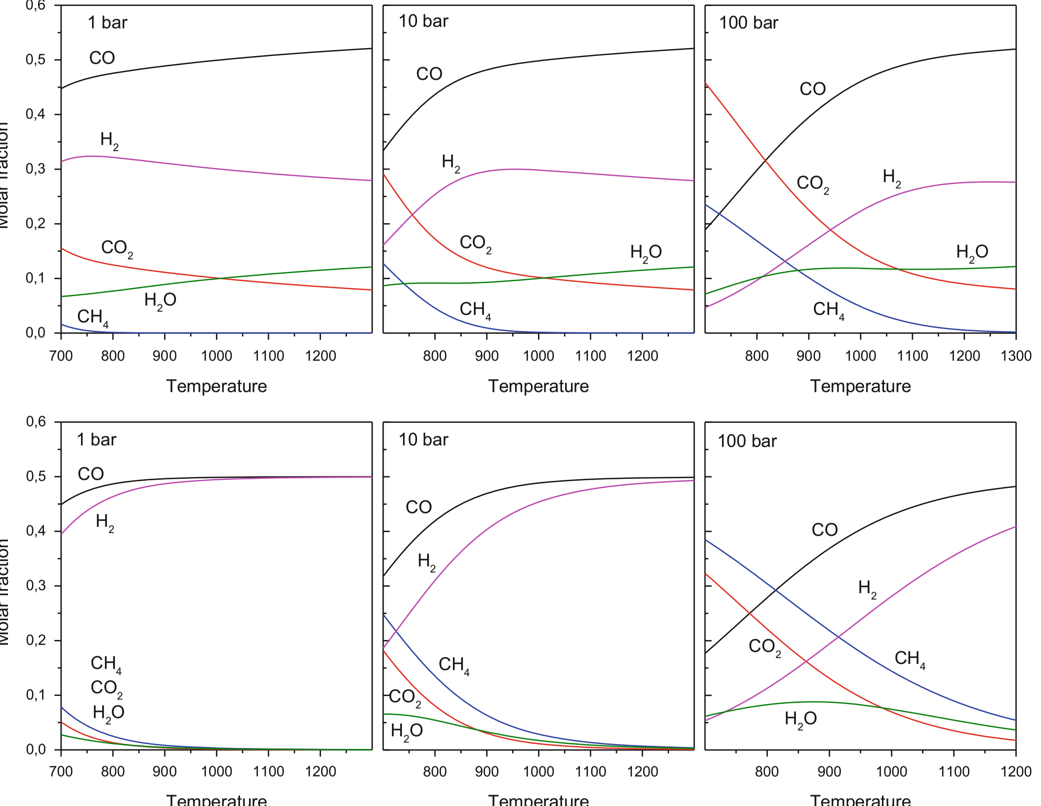

Fig. 4 Syngas composition of main products in dependence of temperature and pressure derived by equilibrium calculations of autothermal ($C_3H_4O_2 + O_2$) and allothermal ($C_3H_4O_2 + H_2O$) biomass gasification (in diagrams above and below, respectively)

lignin. The elemental composition of moisture and ash free (maf) lignocellulose is in a narrow range of 50 wt% carbon, 6 wt% hydrogen, and 44 wt% oxygen, which is practically the same for wood, straw, and other herbaceous types of biomass. Minor constituents such as hetero-atoms containing molecules with nitrogen from proteins, phosphorus, and sulfur or chlorine are usually below 1 wt%.

A good formula representation of maf lignocellulose is $C_6H_8O_4$ or, slightly simplified, $C_3H_4O_2$ with a higher heating value (HHV) of 20 MJ/kg. The formula representation simplifies the formulation of stoichiometric equations and the application of thermodynamic tools. In practice the HHV is somewhat reduced by ash and moisture. Wood contains only 0.5–2 wt% ash, cereal straw and other fast growing biomass 5–10 wt% , and rice straw with 15–20 wt% of ash is an extreme. Moisture below 15 wt% is a pre-requisite for long-term storage of biomass without biological degradation, usually achieved by air drying.

3.1 Mass and Energy Balance of Lignocellulose Gasification

The mass and energy balance for autothermal gasification of dry lignocellulose is outlined in Table 2 with simplified but still typical stoichiometric reaction equations using the simplified formula $C_3H_4O_2$. Stoichiometric combustion requires three molecules of oxygen per formula unit (reaction A). In autothermal gasification, sufficient reaction enthalpy $\Delta_r H$ must be released to heat up the syngas products to the desired gasification temperature, taking into account some heat losses. Experimental results show that about one-third or one molecule of oxygen per formula unit $C_3H_4O_2$ is sufficient for gasification and to heat up the products to ca. 1,200 °C

Table 2 Stoichiometry of autothermal gasification of maf lignocellulose with higher heating value HHV = 20 MJ/kg ($\Delta_c H_i$ = heat of combustion of species i, WGS = water gas shift)

A: Stoichiometric combustion of maf lignocellulose (HHV = 20 MJ/kg)
$C_3H_4O_2 + 3\ O_2 \rightarrow 3\ CO_2 + 0\ H_2 + 0\ CO + 2\ H_2O$
-1440 0 - - 0 (kJ/mol)
$\sum_i \Delta_c H_i$ (kJ) heat of combustion = $\Delta_r H$ heat of reaction = -1440 kJ/mol

B: Autothermal gasification
$C_3H_4O_2 + O_2 \rightarrow CO_2 + 2\ H_2 + 2\ CO + 0\ H_2O$
-1440 0 2·286 2·283 0 (kJ/mol)
$\sum_i \Delta_c H_i = \Delta_r H$ = -304 kJ/mol

C: Autothermal gasification (corrected by the WGS)
$C_3H_4O_2 + O_2 \rightarrow 0.4\ CO_2 + 1.4\ H_2 + 2.6\ CO + 0.6\ H_2O$
-1440 0 1.4·286 2.6·283 0 (kJ/mol)
$\sum_i \Delta_c H_i = \Delta_r H$ = -304 kJ/mol

D: WGS reaction: $CO + H_2O \rightleftarrows CO_2 + H_2$
$\Delta_r H$ = - 41 kJ/mol
WGS equilibrium: $\frac{p(CO_2) \times p(H_2)}{p(CO) \times p(H_2O)} = K_{WGS} = \exp(\frac{4580}{T} - 4.33)$
with equilibrium constant $K_{WGS} \approx 0.3$ at T = 1500 K

gasification temperature. As shown in the table (reaction B), about 21 wt% of the initial bioenergy is used for gasification reactions and heating of products. The heat of the high temperature product gas can be recovered and converted to steam and electricity for a self-sustaining process. The gasification reactions shown in Table 2 are interdepending equilibrium reactions. Reaction B shows the formal autothermal gasification reaction. However, at temperatures above 1,000 °C, the equilibrium of the water gas shift reaction (reaction D) is shifted to the educt side, resulting in higher CO and H_2O contents in the syngas, as shown in reaction C.

3.2 Feedstock Reactivity in Gasification

The reactivity of a solid gasification fuel depends on several parameters such as porosity, specific surface, molecular structure, and catalytic effects of ash components. Compared to coal, biomass and biomass-derived materials such as charcoal produced from wood exhibit a higher reactivity, extending over several orders of magnitude. To a considerable extent, the reactivity can be attributed to the specific surface, which for lignite and biomass char is much higher than that for hard coal. In addition, catalytic effects may play a role as it is known, for example, from potassium contained in the straw char.

Different chemical and physical steps affect the rates of reaction in a certain temperature regime. At lower temperatures, chemical reactions are predominantly rate determining. With increasing temperature the reaction rate rises; however, it becomes limited by pore diffusion of gaseous reactants in the solid particles. At even higher temperatures, surface film diffusion of the gaseous reactants is rate controlling. This can usually be seen by the much lower apparent activation energies compared to the "true" activation energy of the chemical reactions.

3.3 Biomass Pre-treatment

Biomass is usually not directly suited for gasification. Depending on the type of biomass and gasification technology, pre-treatment becomes necessary. Apart from drying and mechanical conditioning such as size reduction, thermal pre-treatment processes allow the conversion of biomass into carbon-rich, energy dense solid and liquid intermediates which are more convenient for handling, transportation, storage, and feeding. Classical carbonization, torrefaction, and fast pyrolysis are suitable processes for such thermal pre-treatment of dry biomass in an oxygen free atmosphere. Their main characteristics are summarized in Table 3. Biomass with high moisture content may be converted by other types of processes, which are currently in different states of development. Solvolytic and hydrothermal liquefaction leads to liquid bio-oils with usually lower oxygen content compared to fast pyrolysis. Hydrothermal and steam-assisted carbonization can be regarded as "wet"

Table 3 Thermal biomass pre-treatment processes

	Carbonization	Torrefaction	Fast pyrolysis
Scheme	Wood → Carbonization → Charcoal, Liquids, Gas	Biomass → Torrefaction → Torgas, Torrefied biomass	Biomass → Fast pyrolysis → Char, Liquids, Gas; Heat carrier loop
Main reactor types	Retorts, piles	Rotating drum, Herreshoff oven, screw reactor, torbed reactor	Screw reactor, rotating cone, fluid bed, ablative reactor
Reaction temperature	400–550 °C	250–300 °C	450–550 °C
Reaction time	10^3–10^5 s	10^3–10^4 s	1–3 s
Yields from dry wood	35 wt% of charcoal, 65 wt% of tar, organic acids and gas	20 wt% of gas, 80 wt% of torrefied material with a heating value of ca. 22 MJ/kg	20 wt% gas, 20 wt% char, 60 wt% liquid condensates including ca. 15–25 wt% of reaction water
Biomass fuel	Wood chips or logs	Particles, pellets, chips	Fine particles (<3 mm)
Issues	State-of-the-art technology	Simple technology, limited scale-up for some reactor types	More complex technology, pilot, demonstration and commercially operated plants available

torrefaction; however, the efficient use or treatment of the by-produced aqueous effluents is a major issue for successful process development.

Torrefaction is a low temperature pyrolysis of lignocellulose carried out at temperatures close to 300 °C. The reaction gases, mainly CO_2 and CO, H_2O, and a little organic vapor are burnt to supply the process energy. Moisture is removed with the gases as steam and the ash remains in the char. The volume of the initial biomass shrinks to almost half and the energy density of the torrefaction char increases correspondingly. Major improvements have been achieved in recent years, for example, within the recently finished EU project SECTOR.

Bio-chars are highly porous, reactive, and prone to self-ignition. For safe handling, storage, and transport of small char particles or powders, either inertization or pelletization is recommended. Pulverized bio-chars have already been proven for firing in power stations together with pulverized coal. For gasification, direct feeding is possible, for example, as a dense char powder stream that can be fed to a gasifier with an inert gas from a pressurized fluid char bed.

By fast pyrolysis carried out at around 500 °C, liquid bio-oil is the main product, consisting of several hundred organic compounds comprising organic acids, aldehydes, ketones, alcohols, and other species. Water is also contained in the bio-oil at up to 25 wt%. Fast pyrolysis is characterized by rapid heating of the biomass, short reaction times of a few seconds, and rapid cooling (quenching) of the organic vapors formed by the pyrolytic reactions. The bio-oil in the commercial pyrolysis

plants available so far is mainly used for heating purposes, and the char is combusted (as is the gas) to produce process and surplus energy.

4 Gasification Technologies

For biomass gasification, a variety of technologies exist. With a view to large-scale production of high quality syngas as required for chemicals and fuels production, mainly the types of gasifiers shown in Fig. 5 have to be considered.

4.1 *Fixed Bed Gasifiers*

In fixed bed reactors, the biomass feedstock is exposed to the gasifying agent in a packed bed that slowly moves from the top of the gasifier to the bottom, where the ash is discharged. By moving through the reactor, the biomass passes distinct reaction and temperature zones for drying, pyrolysis, oxidation, and reduction. The different types of fixed bed gasification reactors are usually characterized by the direction of the gas flow through the reactor and consequently are denoted as up-draft (Fig. 5a), down-draft (Fig. 5b), and horizontal (cross-draft) gasifiers. Depending on the type of feed and product gas application, a multitude of reactor

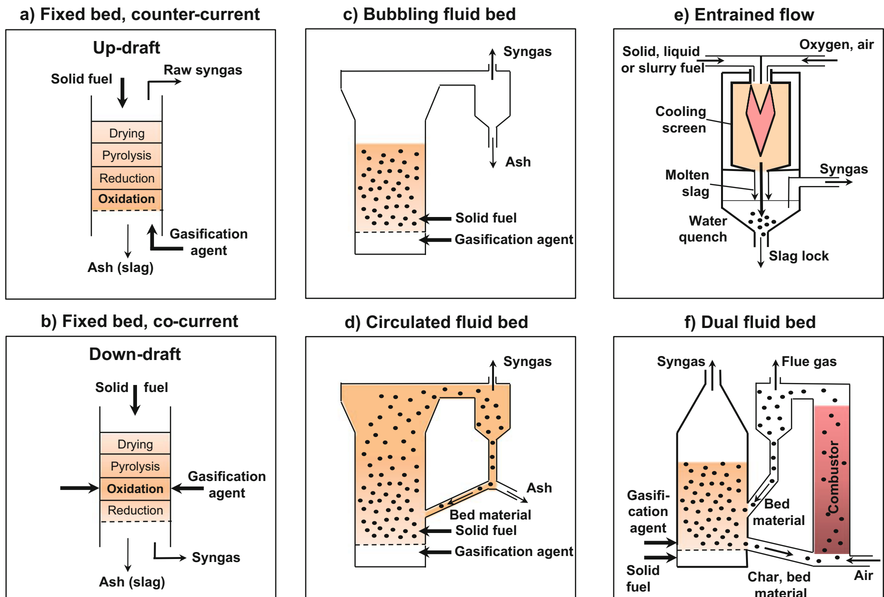

Fig. 5 Main types of gasification reactors for syngas generation from biomass

designs exist. In general, biomass fueled fixed bed reactors are suited for district heat and power production up to thermal fuel capacities of 20 MW_{th}. Producer gas it is not usually used for purposes where clean syngas is required, because substantial gas cleaning would be necessary because of the relatively high amounts of tar forming volatile components (VOC), and entrained ash and dust particles.

4.2 Fluid Bed Gasifiers

In fluid bed gasifiers (Fig. 5c, d, f), biomass particles are rapidly mixed and heated up typically to 700–900 °C by means of a solid heat carrier, usually hot sand and sometimes char or ash, fluidized by air, oxygen, or steam and syngas. The feed material size is in the order of centimeters; pellets and small wood chips may be used. Because of the intense mixing of fuel and bed material particles, the gasification reactions cannot be allocated to distinct local zones as in the case of fixed bed reactors, but occur throughout the whole reaction volume leading to a uniform temperature distribution. The degree of fluidization can be small (bubbling fluid bed, BFB, Fig. 5c) or high (circulating fluid bed, CFB, Fig. 5d). BFB gasifiers have a well-defined interface between the reaction zone in the fluidized bed and the freeboard above. They are a well-known type of reactor also applied to combustion, pyrolysis and chemical reactions, commonly used because of their robust properties but showing tar formation of the order of 1–2 wt%. In a CFB gasifier the higher gas and particle velocities provide even higher heating rates; there is no distinct interface between the fluidized sand bed and the freeboard. Larger, unconverted feedstock particles recirculate in the reactor, whereas fines such as char, ash, and heat carrier particles are entrained out of the reactor and are separated via a hot gas cyclone. The heat carrier is recycled back to the gasifier. The carbon conversion is considerably better than in BFB gasifiers.

The dual bed gasifier follows an allothermal gasification concept, by which air can be used for combustion but without dilution of the produced syngas (Fig. 5f). The gasifier is fluidized with steam as the only gasification agent, resulting in an energy rich syngas. The bed material and some char are frequently transferred into the second reactor, in which the char is combusted for heat generation. If necessary, additional fuel can be supplied to the combustion reactor. The bed material is circulated to transport the heat from the combustor to the gasifier. The heat of the flue gas can be used for heating purposes or electricity production. In all fluid bed gasifiers ash has to be discharged from the system to avoid accumulation in the heat carrier loop.

Fixed and fluid bed gasifiers are typically operated at temperatures below 1,000 °C. Low-melting straw ash can become sticky at ca. 700 °C and can create problems, for example, by agglomeration of bed material. Raw syngas from fixed and fluid bed gasifiers contains tar and methane because of the low gasification temperatures. Its use for combustion can tolerate high methane contents and requires less gas cleaning efforts compared to chemical and microbial applications.

Feed particle size and order of gasification reaction times decrease from about 0.1 m and over 10^3 s for fixed bed gasifiers via ca. 1 cm and 10^2–10^3 s for fluid bed gasifiers down to around 0.1 mm fuel powders, which react in a second or two in an entrained flow gasifier flame.

4.3 Entrained Flow Gasifiers

When syngas use for chemical purposes is intended, it must be of high purity, free of dust, tar, and contaminants in order not to poison the sensitive synthesis catalysts downstream. Unlike most fixed bed and fluid bed gasifiers, entrained flow (EF) gasifiers (Fig. 5e) are able to generate a gas practically free of tar with only little methane at high gasification temperatures above 1,000 °C (see Fig. 4). At residence times of a few seconds, carbon conversion of 95–99 % and even above can be achieved. An EF gasifier can be slurry or particle fed and usually blown by oxygen and, occasionally, steam as the gasification agent. Any solid, slurry, or liquid feed material which can be pumped, sprayed, and atomized pneumatically or mechanically is principally suited. Biomass powders with particle sizes in the order of 10^{-4} m can be fed to the gasifier by a high-density flow feed system. However, this requires periodic operation of a sophisticated system of locks and becomes more complicated when pressure rises.

When biomass and biogenic waste with higher ash and alkali contents is used, the gasifier may be operated at slagging conditions: depending on the ash composition, softening and melting occurs below 1,000 °C. To get a molten slag, EF gasifiers operate above the ash melting point at $>1{,}000$ °C at the expense of more oxygen consumption and correspondingly lower cold gas efficiency, the ratio of energy contained in the product gas over the energy of the fuel input. However, this is at least partly compensated for by the low methane content, which would otherwise reduce the CO and H_2 syngas yield by 4 mol% for every percent of CH_4 according to: $CO + 3\ H_2 \rightleftarrows CH_4 + H_2O$. The liquid slag can be handled, for example, by a reactor equipped with a cooling screen or special ceramic refractory lining, allowing the formation of a liquid slag drained out of the reaction volume. Slagging EF gasifiers can be designed for elevated pressures up to 100 bar and allow for high and economic fuel conversion capacities up to 500 MW_{th} or more.

4.4 Pressurized vs Atmospheric Gasification

Soon after the first commercial application of gasifiers to coal conversion operated at atmospheric pressure, large scale operation of pressurized fixed bed gasifiers was implemented. Operation pressures of 25–40 bar became state-of-the-art, and some gasifiers have been designed for pressures up to 100 bar. Pressure affects different factors, leading to possible benefits such as, for example [3]:

- High specific throughput, leading to high conversion capacities in compact plants
- Increased thermal capacity and efficiency
- Increased methane yield according to Le Chatelier's principle
- Increased methane yield at low temperature operation
- Reduced gas volume to be treated, for example, with regard to gas cleaning
- Saving the work of compression for the subsequent chemical synthesis

The higher specific throughput is relevant not only to plant size and capacity but also to equipment design and cost. As an example, a cost comparison of SNG (substitute natural gas, with high methane contents) production by coal gasification at 30 and 90 bar estimated savings in investment and specific production costs of around 10% in the high pressure case [4]. The reason was mainly attributed to the higher thermal efficiency of gasification at higher pressure. Also, the gas volume to be treated in gas cleaning and conditioning is reduced (40 m^3 at 80 bar instead of 700 m^3 at ambient pressure, estimated by real gas considerations at 800 °C).

Different types of high pressure gasification reactors exist: entrained flow reactors as developed by Shell, Siemens, Linde, or ThyssenKrupp Uhde as well as fluid bed (Foster Wheeler, ThyssenKrupp Uhde) or fixed bed reactors (Air Liquide), all with their specific designs related to operation at elevated pressures. For biomass conversion these reactors are not state-of-the-art today. Technology readiness levels from pilot to demonstration status have been attained so far, still requiring R&D on optimization and further development. Among others, feedstock preparation and high pressure feeding are most important issues. Fuel supply under elevated pressures is facilitated by lock hopper systems or pneumatic devices for solids whereas, for liquids and slurries, pumps and screw feeders can be used.

The effect of pressure on reactor dimensions, synthesis gas, and thermal capacity can be seen from Table 4, according to an example given by Schingnitz in [5], considering the GSP type of EF gasifiers as first realized at the Gaskombinat Schwarze Pumpe in Germany. By increasing the pressure from 25 to 80 bar, the reactor performance is increased by a factor of nearly two.

4.5 Economy of Scale

When complex technology is used, economy of scale plays an increasingly important role, particularly in high temperature processing with regard to heat losses. These are determined mainly by the volume:surface ratio of the reactor, which is reduced with increasing dimensions. At the same time, cold gas efficiency and overall performance is increased. Doubling reactor diameter and height allows for an eight times higher thermal fuel and syngas capacity (Table 4). For economy of scale reasons, syngas production and its further processing to synfuels demands large scale facilities; for comparison, a crude oil refinery has an input capacity in the order of 10 Mt/a (megatons per annum).

On the other hand, biomass usually exhibits low volumetric energy densities and has to be harvested and collected from large, distributed areas. Major sources of

Table 4 Scale-up example for EF gasifiers according to [5]

	Variant I	Variant II	Variant III
Reactor dimensions diameter × height	2m × 3.5m	2.9m × 5.3m	3.7m × 6.7m
Cylindrical surface area/m^2	22	48	78
Volume/m^3	11	35	72
$p = 25$ bar			
Thermal fuel capacity/MW_{th}	130	400	800
Syngas flow rate/Nm^3/h	50,000	160,000	320,000
$p = 80$ bar			
Thermal fuel capacity/MW_{th}	225	700	1,400
Syngas flow rate/Nm^3/h	86,000	280,000	560,000

biomass are agricultural and forestry products and residues, including purpose grown bioenergy crops, residues from land cultivation, industrial waste, and, to a certain extent, municipal solid waste. Furthermore, chemical composition and the content of minerals and other inorganic material, usually denoted as the ash content, may vary significantly. Regarding this multitude of potential materials, feed tolerant and flexible pre-treatment technologies need to be applied to achieve high throughput capacities, improving economic viability of the plants. Pre-treatment processes are suited to provide more homogeneous feed materials of high energy density as gasification fuels. Such a pre-treatment may be carried out in regionally distributed plants, the products of which are transported to larger conversion facilities for syngas and synfuel production.

5 Syngas Conditioning

Typically, a H_2:CO ratio in the order of 1 or lower is produced by biomass gasification, which cannot be used directly for all syntheses. By the WGS reaction the ratio has to be adjusted to the specific requirements of the synthesis process. The syngas produced by gasification contains impurities, depending on the type of feedstock and

Table 5 Examples of maximum contamination levels tolerated by methanol synthesis [6]

Contaminant	Level	
Tars	<0.1	mg/Nm^3
CH_4	<3	vol%
NH_3	10	ppm
HCN	0.01	ppm
Total sulfur	0.5	ppm
Halides	0.001	ppm

conversion technology applied. Downstream chemical synthesis demands cleaning down to ppm level for substantial raw syngas (Table 5). Typical organic contaminants are volatile (VOC) and tar forming components such as BTX (benzene, toluene, and xylenes) and char particulates. Inorganic nitrogen-, sulfur-, and chlorine-containing impurities are ammonia (NH_3), hydrogen cyanide (HCN), hydrogen sulfide (H_2S), carbonyl sulfide (COS), and hydrogen chloride (HCl), as well as volatile metals (regarding biomass in particular Na and K), dust, and soot. Larger, condensable organic molecules, summarized as tars, being produced in low temperature gasification processes reduce the primary syngas yield and may cause fouling of the downstream equipment, coat surfaces, and plug pipes, pores in filters, and sorbent materials. Other contaminants are corrosive or poison the catalysts in the following synthesis stages downstream. For raw syngas cleaning, conventional technology is available [6]. Tar constituents and BTX may be removed either by thermal or catalytic cracking or by scrubbing with an oil-based medium followed by gasifier recycle. The other above-mentioned impurities can be removed by standard wet gas cleaning technologies. Using absorbing liquids such as refrigerated methanol or amines, for example, in the Rectisol or Selexol process, CO_2 and sulfur compounds are successively removed in separate fractions, resulting in an almost pure CO_2 and an H_2S/COS enriched Claus gas fraction suitable for sulfur production. In advanced "dry" hot gas cleaning, the residual contaminants are removed by solid chemical sorbent materials at elevated temperatures up to 800 °C. If gasification is conducted at pressures required in the subsequently following synthesis, dry pressurized gas cleaning methods are expected to provide significant energetic benefits.

6 Syngas Derived Products

A variety of chemicals and fuels, such as hydrocarbons, methanol, hydrogen, and methane, are accessible by different pathways (Table 6). An additional range of fuels and chemicals can be obtained via methanol, yielding hydrocarbon fuels, olefins, and oxygenates such as ethers, acetic acid, and acetic anhydride. Most of the reactions are not completely selective; integration of additional process steps is necessary to use or recycle various by-product streams. Some important direct and indirect syngas conversion reactions are presented in the following. Combinations of a chemical synthesis and a subsequently following microbial conversion may also be promising, such as fermentation of syngas [7, 8] or co-fermentation of (unconverted) syngas and methanol.

Table 6 Syngas reactions to synfuels

Reaction to. . ..	Formal reaction equation	Equation
Methanol	$CO + 2\ H_2 \rightleftarrows CH_3OH$	(1)
Ethanol, higher alcohols	$2\ CO + 4\ H_2 \rightleftarrows C_2H_5OH + H_2O$ $CH_3OH + n\ CO + 2n\ H_2 \rightleftarrows C_{n+1}H_{2n+3}OH + n\ H_2O$	(2) (3)
Dimethyl ether	$3\ CO + 3\ H_2 \rightleftarrows CH_3OCH_3 + CO_2$ $2\ CH_3OH \rightleftarrows CH_3OCH_3 + H_2O$	(4a) (4b)
Gasoline and olefins	$n\ CH_3OCH_3 + n\ CH_3OH \rightleftarrows$ "$(CH_2)_{3n}$" $+ 2n\ H_2O$ $n\ CH_3OCH_3 \rightleftarrows$ "$(CH_2)_{2n}$" $+ n\ H_2O$	(5) (6)
Hydrocarbons (Fischer–Tropsch)	$n\ CO + (2n+1)\ H_2 \rightleftarrows C_nH_{2n} + n\ H_2O$	(7)
Hydroformylation	$CO + H_2 + C_nH_{2n} \rightleftarrows C_nH_{2n+1}CHO$	(8)
Hydrogen	$CO + H_2O \rightleftarrows CO_2 + H_2$	(9)
Methane	$CO + 3\ H_2 \rightleftarrows CH_4 + H_2O$	(10)
Polyoxymethylene	$CH_3OCH_3 + n\ HCHO \rightleftarrows CH_3(CH_2O)_nCH_3$ n = 1–6	(11)

6.1 Methanol

Methanol is produced from syngas at temperatures between 220 and 275 °C and pressures ranging from 50 to 100 bar using $Cu/ZnO/Al_2O_3$ catalyst systems. Major methanol technology suppliers today are ICI, Air Liquide, and Mitsubishi. Processes with higher selectivity toward methanol are also known but require more severe conditions of up to 300 bar and 380 °C. The overall reaction equation is given in a simplified way in equation (1) of Table 6. Methanol can be used in fuel blends with gasoline containing up to 20 vol% of methanol in internal combustion engines without much modification. However, in the European Union (EU) only 3 vol% are allowed for gasoline blends, mainly because of its toxicity. Another option is the use of methanol in fuel cells, either directly in Direct Methanol Fuel Cells or indirectly as a hydrogen source after reforming. In addition, methanol-derived fuels such as dimethyl ether, methyl *tert*-butyl ether, and biodiesel as fatty acid methyl esters play an important role. The global production capacity of methanol of currently around 80 Mt/a for chemical use is still growing, predominantly for primary conversion to formaldehyde, acetic acid, methyl methacrylate, methyl chloride, and methylamine, which again are raw materials for secondary conversion to important further chemical products.

6.2 Ethanol and Higher Alcohols from Syngas

Direct synthesis of ethanol from syngas ((2) of Table 6) is intensely investigated at present. Another approach is the homologization of methanol, that is, the reaction of methanol with syngas to yield ethanol ((3) of Table 6), by which alcohols with longer carbon chains can also be formed. However, the selectivities toward ethanol are still moderate and there is a lack of highly efficient catalysts for these reactions.

6.3 Dimethyl Ether

Dimethyl ether (DME) can be obtained from direct synthesis according to (4a) or via dehydration of methanol (4b). The formation of DME is thermodynamically favored at $CO:H_2$ ratios around 1:1, which are typically expected from biomass gasification. From a mechanistic point of view, direct synthesis proceeds also via methanol formation and subsequent release of water but without intermediate isolation of methanol. The process can be designed to yield both methanol and DME. Mixed catalysts are employed consisting of the established methanol catalysts and dehydration catalysts. The latter are solid-acid catalysts, for example, alumina, silica-, phosphorus- or boron-modified alumina, or HZSM-5 zeolites. Because DME properties are similar to those of liquefied petrol gas (LPG), it can be used in typical LPG applications, for example, power generation and domestic or transportation fuels. DME can be admixed to LPG up to around 20 vol%. Compared to LPG, the cetane number is much higher and therefore DME is a suitable, clean fuel for diesel engines. Because of missing carbon–carbon bonds, the combustion of DME leads to practically soot-free exhaust gases. Because of its physico-chemical properties, more use of DME is made as refrigerant, aerosol propellant, solvent, and extraction agent.

6.4 Methanol to Gasoline (MtG)

Conversion of methanol to gasoline (MtG) is accomplished by zeolitic ZSM-5 catalysts via either fluid or fixed bed technologies. The former was demonstrated by Mobil, Union Rheinische Braunkohlen Kraftstoff AG, and Uhde in a pilot plant at UK Wesseling and the latter was operated successfully by Methanex in New Zealand licensed by ExxonMobil. The Mobil process yields around 38 wt% of gasoline, 4 wt% of LPG, approximately 58 wt% of water, and a small amount of fuel gas. The underlying chemistry is complex and initiated by the formation of DME through dehydration of methanol (4b). The following chain growth and cyclization reactions proceed via further release of water and can be described, in a very simplified manner, by (5) and (6) of Table 6. These reactions involve not only reactions of DME with itself ((6) of Table 6) but also reactions of DME with methanol ((5) of Table 6). The resulting gasoline type of fuel exhibits a high octane number thanks to the substantial amount of aromatic components. However, direct production of longer chain alkanes for diesel and jet fuel using typical MtG catalysts is not possible today. A similar process is the TIGAS process (Topsøe Integrated GAsoline Synthesis) developed by Haldor Topsøe and demonstrated at a pilot plant in Houston. It is, in principle, an improved MtG process combining methanol, DME, and gasoline production in a single synthesis loop, thus circumventing intermediate production and storage of methanol. Different syngas compositions can be employed because methanol/DME synthesis is flexible in terms of syngas specifications.

6.5 Methanol to Olefins (MtO)

Ethylene and propylene can be produced from syngas via methanol by using medium- or small-pore silicoaluminum phosphate molecular sieves (SAPO). The smaller pore size, for example, of the UOP catalyst SAPO-34 favors the formation of the desired light olefins with close to 90% yield of ethylene and propylene. The selectivity of a ZSM-5 catalyst toward propylene formation is higher, but with larger amounts of longer hydrocarbon chains as by-products. Reaction temperatures of 350–550 °C are used. The MtO process can be combined with the MtG technology as realized in the so-called MOGD process (Mobil Olefin to Gasoline/Distillate). Employing this process, olefins are synthesized in the first step followed by olefin oligomerization to gasoline or diesel using a ZSM-5 catalyst. Hence, this route also affords, unlike the MtG process, the production of synthetic diesel or jet fuel.

6.6 Fischer–Tropsch Synthesis

By means of the Fischer–Tropsch (FT) process ((7) of Table 6), syngas is directly converted into hydrocarbons. Related processes are well-established and currently operated, for example, by Sasol (Sasol Advanced Synthol, SAS process in Secunda, South Africa) and Shell (Middle Distillate Synthesis, MDS process in Bintulu, Malaysia and in the PEARL plant in Qatar) by using coal and natural gas, respectively. FT syntheses can be conducted in slurry-phase, fixed bed, and fluid bed reactors, mainly depending on the catalysts and reaction temperatures employed. With respect to reaction temperatures, one can distinguish between low- and high-temperature FT technologies (LT-FT and HT-FT). LT-FT synthesis is operated at temperatures around 220 °C and yields primarily long-chain hydrocarbons such as paraffin and waxes whereas HT-FT processes, operating at temperatures around 340 °C, produce mainly naphtha and olefins. Long-chain products are hydrocracked in the next step to yield diesel in very high quality. Today, LT-FT syntheses combined with slurry-phase reactors are the preferred option. Hydrocarbon chain growth proceeds at the surface of cobalt- and/or iron-based catalyst systems, for example, with Fe-, Fe/Co-, Fe/Co-Spinel-, Co/Mn-Spinel-, or Cu-doped Co catalysts. Iron catalysts are cheaper than cobalt catalysts and more flexible as well as more resistant with respect to syngas composition and quality. They catalyze the WGS reaction and therefore tolerate lower H_2:CO ratios in the syngas. Co catalysts show best performances at a H_2:CO ratio of 2:1 and feature longer lifetimes as well as higher selectivities than iron catalysts.

6.7 Hydroformylation

Hydroformylation or oxo synthesis is an important industrial process for the production of linear and branched aldehydes from alkenes in homogeneous reactions catalyzed by Co or Rh catalysts ((8) of Table 6). The individual process designs and catalysts used depend on the chain length of the olefin to be hydroformylated, and the catalyst metal center and ligands. Industrial processes in place today are based on cobalt catalysts for the production of medium- to long-chain aldehydes, whereas rhodium-based catalysts are mainly used for the conversion of propylene.

6.8 Hydrogen

Hydrogen can be produced via gasification of biomass by a downstream WGS reaction of the syngas ((9) of Table 6) converting carbon monoxide with water into additional hydrogen. Crude bio-hydrogen produced in that way has to be cleaned, compressed or liquefied, or stored on solid state materials, and can be used as fuel for specialized combustion engines or fuel cells. Converting biomass to hydrogen allows for a maximum conservation of energy originally contained in the feedstock, because the shift reaction is less exothermic than the above-mentioned reaction pathways.

6.9 Methane

Methane is usually produced from biomass by anaerobic digestion, but can also be obtained from syngas, and allows for the use of a wider variety of biomass types. Methane can be obtained directly in the syngas with higher yields at low temperature gasification or by conversion of the syngas formed. The increase of methane yield to produce a high caloric town gas was one important goal in coal gasification at elevated pressures in Germany in the post-war decades. Synthetic natural gas from biomass or Bio-SNG can be used, after cleaning, compression, or liquefaction, e.g., as fuel for modified spark ignition engines, and exhibits high octane numbers. Recently, SNG production has been discussed and developed in the context of energy storage in fluctuating renewable energy production systems by power-to-gas technologies. From surplus electrical power, hydrogen is generated from water by electrolysis and subsequently used for CO_2-hydrogenation to produce methane according to reverse (9) and (10) of Table 6.

7 Recent Process Developments

The longest tradition in the production of synfuels and chemicals can be found in coal conversion technologies. However, coal was not cost-competitive for a long time compared to crude oil refining. Recently, new production facilities have been erected, for example, in China with large production capacities to produce methanol, propylene, DME, and SNG from coal. Natural gas is also utilized for FT synthesis, for example, in oil- and gas-producing countries such as Qatar, Nigeria, or Malaysia. Increasing interest is also devoted to biomass utilization in the context of renewable energy and climate change issues. The value of a biofuel has to be scored in terms of its CO_2 reduction potential. In the EU, the greenhouse gas emission saving from the use of biofuels and bio-liquids should be at least 50% and even 60% in 2018 for those installations in which production started on or after January 2017. However, comparable regulations for the production of chemicals and materials do not exist in the EU.

In Table 7 a selection of pilot and demonstration plants in Europe is compiled, giving details of the range of technologies, conversion capacities, and their state of development. Because of the importance of Scandinavian wood industries, the main efforts can be observed in this region. Many more examples of synthesis gas utilization processes are given in the maps available online from the IEA Bioenergy and the European Biofuels Technical platform, even though mainly dedicated to bioenergy production. The examples given in Table 7 show the multitude of options

Table 7 Current synfuel pilot or demo projects

No.	Project	Feedstock	Pre-treatment + gasification features	Synthesis, products
A	Växjö Värnamo Biomass Gasification Centre, S	Forest biomass	Foster Wheeler pressurized CFB + hot gas filtering	Heat and power, clean syngas
B	Güssing Renewable Energy Multifuel Gasification, A	Wood	Repotec dual fluid bed also referred to as fast internally circulating fluid bed (FICFB), 8 MW_{th}, atm	CHP, SNG (1 MW) and FT plant (slip stream)
C	BioDME, S	Black liquor	Chemrec EF, 3 MWt_h, 30 bar	DME, methanol
D	BioTfuel, F	Forest biomass	Torrefaction + Uhde Prenflow EF, 15 MW_{th}	FT-products
E	bioliq®/KIT, D	Lignocellulose	Air Liquide fast pyrolysis, 2 MW_{th} + Air Liquide EF, 5 MW_{th}, 80 bar	DME, gasoline
F	GoBiGas, S	Forest biomass	Metso/Repotec dual bed, 20 MW	Bio-methane
G	NSE Biofuels Varkaus, S	Forest biomass	CFB, 12 MW (5 MW_{th} for synfuel application)	Heat for a lime kiln, FT-products (slip stream)

to set up a process chain regarding feedstock, pre-treatment, gasification, and synthesis technology.

7.1 Växjö Värnamo Biomass Gasification Centre (A)

The first pressurized CFB pilot gasifier for biomass, located in Värnamo, was designed and built by Foster Wheeler and SYCON and commissioned in 1993–1996. The plant was run until 1999 and then mothballed in 2000 after successful operation as a Biomass Integrated Gasification Combined Cycle (BIGCC) demonstration plant. Different types of wood, bark, straw, and refuse-derived fuels were used as feedstocks. Of the total thermal biomass input of 18 MW_{th}, 6 MW_{el} were fed into the electricity grid and about 9 MW_{th} were supplied to the Värnamo district heating network. Operated at 18 bar, the wood chips were forced into a lock-hopper and screw-fed into the air-blown gasifier; average gasification temperatures were slightly below 1,000 °C, a downstream hot gas candle filter removing particulates. The EU CHRISGAS project (2004–2007) demonstrated biomass gasification followed by gas upgrading via hot gas cleaning to remove particulates, steam reforming of tar and volatile organic components, and WGS reaction to enhance the hydrogen yield with regard to biofuel production.

7.2 Güssing Renewable Energy Multifuel Gasification, Austria (B)

The allothermal dual bed gasifier, designed and built by Repotec company, generates concentrated syngas undiluted with N_2 since 2002. A typical syngas composition is 40 vol% H_2, 25 vol% CO, 20 vol% CO_2, 10 vol% CH_4, and 4 vol% N_2. The CHP plant has a fuel capacity of 8 MW_{th} and an electrical output of about 2 MW_{el}. The Güssing plant was extended toward polygeneration concepts, where several products are generated simultaneously. After raw syngas cleaning, most of the CO and H_2 have been converted into a biosynfuel mix in a single pass through an FT synthesis reactor (0.5–1 L/h). Unconverted syngas, mainly methane, was available for downstream power or electricity generation by combustion. There is no need to adjust the H_2:CO ratio, especially when an Fe catalyst is used. The Güssing experiments were performed in a raw syngas slipstream and served mainly to develop, test, and evaluate suitable polygeneration concepts within the EU RENEW project (2003–2008). Within the EU BioSNG project (2006–2009) a 1-MW_{th} methane production was integrated and operated to demonstrate Bio-SNG production from solid biofuels and its integration into the existing natural gas infrastructure (i.e., fuel station for vehicles, natural gas grid). The Güssing gasifier has meanwhile operated for ca. 80,000 h. Meanwhile, a 20 MWgas SNG plant is operated within the Gotherbūrg Biomass Gasification Project (GoBiGas) in Sweden, also based on Repotec's gasification technology.

7.3 BioDME at Pitea, Sweden (C)

Black liquor, a waste stream of the Kraft pulping process, is a black, aqueous solution containing ca. 5 wt% of inorganic cooking chemicals and ca. 10 wt% lignin-derived organic material, with about half of the initial bioenergy. Conventionally, the liquor is concentrated by about one order of magnitude and is then combusted in boilers to recover the cooking chemicals to recycle and to supply the pulping process with steam and electricity. Integration of gasification into the pulp mill process allows for more efficient energy recovery and a considerable reduction of the synthetic biofuel production cost. The BioDME pilot facility at Pitea, Sweden is integrated into the Smurfit Kappa Pulp Mill. Successful demonstration of the process was achieved within the EU BioDME project (2008–2012) and is now part of the Swedish gasification center hosted by Lulea University of Technology. The EF gasifier technology has been developed by Chemrec, Haldor Topsøe contributing the DME synthesis technology. Volvo performed truck tests with DME as an environmentally friendly diesel fuel; more than one million truck kilometers have been accumulated. The EF gasifier consists of a pressure resistant vertical steel cylinder with a top burner, by which black liquor concentrate with a feed capacity of up to 4 tons/h is pneumatically atomized with pure oxygen. Gasification temperature and pressure are 1,000 °C and 30 bar, respectively. The 200–300 °C lower gasification temperatures compared to other EF gasifiers for lignocellulose are possible because of the lower melting slag of the pulp cooking chemicals. The slag is quenched in a water bath at the gasifier bottom and batch-wise discharged with a lock hopper. Organic vapors are removed from the syngas first with two serial charcoal beds; further purification is achieved with a conventional MDEA amine absorption tower with stripper for regeneration. A WGS unit is used to adjust the desired H_2:CO ratio. After syngas compression, the residual sulfur compounds and other impurities are removed to parts per billion trace levels in a guard bed to prevent catalyst poisoning. An improved two-stage synthesis, first at 240 °C then at a thermodynamically more favorable lower temperature, eliminates the need for recycling of unconverted syngas. Catalytic dehydration to DME is integrated with a design production capacity of 4 tons/day DME.

7.4 BioTfuel Project Dunkirk, France (D)

The aim of the BioTfuel project is to construct and operate a biosynfuel pilot facility with a design capacity of about 3 tons/h. To ensure a continuous full load operation, co-processing with fossil fuels up to 100% is possible. Partners with complementary expertise are Axens, CEA and IFP Energies nouvelles, Sofiprotéol, ThyssenKrupp Uhde, and Total as the project leader. As feedstock, wood and non-edible residues from agriculture as well as fossil fuels are planned. The dried biomass is crushed and torrefied at Sofiprotéols facilities at Venette, France; the brittleness of the char simplifies milling to a fine powder, which is a suitable feed

for pressurized EF operation. The torrefaction char is then delivered to the central gasifier site at Dunkirk. There, the fine powder of 50–200 μm size or pulverized fossil fuels is transferred by means of an inert gas from a pressurized fluid bed into the ca. 15-MW_{th} pressurized entrained flow gasifier. The PRENFLOW-type slagging and oxygen-blown gasifier is supplied by ThyssenKrupp Uhde and is equipped with a membrane wall designed for high gasification temperatures of 1,200–1,600 °C at 30–40 bar pressure. At the exit of the gasifier chamber, the hot syngas is cooled to ca. 220 °C in a water quench; the water vapor saturation is needed for the WGS reaction downstream. Though there has been much experience with the PRENFLOW technology, for example, from the large IGCC plant in Puertollano, Spain, the adaptation for reliable injection of variable biomass-to-fossil fuel feed mixtures requires attention. The expected H_2:CO ratio of 0.5–0.7 in the raw syngas is increased to about 2 via the WGS reactor for the intended FT synthesis, combined with a conventional sour gas removal unit. A separated testing line for gas purification is available as a 5–10% side stream of the main raw syngas flow. The FT synthesis makes use of a Co catalyst in a slurry bubble column reactor with subsequent hydro-cracking and hydro-isomerization, mainly to yield diesel, jet fuel, and naphtha. The parallel development work to be carried out at the Dunkirk and Venette sites is coordinated and guided by Bionext, aiming at an accumulation of sufficient experience for the design of biosynfuel plants with a biomass capacity of 1 Mt/a in 2020.

7.5 Bioliq® Project at Karlsruhe Institute of Technology (KIT) (E)

The bioliq® process has been designed to overcome the logistical limitations when using a broad range of different types of biomass for large, industrial-scale processing [9]. The forest, agricultural, and other types of bio-residues are favored as feedstocks because they don’t compete in food and feed production. They are widely distributed and usually exhibit low volumetric energy densities. Therefore the process has been split up, first into a pre-treatment by fast pyrolysis conducted in a number of regionally distributed plants to increase the energy density of the biomass. The intermediate product, termed biosyncrude, is produced by mixing the pyrolysis char and liquid condensates, thus maintaining ca. 85% of the original bioenergy. The biosyncrude is then collected from the de-centralized plants and converted into synthesis gas which is further processed to synthetic fuels and chemicals in an industrial complex of reasonable economic size.

At KIT, a 2–5-MW_{th} pilot plant has been erected for process demonstration and as a research and development platform for further process improvement and optimization (Fig. 6). The separate process steps were commissioned stepwise until 2013, and a joint operation producing synthetic gasoline from biomass for the first time was achieved in 2014. In the fast pyrolysis plant with a biomass feed

Fig. 6 Image of the bioliq® pilot plant at KIT. From *left* to *right*: fast pyrolysis plant, biosyncrude storage, EF gasifier, gas cleaning section, and synthesis plant in outdoor construction

capacity of 500 kg/h (2 MW_{th}) biomass is contacted with hot sand at 500 °C in a twin screw reactor. As the sand as heat carrier is recycled and re-heated, the char formed after pyrolysis is separated from the hot pyrolysis vapors, which are then liquefied in two condensation stages. Although from wood a single homogeneous condensate can be produced, wheat straw and other ash-rich feedstocks usually lead to two condensates: an organic phase and a highly water-containing aqueous phase. From these, biosyncrude is produced by adding the pyrolysis char in a colloidal mixer and milling system [10]. Liquids or slurries can more easily be fed to a pressurized EF gasifier and do not require additional fluidization agents as in the case of feeding solid particles. In the bioliq® gasification plant the highly viscous biosyncrude is fed to a 5-MW_{th} oxygen blown high pressure EF reactor by a progressive cavity pump. The slagging reactor operated at temperatures up to 1,600 °C and pressures of up to 80 bar is equipped with an internal cooling screen, particularly suited to the conversion of ash-rich feeds and to fast start up and shut down procedures. The molten slag drops down into the quench water reservoir and is removed as solid slag. Then the tar-free, low-methane raw syngas is passed through a water quench, which is later rebuilt into a water injection quench, by which the gas temperature can be adjusted to an optimum temperature for downstream gas cleaning. A high pressure, high temperature process for gas cleaning is being developed at KIT. Temperatures of 500–800 °C can be applied and are adjusted to optimum operation temperatures, where energy savings can be expected compared to the conventional low temperature gas cleaning processes. Fines are

separated by ceramic filter elements and sour gases (HCl, H_2S, COS) are retained by fixed bed sorption. In a catalytic reactor, NH_3, HCN, and organic trace compounds are decomposed. Another sorption bed is added as a safeguard. Prior to synthesis, CO_2 and water are separated from the purified gas. For synthesis, a 700-Nm^3/h slip stream (2 MW_{th}) of the syngas is used. First, DME is produced in a single step from syngas by simultaneous methanol formation, methanol dehydration, and WGS reaction using a mixture of commercial catalysts for methanol production and dehydration. Then gasoline is produced based on the MtG process. The raw product of the fuel synthesis is separated from the non-converted syngas for recycling and is distilled to receive the main product, high octane gasoline, along with some light and heavy hydrocarbon compounds.

8 Process Efficiency and Economics

For several syngas-based process chains and production scenarios, techno-economic and life cycle assessments have been conducted. Most examples are found for different BtL process chains for synthetic fuel production, of which some results are briefly discussed in the following, together with some general remarks.

8.1 Process Efficiency

The main task of a conventional petroleum refinery is the separation of the hydrocarbon constituents of crude oil by distillation. Except for the FCC cracker and the removal of small amounts of S, N, O, and Cl hetero-atoms by hydrogenation, only limited chemical processing is involved. About 7% of the energy content of the oil is consumed in the refinery processes by combustion of poor-quality products; approximately 80% of the energy can be recovered in the form of liquid fuels and chemical feedstocks. In contrast, conversion of lignocellulose biomass to synthetic fuels and chemicals in a BtL process is facilitated in a sequence of chemical transformations. Each of the chemical reactions must be exergonic to proceed with negative Gibbs enthalpy and, in most cases, are exothermal. The heat of the gasification reaction remains as sensible heat of the hot raw syngas and amounts to 10–20% of the initial bioenergy, depending on O_2 consumption. The heat of reaction of FT synthesis amounts to 20–25% of syngas energy. With typical FT synthesis temperatures of around 200 °C, this energy cannot be used very efficiently. However, a substantial part of the released heat can be used internally for process heat and electricity production [11].

Based on stoichiometric equations and calorific values of the substances involved, theoretical maximum energy yields of around 55% or 60% are achievable for hydrocarbons and methanol production from lignocellulosic biomass,

respectively. The practical efficiency depends on the type of biomass and targeted products as well as on conversion technology and capacity. In work conducted at TU Bergakademie Freiberg, different process concepts have been studied with regard to efficiency and production costs [12]. Among the gasification technologies considered were the FICFB reactor (B in Table 7), a pressurized CFB reactor (Bergakademie Freiberg), and the EF reactor for biosyncrude gasification of KIT (E in Table 7). Biogas production by fermentation followed by steam reforming was also considered for syngas production. For a given conversion capacity and feedstock, the production of methanol the estimated efficiencies range between 23% and 49%, whereas for production of hydrocarbons by FT synthesis efficiencies of 28–44% are calculated. Even though a direct comparison is difficult, this comparative study showed that, for a certain product, each syngas technology performs differently.

The bio-based coverage of process energy demand is of prime importance for biofuel production. Therefore other studies lead to lower energy efficiencies related to the products, but are practically self-sufficient in terms of energy supply with a maximum CO_2 reduction potential by saving the use of additional fossil fuels. In the recently finished EU BioBoost project an overall life cycle assessment estimated a CO_2 reduction potential of 82% for a synthetic fuel production scenario based on the bioliq® process [13].

Different efficiencies are also estimated within one selected process chain. As an example, for the bioliq® process converting straw into FT-products, overall efficiencies have been estimated in the range of 39 ± 6%, considering a number of studies also used for the cost estimated as discussed below. In some cases, even surplus energy was estimated from the process heat.

8.2 Economic Considerations

Commercial synthesis gas biorefineries for the production of synfuels and chemicals are not yet present on the market. The production of synthetic fuels and chemicals requires complex and cost-intensive technologies and has to take advantage of economies of scale. In Fig. 7 the results of various techno-economic studies on the bioliq® process, based on the more commonly reported FT synthesis, are depicted. The decrease of production costs from ca. 1.9 to 0.9 €/L of fuel with increasing conversion capacity can be observed, even though considerable deviations occur. These are mainly based on different total capital cost estimates and diverse cost models applied. Costs for biomass supply, biosyncrude transport, and other logistic factors are usually not directly taken into account but are indirectly contained e.g., in the biomass feedstock prices. One exception is the study carried out within the EU BioBoost project, where different pathways proceeding via de-centralized production of an intermediate energy carrier, and central further conversion to fuels, chemicals, heat, and power have been analyzed. Within the project a logistic model has been developed to optimize biomass supply and

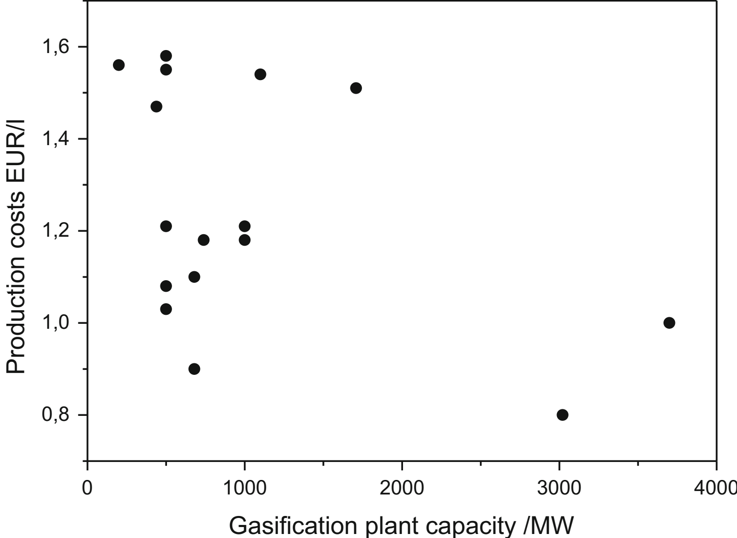

Fig. 7 Specific production costs of synthetic biofuels vs gasification plant fuel capacity taken from different techno-economic studies on the bioliq® process

conversion chain scenarios with regard to minimum production costs on a EU-wide level, taking into account promising biomass supply regions and prices, transportation network and distances, as well as the number, location, and conversion capacity of de-centralized and central conversion sites. According to a simulation example for the bioliq® concept, costs for biomass transport are ca. 10%, those for biosyncrude transport less than 5%, and the costs for the feedstock as well as for de-centralized and central processing are in the same region of around 30% each. The resulting data point in Fig. 7, located at 1,100 MW and 1.56 €/L, relates to such a simulation, including several local and central plants of varying size, being based on straw utilization in South East Europe [13].

9 Conclusions

The variety of pilot and demonstration projects on syngas biorefineries is on the way to verify the technical and economic feasibility of biomass-based thermochemical syngas processes. Benefits for the further development can be taken from the experience of already established coal and gas conversion technologies. However, specific differences exist in biomass conversion, requiring appropriate adaptation and improved solutions. Issues to be addressed with regard to the broad range of potential feedstocks differing in their composition, consistency, and availability are related to the their pre-treatment, feeding, and operation of gasifiers, raw syngas

cleaning, and conditioning, according to the requirements of the chemical syntheses applied.

It is not possible to separate the fuels and chemicals business. Considering a BtL production complex, not only the most valuable mix of fuels and chemicals has to be produced but also co-production of heat and power has to be facilitated to achieve sufficient energetic efficiencies. The implications of complex polygeneration should be considered in the context of emerging thermochemical biorefineries: combinations of thermochemical, biochemical, and physicochemical biorefineries are part of the future organic chemical industry converting the available multitude of bio-feedstocks into a broad, flexible spectrum of valuable organic platforms and fine chemicals, high performance synthetic fuel components, and materials, in addition to energy in the form of heat, high-pressure steam, or electricity. Already today, advantage can be made by integration of biomass conversion plants into the existing infrastructure of chemical plants or oil refineries.

Regarding biomass as a feedstock, new product routes are desirable, allowing maintaining of oxygen molecules in the final products, as they are already naturally contained in the biomass feed stock. In this way, the energetic and carbon efficiency would be increased considerably. Examples are high performance fuel components or additives, oxygen-containing monomers for polymers, such as polyoxymethylene; see (11) in Table 6. A syngas biorefinery would also benefit from integrating CO_2 utilization via external hydrogen supply, supplied by other renewable resources. In this way the carbon efficiency could be improved even more. As biomass is the only long term renewable carbon resource, its most efficient and economic use remains one of the most challenging issues in biomass research and development.

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Syngas Biorefinery and Syngas Utilization

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Abstract Autotrophic acetogenic bacteria are able to capture carbon (CO or CO_2) through gas fermentation, allowing them to grow on a spectrum of waste gases from industry (e.g., steel manufacture and oil refining, coal, and natural gas) and to produce ethanol. They can also consume syn(thesis) gas (CO and H_2) made from the gasification of renewable/sustainable resources, such as biomass and domestic/ agricultural waste. Acetogenic gas fermentation can, therefore, produce ethanol in any geographic region without competing for food or land. The commercialization of the process is now at an advanced stage. The real potential of acetogens, however, resides in their capacity to produce chemicals and fuels other than ethanol. This requires the redesign and implementation of more efficient metabolic pathways, adapting them to high performing manufacturing processes. Respective species, their bioenergetics, the genetic tools developed for their metabolic engineering, culture techniques and fermenter set-ups, as well as the commercialization, are comprehensively described and discussed in this chapter.

Keywords Autotrophic acetogens, CO, CO_2, Syngas, Wood-Ljungdahl pathway

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1 Introduction

The anaerobic conversion of CO_2 and H_2 to acetate in digested sludge was first described by Fischer and colleagues [1]. Later, Wieringa isolated a pure culture of *Clostridium aceticum*, which thus became the first known autotrophic acetogen [2–4]. As the organism seemed to be lost during World War II, the biochemical reactions of acetogenesis were elucidated using *Moorella thermoacetica* (formerly *Clostridium thermoaceticum*). In honor of the scientists mainly involved in this work, this metabolic feature was named the Wood–Ljungdahl pathway. In 1977, the sodium-dependent *Acetobacterium woodii* was isolated [5], and in 1981 a spore preparation of *C. aceticum* was found in a laboratory fridge of Barker (University of California Berkeley), which could be successfully revived [6]. Since then, numerous mesophilic and thermophilic autotrophic acetogens have been isolated and characterized [7–10]. Based on the presence of Wood–Ljungdahl pathway genes, more bacteria might possess the ability of autotrophic acetogenesis, but this needs to be verified experimentally.

The ability to capture carbon directly in the form of carbon monoxide (CO) and carbon dioxide (CO_2), present in syn(thesis) gas (which is used for certain reactions in the chemical industry but is also a waste by-product of many industrial processes), through anaerobic fermentation gives many anaerobic acetogens great potential for use as microbial production platforms for a range of high value commodity chemicals and fuels. Thus, in recent years, acetogens have attracted significant attention as the process organism for the biotechnological production of fuels and chemicals from industrial waste gas streams [11]. This emerging technology allows sustainable, high volume production of fuels and commodities independent from food-based substrates. The technology has been successfully demonstrated by a few companies at pilot and demo scales and the first commercial units have been announced.

The development of these microbial production platforms has historically been hampered by a lack of available genomic sequences and genetic tools, although recent advances in sequencing technologies and subsequent implementation of genetic methods have made these organisms far more accessible for directed mutagenesis. Closed genome sequences have been published for *A. woodii* [12], *M. thermoacetica* [13], *Clostridium ljungdahlii* [14], *Clostridium autoethanogenum* [15], *Clostridium carboxidivorans* [16], *C. aceticum* [17], *Eubacterium limosum* [18], and *Clostridium difficile* [19].

Key to development of a microbial platform is the establishment of robust and reproducible procedures for DNA transfer into the organism. The delivery of plasmids into an organism whose genome sequence has been elucidated allows the development of targeted group II intron-based mutagenesis and directed homologous recombination strategies to facilitate individual gene knockout and complementation studies, as well as expression of heterologous genes.

2 Anaerobic Autotrophs

Many mesophilic and thermophilic autotrophic acetogens are currently known. However, not all have been investigated in detail and only a few are currently used in or for developing industrial applications. These latter ones are detailed in the following paragraphs.

2.1 Clostridium aceticum

C. aceticum was isolated in 1936 from sludge from a canal in the Netherlands and characterized with respect to morphology, nutritional requirements, growth pattern, and product formation. Its remarkable metabolic activity is the conversion of four molecules of hydrogen and two molecules of carbon dioxide into one molecule of acetate and two molecules of water. Heterotrophic substrates can also be used

[2–4]. *C. aceticum* was the first autotrophic acetogen cultivated as a pure culture. CO can also serve as a carbon source [20]. After the war the culture seemed to be lost, but was later found in a laboratory culture collection in California [6]. At about the same time, attempts to re-isolate the organism were also successful [21]. The reason for various earlier failed attempts at its re-isolation was most probably the alkaline pH optimum of 8.3. The complete genome sequence of *C. aceticum* was recently published [17]. Interestingly, the bacterium does not contain genes for quinone synthesis [22] although cytochromes have been detected [6]. Thus, no electron transport chain via cytochromes and quinones is possible. Instead, *C. aceticum* harbors an Rnf (designation stems from *Rhodobacter* nitrogen fixation) complex, which might be acting as a proton pump in this organism [22] (see Sect. 3).

2.2 Acetobacterium woodii

A. woodii was isolated in 1977 from black sediment from an oyster pond in Woods Hole, MA [5]. It can grow on CO_2 plus H_2, producing acetate (as *C. aceticum*). The heterotrophic substrate range is rather narrow, being limited to some sugars, organic acids, and O-methylated aromatic compounds. The bacterium is Na^+-dependent [23], uses an Na^+-dependent ATPase [24], and generates an Na^+-gradient across the cytoplasmic membrane by means of an Rnf complex [25]. Its genome has been completely sequenced [12] and its energy metabolism belongs to the best understood among acetogens [26]. *A. woodii* is the model organism for Na^+-dependent autotrophic acetogens.

2.3 Clostridium ljungdahlii

C. ljungdahlii was isolated from chicken yard waste as an organism being capable of using syngas (mainly a mixture of CO plus H_2) as sole carbon source [27]. Its genome was completely sequenced, indicating that this bacterium is one of the most versatile acetogens with respect to substrate utilization [14]. In addition to acetate, it produces large amounts of ethanol and smaller amounts of 2,3-butanediol and lactate [28]. The genes responsible for ethanol and 2,3-butanediol production have been identified as well as the function of the Rnf complex as a proton pump [29–31]. Together with *C. autoethanogenum*, *C. ljungdahlii* developed into a model organism for H^+-dependent autotrophic acetogens. Strains of *C. ljungdahlii* are industrially used by INEOS Bio (see Sect. 7.2).

2.4 Moorella thermoacetica

Whereas all other bacteria mentioned in Sect. 2 are mesophilic, *M. thermoacetica* is a moderate thermophile (optimal growth temperature 55°C). It was isolated under heterotrophic conditions from horse manure [32]. *M. thermoacetica* served as the model organism for elucidation of the enzymology of the Wood–Ljungdahl pathway [8] (see Sect. 3). Much later it was discovered that the organism is also capable of autotrophic growth [33]. It does not contain an Rnf complex but instead possesses cytochromes and quinones as well as an energy-conserving hydrogenase (Ech) for generation of a proton gradient [13, 26, 34]. Nitrate and nitrite can be used as terminal electron acceptors [35, 36]. With nitrate, H_2-dependent growth yields are higher than those with CO_2. In the presence of nitrate, cytochrome synthesis is repressed [37].

2.5 Butyribacterium methylotrophicum

B. methylotrophicum is a catabolically versatile, mesophilic, spore-forming anaerobe that was isolated from a sewage digester in Marburg, Germany [38]. Heterotrophic growth is possible with sugars, organic acids, and C1-compounds such as methanol. Autotrophic growth relies on $CO_2 + H_2$ gas mixtures. An adapted strain, the so-called CO strain, can also grow on CO and syngas [39]. It is a mutant that expresses higher levels of ferredoxin: NAD^+ oxidoreductase, which is not inhibited by NADH [40]. Products are acetate and butyrate, but, at decreasing pH values, increasing amounts of butanol and ethanol are formed [41, 42]. Lactate has also been described as a product [43]. In the EU, *B. methylotrophicum* is classified as risk group 2. A genome sequence is not currently available.

2.6 Eubacterium limosum

E. limosum was isolated on methanol as a substrate, inoculated with sheep rumen fluid and sewage sludge [44]. Products from methanol are acetate, butyrate, and caproate. Autotrophic growth with $CO_2 + H_2$ or CO as sole carbon and energy source is also possible. Under these conditions, no caproate is formed [45]. In defined media, butyrate is produced from CO [46, 47]. An energy conservation model has been presented, suggesting that the energetic benefit when growing on CO might be a reason that butyrate is only formed on CO and not on $CO_2 + H_2$ gas mixtures [48]. Complete and draft genomes are available for two *E. limosum* strains, the latter also producing butanol [18, 49].

2.7 Clostridium autoethanogenum

C. autoethanogenum was isolated from rabbit feces using CO as sole carbon and energy source. It produces acetate, ethanol, and CO_2 from CO [50]. Other natural products are lactate and 2,3-butanediol [28]. It can also grow well on syn(thesis) gas. This organism is used industrially for ethanol production from steel mill exhaust gases by LanzaTech (see Sect. 7.2). The genome sequence has been determined [51, 52].

2.8 Clostridium coskatii

C. coskatii was first described in a poster at the 60th annual Meeting of the Society for Industrial Microbiology in San Francisco, August 1–5, 2010 [53]. The bacterium was isolated from estuary sediment collected from the Coskata-Coatue Wildlife Refuge in Nantucket, MA. *C. coskatii* produces ethanol as a primary product from CO or CO_2 + H_2. The organism is closely related to *C. autoethanogenum*, *C. ljungdahlii*, and *Clostridium ragsdalei*. The organism has been patented by Coskata, Inc. for ethanol production from CO-containing gas mixtures [54, 55].

2.9 Clostridium ragsdalei

C. ragsdalei strain P11 produces acetate, ethanol, and butanol when using CO as a substrate. Under these conditions, ethanol is the major product. However, when grown on fructose, acetate is the dominant fermentation product and no butanol is formed [56]. Optimization of the trace elements nickel, zinc, selenium, and tungsten improved growth and ethanol production of *C. ragsdalei* [57]. The organism is also able to reduce certain fatty acids to their corresponding alcohols. Formation of propanol, butanol, pentanol, and hexanol from propionate, butyrate, pentanoate, and hexanoate has been reported. In addition to these primary alcohols, acetone could be reduced to isopropanol [58].

2.10 Clostridium carboxidivorans

C. carboxidivorans strain P7 was isolated from sediment of an agricultural settling lagoon at Oklahoma State University under a CO atmosphere [59]. The organism stained Gram-positive and produced acetate, ethanol, butyrate, and butanol from CO. Low partial pressure of CO in the headspace led to increased butanol and also hexanol production [60]. The genome sequence has been determined [16].

2.11 Thermoanaerobacter kivui *(Formerly* Acetogenium kivui*)*

T. kivui was isolated from sediment samples from Lake Kivu, Africa. The enrichment culture was incubated at 60°C under an atmosphere of 20% CO_2 and 80% H_2. Acetate was the sole product. Heterotrophic growth with mannose, glucose, fructose, and pyruvate was also possible. Formate allowed only weak growth [61]. CO as sole energy source did not allow growth, although in combination with H_2 doubling times of 2.7 h were observed [33]. Although one report states the importance of Na^+ for autotrophic growth of *T. kivui* [62], genome sequencing and analysis as well as respective experimentation revealed a proton-dependent bioenergetic system. An Rnf membrane complex is not present, but instead an energy-conserving hydrogenase (Ech) [63].

2.12 Alkalibaculum bacchi

A. bacchi was isolated from soil under a CO atmosphere in the presence of 2-bromoethanesulfate (for inhibiting methanogenesis). In addition to gas mixtures such as H_2 + CO_2 and CO + CO_2, a number of heterotrophic substrates (sugars, organic acids, alcohols, trimethylamine) can be used for growth with ethanol and acetate as main products. The bacterium is remarkably alkali-tolerant (up to pH 10.5) [64]. In mixed culture with *Clostridium propionicum*, a syngas fermentation resulted in production of ethanol and propanol plus minor amounts of butanol [65]. Added carboxylic acids (propanoic acid, butyric acid, hexanoic acid) could be reduced to their corresponding primary alcohols [66].

2.13 Blautia producta *(Formerly* Peptostreptococcus productus *and* Ruminococcus productus*)*

A strain of *B. producta* (U-1) was isolated from anaerobic sewage digester sludge under an atmosphere of 50% CO. Under optimal conditions, a doubling time of 1.5 h was recorded. Autotrophic growth on CO_2 + H_2 (significantly slower) and heterotrophic growth on a variety of substrates was also possible. Acetate was the main fermentation product [67]. When grown on sugars, strain U-1 also produced lactate, succinate, and formate [68]. Another *B. producta* isolate (strain Marburg) was also able to grow on CO, although the type strain is unable to do so [69]. A number of Wood–Ljungdahl pathway enzyme activities could be measured [70]. The active carbon species deriving from CO oxidation is CO_2 rather than bicarbonate [71].

2.14 Clostridium difficile

C. difficile is a dangerous pathogen, representing a considerable threat to the North American and European healthcare systems. Infection rates are still increasing and numerous nosocomial outbreaks have been reported [72]. Originally, all bacteria pathogenic against humans were considered heterotrophs [73]. It therefore came as a surprise that *C. difficile* and phylogenetically closely related isolates were capable of autotrophic growth on CO_2 plus H_2 [74, 75]. The arrangement of genes encoding the enzymes for the Wood–Ljungdahl pathway in *C. difficile* is identical to the operon structure in *C. ljungdahlii* [75]. *C. difficile* is thus the first known autotrophic human bacterial pathogen, but as a risk group 2 organism it is not considered for biotechnological applications.

3 Wood–Ljungdahl Pathway and Bioenergetics

The reductive acetyl-CoA- or Wood–Ljungdahl (WL) pathway is probably the oldest carbon assimilation pathway on Earth [12, 76, 77]. It is found in acetogens, methanogens, and strictly anaerobic sulfate-reducing bacteria and archaea [77]. It consists of two parts, the methyl and the carbonyl branch (Fig. 1). In the former, one molecule of CO_2 (or CO) is bound to the coenzyme tetrahydrofolate (in bacteria) and then reduced in several steps to a methyl group, which is transferred to an iron-sulfur-corrinoid protein (FeSCo-P). FeSCo-P serves as the methyl donor for the key enzyme of the pathway, the nickel-containing bifunctional CO dehydrogenase/ acetyl-CoA synthase. In the carbonyl branch, another molecule of CO_2 is reduced to CO, which is also bound to acetyl-CoA synthase. There, methyl and carbonyl groups are fused to an acetyl residue and, combined with coenzyme A (CoA), are converted into acetyl-CoA. This compound can be used anabolically for biosyntheses or catabolically for formation of acetate by phosphotransacetylase (Pta) and acetate kinase (Ack), thereby yielding one molecule of ATP by substrate level phosphorylation. Looking at the energetics of the pathway, it becomes clear that one ATP is required for formyl-THF formation although only one ATP is gained from acetate formation. So, the net balance is zero, and additional energy-conserving mechanisms must exist.

For this purpose, different mechanisms are employed by acetogens. Although the biochemical reactions of the WL pathway have been elucidated using *M. thermoacetica* until the late 1980s, *A. woodii* was the first acetogen whose energetics were well-understood [26]. The breakthrough was the discovery of flavin-based and ferredoxin-dependent electron bifurcation that can be coupled to proton or Na^+ gradient generation and thus to ATP formation [78, 79]. So far, four such flavin-based electron-bifurcating enzyme complexes have been discovered in autotrophic acetogens (Table 1). Four more such reactions are known from *Clostridium kluyveri*, *Clostridium acidurici*, and methanogenic archaea [86], which,

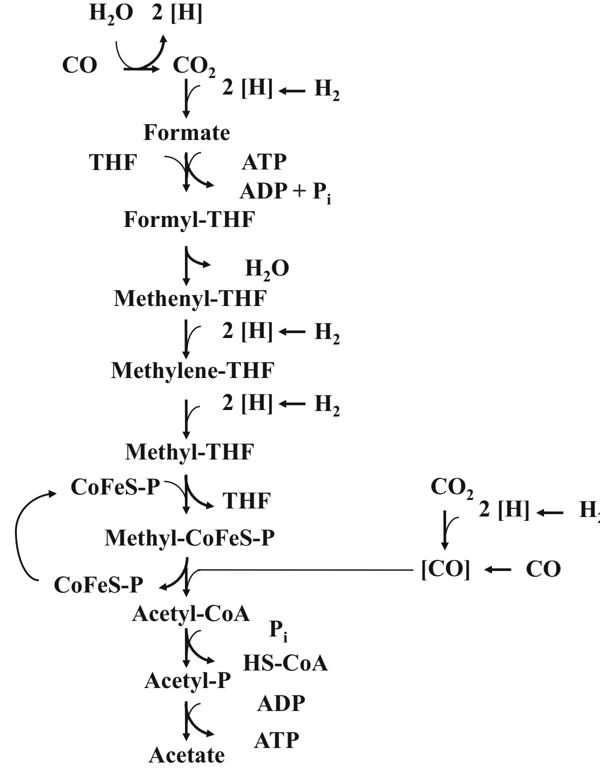

Fig. 1 Wood–Ljungdahl pathway employed by autotrophic acetogenic bacteria. *CoFeS-P* corrinoid-iron-sulfur protein, *2 [H]* reducing equivalents, *Pi* inorganic phosphate, *THF* tetrahydrofolate

Table 1 Flavin-based and ferredoxin-dependent electron bifurcation and confurcation enzyme complexes in autotrophic acetogens

Enzyme complex	Reaction catalyzed	Organism	References
NAD^+-specific [FeFe]-hydrogenase (HydABC(D))	$3\ H^+ + NADH + Fd^{2-} \rightleftharpoons 2\ H_2 + NAD^+ + Fd_{ox}$	*A. woodii* *M. thermoacetica*	[80, 81]
ferredoxin:$NADP^+$ oxidoreductase (NfnAB)	$2\ NADP^+ + NADH + Fd^{2-} + H^+ \rightleftharpoons 2\ NADPH + NAD^+ + Fd_{ox}$	*M. thermoacetica*	[82]
caffeyl-CoA reductase (CarCDE)	caffeyl-CoA + 2 NADH + Fd_{ox} → 2,3-dihydroxyphenylpropionyl-CoA + 2 NAD^+ + Fd^{2-}	*A. woodii*	[83]
$NADP^+$-specific [FeFe]-hydrogenase/ formate dehydrogenase (HytA-E/FdhA)	$NADPH + Fd^{2-} + H^+ + 2\ CO_2 \rightleftharpoons NADP^+ + Fd_{ox} + 2$ formate	*C. autoethanogenum*	[84]
Lactate dehydrogenase/electron-transfer flavoprotein	Lactate + Fd^{2-} + 2 NAD^+ → pyruvate + Fd_{ox} + 2 NADH	*A. woodii*	[85]

however, might only be the tip of the iceberg, as several other reactions might be candidates for such a mechanism. In principle, respective enzymes couple the exergonic reduction of a substrate with NADH to the endergonic reduction of ferredoxin with NADH. Similarly, flavin-based electron confurcation has been demonstrated in *A. woodii* in the case of lactate utilization (Table 1). Endergonic lactate oxidation with NAD^+ as oxidant is driven at the expense of simultaneous exergonic electron flow from reduced ferredoxin to NAD^+ [85].

Key to energy conservation under autotrophic conditions is the generation of reduced ferredoxin from H_2. This reduced ferredoxin can be reoxidized at the membrane-bound Rnf complex, simultaneously reducing NAD^+ to NADH. This exergonic reaction is coupled to pumping of either H^+ or Na^+ across the membrane. A proton gradient is, for example, formed in *C. ljungdahlii* [29] and then used for ATP generation by an H^+-dependent ATPase. On the other hand, the Rnf complex of *A. woodii* pumps a sodium cation [87] and uses the Na^+ gradient by an Na^+-dependent ATPase for ATP formation [24].

In *M. thermoacetica*, cytochromes and quinones are present, indicating the presence of an electron-transport chain across the membrane. Such a chain might also involve iron-sulfur and flavoproteins, which are present in *M. thermoacetica* as well. A tentative scheme for generation of a proton gradient has been proposed [34].

A third possibility for energy conservation is the presence of an energy-conserving hydrogenase (Ech) catalyzing the reversible oxidation of reduced ferredoxin with protons to hydrogen, thereby generating a proton or Na^+ gradient. Such membrane-associated enzymes have been found in several H_2-consuming, as well as H_2-producing, archaea and bacteria [79], for example, in *M. thermoacetica*. As this organism also forms cytochromes and quinone, the physiological role of the two systems still requires elucidation. Are both contributing to the generation of an ion gradient or only one and, in that case, which one? A recent hypothesis proposed to separate acetogens based on the presence of either *rnf* or *ech* genes [26]. Although in *M. thermoacetica* this proposal cannot yet be clearly verified (cytochrome plus quinone and also *ech* genes), in *T. kivui* (a thermophilic autotrophic acetogen) only *ech* genes could be detected [63]. Further genome comparisons revealed that indeed the presence of *rnf* and *ech* genes in autotrophic acetogens seem to be mutually exclusive [22].

Finally, the reduction step from methylene-tetrahydrofolate (THF) to methyl-THF is highly exergonic and irreversible under physiological conditions ($DG0' = -22$ kJ/mol) [88]. This methylene-THF reductase could thus be another site, which is coupled to electron transport or electron bifurcation, as speculated for *M. thermoacetica* [34] and *C. ljungdahlii* [14]. Indeed, electron bifurcation with endergonic reduction of a still unknown receptor has been shown in *M. thermoacetica* [89], whereas in *A. woodii* an additional energy conservation during this reaction has been excluded [26].

In general, autotrophic acetogens do not possess many possibilities for ATP generation and are operating at the thermodynamic limit of life. This is an important aspect for metabolic engineering of novel pathways in these organisms. Syntheses, demanding a high amount of ATP, are unlikely to function well in this group of bacteria.

4 Genetic Methods and Metabolic Engineering

The full potential of acetogens as a chassis for the production of a wide range of chemicals and biofuels may be realized through the implementation of metabolic engineering strategies. These are reliant on the availability of effective gene tools and gene transfer procedures. The first anaerobic acetogen to undergo genetic modification studies successfully was *C. difficile* [90], although at the time its classification as an acetogen was not known, and mutation studies were primarily concerned with elucidation of virulence pathways and mechanisms. Plasmid transfer by conjugative methods from *Escherichia coli* donors was demonstrated using an indigenous Gram-positive replicon (pCD6) from *C. difficile* [90]. Subsequently, a range of directed mutagenesis methods were developed and implemented in *C. difficile*, including the ClosTron [91, 92], which utilizes a directed mobile group II intron from the *ltrB* gene of *Lactococcus lactis*, generating targeted insertional mutants, selectable by acquisition of antibiotic resistance. The nature of the mechanism of insertion of the intron means that such insertional mutants are completely stable, and host antibiotic resistance can only occur through chromosomal insertion of the group II intron. ClosTron technology allows for the rapid generation of insertional gene knockouts and has been found to be widely applicable within the genus *Clostridium*, including the homoacetogens for which a DNA transfer method, electroporation, or conjugative plasmid transfer from *E. coli* donors, has been established. Implementation of the ClosTron has been demonstrated in both *C. ljungdahlii* [unpublished data] and *C. autoethanogenum* [89].

The first anaerobic acetogen for which a genetic modification system was specifically designed with a view to creating a microbial platform for chemical synthesis was *C. ljungdahlii.* A relatively low frequency electroporation transformation procedure was described using the *Clostridium–E. coli* shuttle vector pIMP1, and heterologous expression of the *Clostridium acetobutylicum* butanol synthesis pathway genes *thlA, hbd, crt, bcd, adhE*, and *bdhA* (encoding thiolase, 3-hydroxybutyryl-CoA dehydrogenase, crotonase, butyryl-CoA dehydrogenase, bifunctional butanol/butyraldehyde dehydrogenase, and butanol dehydrogenase, respectively) was demonstrated [14]. This recombinant strain was shown to be capable of producing butanol, albeit at a low (2 mM) concentration in the exponential growth phase in batch culture. The low concentrations of butanol observed were shown to be caused by the organism's ability to metabolize 1-butanol to butyrate. Nevertheless, this proof of principle experiment represented an important step towards the establishment of the first acetogenic microbial platform, with a system capable of heterologous expression of metabolic pathway genes, although knockouts of native genes had not yet been demonstrated.

Subsequently, the transformation procedure was optimized, and frequency was improved to a level where homologous recombination methods for directed chromosomal knockouts using suicide plasmids became possible [30]. A chromosomal 'clean' deletion of the gene *fliA*, encoding a putative sigma factor involved in flagella biosynthesis, demonstrated the growing potential for *C. ljungdahlii* as a

model platform organism for gas fermentation based systems. More recently, it was shown that heterologous genes could be introduced in a stable manner into the chromosome from a plasmid through isolation of double crossover mutants using homologous recombination cassettes, delivered by suicide vectors, in this instance encoding enzymes required for butyrate production as proof of principle [93]. After a number of metabolic pathway modifications, this recombinant strain developed was shown to redirect carbon and electron flow significantly towards the production of butyrate. Carbon and electron yields in butyrate were approximately 50% with H_2 as the electron donor, and 70% with CO [93]. This development represents a crucial component of the genetics toolkit, as it allows the generation of production strains, with metabolic pathways modified through expression of synthetic and heterologous genes, which require no antibiotic selective pressure for maintenance.

A useful recent addition to the tools available for manipulation of genetic pathways was the implementation of an inducible promoter system originally shown to be effective in *C. perfringens* [94], and later in the solventogenic bacterium *C. acetobutylicum* [95], based around the native lactose operon present in *Clostridium perfringens*. Inducible promoters have a broad range of applications, including gene complementation studies, adjustable modulation of protein expression, and transposon mutagenesis studies. This system consists of the constitutively expressed transcriptional activator *bgaR*, encoding a protein that binds to and activates the *bgaL* (β-galactosidase) promoter when in the presence of lactose. Adaptation of this system from *C. acetobutylicum* involved the exchange of a Gram-positive replicon on the plasmid for one that had previously been shown to function well in *C. ljungdahlii,* and the system was exemplified through inducible upregulation of the native *adhE1* gene (encoding a bifunctional ethanol/acetaldehyde dehydrogenase), such that expression was shown to be 30 times higher than in the wild type organism [96].

The combined ability to modify native pathways through directed clean mutagenesis, and introduce stable heterologous genes into the chromosome, alongside the development of an inducible promoter system, further reinforces the importance of *C. ljungdahlii* as a forerunner model platform acetogen for the production of high value chemicals from synthesis gas.

A. woodii was the first autotrophic acetogen to be investigated in detail, and as such the native organism is well-characterized, with its energy conservation pathways amongst the best understood of all acetogens [26, 97, 98]. Plasmid transfer has been demonstrated into *A. woodii* via both conjugative transfer from an *E. coli* host strain and by electroporation [99]. The electroporation procedure was improved through adaptation of the refined protocol for *C. ljungdahlii* [14], and plasmid-based heterologous expression of selected theoretical bottlenecks in the Wood–Ljungdahl pathway was employed to increase metabolic flow and thus yields of acetate produced by the first engineered strain of *A. woodii* [100]. In a pH-controlled batch process, acetate concentrations in the recombinant strains reached a maximum of 51 g/L after 3.7 days, compared to the reference strain whose acetate concentrations reached 44.7 g/L under equivalent conditions [100]. Further genetic tools are currently in development for this organism.

A comprehensive range of tools for the moderately thermophilic acetogen *M. thermoacetica* has also recently been implemented, making use of a uracil auxotrophic mutant strain as a screening tool for successful double crossover homologous recombination events, and consequent insertion of heterologous genes into the host chromosome [101]. The system was developed through elucidation of a successful electroporation strategy which allowed integration of a methylated vector containing a knockout cassette targeting the gene *pyrF*, part of the uracil biosynthesis pathway. A double crossover deletion mutant was obtained through serial screening of isolated single colonies, and its uracil auxotroph phenotype confirmed. This strain can become the base strain for chromosomal insertion of synthetic and heterologous genes using plasmids which couple the repair of *pyrF* and alleviation of uracil auxotrophy to the insertion of foreign DNA. A lactate dehydrogenase gene from *Thermoanaerobacter pseudethanolicus* was inserted into the chromosome of *M. thermoacetica* under control of a native promoter as a proof of concept experiment, and synthesis of lactate was observed in the organism for the first time. When grown on basal media supplemented with fructose, lactate concentrations of 6.8 mM were observed in batch culture, whereas the wild type organism was unable to produce a detectable concentration.

Subsequently, a novel strain of *M. thermoacetica* (Y72) was isolated [102], and its frequency of transformation was shown to be approximately 20 times that of the ATCC 39073 strain, hypothesized to be because of the reduced number of copies of the native restriction–modification system. More recently, a novel thermostable kanamycin resistance marker (kan^R), derived from a plasmid harbored by *Streptococcus faecalis*, was shown to be functional within *M. thermoacetica* [103], further expanding the rapidly growing genetic toolkit available for those wishing to develop a thermophilic acetogenic platform.

A further method likely to figure prominently in acetogens in the coming years is a method, now termed Allele-Coupled Exchange (ACE), which allows the rapid insertion of heterologous DNA of any size or complexity into the genome [104]. The system is designed so that, following integration of the plasmid by single-crossover recombination, the desired second recombination event leads to a plasmid-borne allele becoming 'coupled' to a genome-located allele, and the creation of a new selectable allele that facilitates the isolation of double-crossover cells. The order of recombination events is dictated by the use of highly asymmetric homology arms. A long homology arm (e.g., 1,200 base pairs) directs the first recombination event (plasmid integration) and a much shorter homology arm (e.g., 300 base pairs) directs the second recombination event (plasmid excision). A number of different genetic loci may be used to insert heterologous DNA via ACE. One of the most useful exemplifications of the method exploits the native *pyrE* gene. During the procedure this gene is inactivated by replacement of the wild-type allele with a mutant allele lacking approximately 300 base pairs from the 3′ end of *pyrE*. The *pyrE* gene encodes orotate phosphoribosyltransferase, which, in common with PyrF, is an enzyme involved in pyrimidine biosynthesis. One of its most useful features is that it can be used as both a positive and a negative selection marker. This is because the presence of a functional allele is essential in the absence

of exogenous uracil, whereas the presence of a non-functional allele renders cells sensitive to 5-fluoroorotate (FOA). Accordingly, a heterologous *pyrE* gene can be used as a counter-selection marker in a *pyrE* minus background in an equivalent manner to *pyrF* [105]. Its use as a counter-selection marker was demonstrated in two different strains of *C. difficile* using a heterologous *pyrE* allele from *Clostridium sporogenes* [106]. Crucially, however, the design of the created uracil auxotroph strain is such that its mutant *pyrE* allele can be rapidly restored (2 days in the case of *C. difficile*) to wild-type using an appropriate ACE correction vector. This allows any specific in-frame deletion mutant made to be characterized in a clean, wild-type background. Furthermore, this facility provides the parallel opportunity to complement the mutant at an appropriate gene dosage through insertion of a wild-type copy of the inactivated gene, under the control of either its native promoter or the strong P_{fdx} promoter (derived from the ferredoxin gene of *C. sporogenes*), concomitant with restoration of the *pyrE* allele back to wild-type [106]. The suite of ACE vectors needed for the manipulation of the genomes of *C. ljungdahlii* and *C. autoethanogenum* have now been assembled and exemplified in both acetogens [unpublished data].

5 Fermentation

5.1 Fermentation Overview and Routes

Gas fermentations are fundamentally different from sugar fermentations in that the gaseous substrate has to be supplied continuously at high rates, and cannot be added to the media before the start of a fermentation run. As such, gas fermentations are most suitable as fed-batch or continuous process, whereas sugar fermentations are typically operated as batch or fed-batch processes. Continuous sugar fermentations are typically hampered by contamination problems, with other microorganisms thriving on the sugar substrate. Given that only a few organisms can effectively grow on one-carbon substrates and CO is toxic or at least inhibitory to most microorganisms, the threat of microbial contamination does not pose as great a limitation for gas fermentations. The product spectrum of gas fermentations is dictated by some degree by which substrate combination is used.

5.1.1 CO, CO + H_2, and CO/CO_2 + H_2

Most gas fermentation work to date has been carried out on CO-containing gas streams. The reduced substrate CO acts as both carbon and energy source, thus providing sufficient energy to synthesize even reduced products such as ethanol, butanol, 2,3-butanediol, or isopropanol.

5.1.2 $CO_2 + H_2$

In contrast to CO, CO_2 can only act as carbon source but not as energy source, and H_2 is required for fixation of CO_2. Most reports on fermentations with CO_2 and H_2 describe acetic acid as sole fermentation product, but production of ethanol [89] or other products such as acetone [9] has also been described.

5.1.3 Microbial Electrosynthesis (MES)

CO_2 fixation has also been demonstrated in the absence of hydrogen when an electric current is supplied. In this so-called microbial electrosynthesis (MES) concept, the bacteria grow on a cathode. This has been shown for several acetogenic species including *C. ljungdahlii*, *C. aceticum*, *M. thermoacetica*, and two *Sporomusa* species with a high efficiency of over 80% [107]. *Acetobacterium woodii*, which is sodium- rather than proton–dependent, was unable to consume current. There are several excellent reviews that cover all aspects of microbial electrosynthesis in detail [108–111].

5.1.4 Acetogenic Mixotrophy

As a route to very energy intense products (e.g., isoprene) and still having maximized carbon utilization, a concept called acetogenic (anaerobic, non-photosynthetic; ANP) mixotrophy has been proposed where gases and carbohydrates are consumed at the same time [112].

5.1.5 Carboxylic Acid Conversion

Acetogens such as *C. autoethanogenum, C. ljungdahlii*, and *C. ragsdalei* have been demonstrated to convert effectively a range of carboxylic acids as acetic acid, propionic acid, butyric acid, valeric acid, and caproic acid into their respective alcohols in the presence of CO [113–115]. This may be integrated with a carboxylate fermentation platform [116].

5.2 Fermentation Control Parameters and Optimization

Parameters that can be used to monitor gas fermentations differ from those for aerobic fermentations. Although, in aerobic fermentations, dissolved oxygen (dO_2) is a key parameter to monitor and control the process, this cannot be used in gas fermentations because of the lack of readily available technologies for the measurement of dissolved CO and routine indirect assays are arduous. Instead, one

needs to rely on indirect gas measurements to monitor the fermentation in addition to biomass and metabolite as well as oxidation reduction potential (ORP) measurements to track the progress of a fermentation run.

Inlet and outlet gas measurements can give a direct indication of the fermentation status and show whether the microbes are readily utilizing the feed gas. As an example, in a fermentation of CO-rich gas to ethanol and acetate, the CO_2/CO ratio can give an indication of the metabolic outcome of the supplied gas:

$$4CO + 2H_2O \rightarrow 1CH_3COOH + 2CO_2 \quad CO_2/CO\,\text{ratio} = 0.50 \quad - \quad \text{Acetate as a major product}$$

$$6CO + 3H_2O \rightarrow 1CH_3CH_2OH + 4CO_2 \quad CO_2/CO\,\text{ratio} = 0.66 \quad - \quad \text{Ethanol as a major product}$$

Gas supply to the culture can be altered by changing the parameters that control gas to liquid mass transfer, including gas feed rate, liquid agitation rate, or pressure. In addition, typical control parameters such as temperature and pH must be controlled to maintain the state of the fermentation and the metabolite profile.

5.2.1 pH

pH is one of the key parameters that needs to be controlled during a fermentation. Acetogens, as do other organisms, have a pH range in which growth is optimal and the cells are metabolically active. Given the phylogenetically diverse nature of acetogens [117], there are both acetogens that have a low pH optimum and those that prefer a higher pH range (Fig. 2).

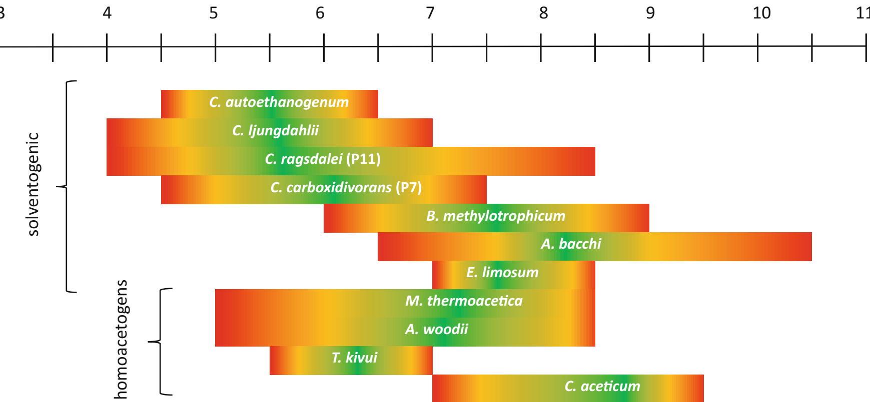

Fig. 2 Reported pH optimum for acetogens considered for industrial applications. *C. autoethanogenum* [50], *C. ljungdahlii* [27], *C. ragsdalei* (P11) [118], *C. carboxidivorans* (P7) [59], *B. methylotrophicum* [38], *A. bacchi* [64], *E. limosum* [119], *A. woodii* [5], *M. thermoacetica* [120], *T. kivui* [61], and *C. aceticum* [6]

Typically, solventogenic acetogens have a lower pH optimum than those that only produce acetic acid (homoacetogens), although this is not always true (see, e.g., *A. bacchi*). At lower pH, acetic acid is more toxic for the cells as more undissociated acid is present which can pass through the membrane and enter into the cell, where it can dissociate again and disrupt the proton gradient across the membrane. The maintenance of this proton gradient is required for energy conservation and several transport mechanisms.

As such, lowering the pH in the medium can lead to a shift from acidogenesis to solventogenesis, allowing increased production of ethanol and other highly reduced products [121, 122]. This was investigated by Gaddy and Clausen using *C. ljungdahlii* growing in a two-stage Continuous Stirred-Tank Reactor (CSTR) system, where the pH of the first reactor was set to pH 5 to promote cell growth and that of the second reactor to pH 4–4.5 to promote ethanol production [123]. A similar strategy was recently also investigated for *C. autoethanogenum* [124] and it has been demonstrated that a set-up with a smaller first stage and a larger second stage could also be a feasible option [125]. In the case of *C. autoethanogenum,* a pH around 4.75 was found optimal for ethanol formation [126, 127], whereas for *C. ragsdalei* a pH below pH 6 was not associated with high ethanol production [128].

Routine and continuous online monitoring the pH trend during the fermentation gives an instant understanding of the state of the fermentation process, as it is an indication of the metabolites the bacteria are producing. For example, a drop in the pH would indicate acetate production. Maintaining a relatively constant pH is important and adjustment of the fermentation pH is therefore critical to avoid a crash.

The pH range of the organism should also be considered when introducing and optimizing fermentation conditions for heterologous enzymes to match the pH optimum best.

5.2.2 Temperature

Temperature is also an important parameter as it influences the microbial activity as well as the gas solubility, which increases with decreasing temperature [128]. Most acetogens are mesophilic that grow best between 30 and 40°C, but there are also thermophilic acetogens such as *M. thermoacetica* [120] or *T. kivui* [61] that grow best between 55 and 75°C.

Lowering the temperature may also help to increase tolerance towards solvents. For *C. ragsdalei* (P11), ethanol production was higher at a temperature of 32°C than at its optimum growth temperature of 37°C [128].

5.2.3 Gas Supply

Both CO and H_2 are not very soluble gases. Although different reactor designs are being developed to address the mass transfer issue (see Sect. 6), most fermentation

development work is carried out either in bottles or in CSTRs. The gas supply in such systems can be increased via the gas feed rate or loading, agitation or shaking, or by pressure.

The partial pressure has a big influence on the microbial growth and metabolism [129]. In a study with *C. carboxidivorans* (P7), an increase in partial pressure of CO (P_{CO}) from 0.35 to 2.0 atm led to a maximum cell concentration, an increase in ethanol production, and a decrease in acetate production [130]. In organisms that are more sensitive to CO, such as *Blautia producta* [131] and *E. limosum* [45], a prolonged doubling time has been observed when the partial pressure of CO was increased. Up to a pressure of 1.6 atm, a linear relationship between the reaction rate and CO partial pressure was observed, but at a pressure of 2.5 atm the culture failed to utilize CO after an initial period of CO uptake [132]. It was hypothesized that this might be because of CO toxicity, caused by insufficient cell concentrations resulting from a failure to keep the reaction at a mass transfer limit stage. Therefore, high CO pressure could be applied once a sufficient cell concentration is achieved. By gradually increasing the pressure applied to a culture, these researchers were able to achieve a CO partial pressure of 10 atm [132].

A model for hydrogen partial pressure (pH_2) for syngas fermentation has been established for *C. ragsdalei* (P11) [133]. In *A. woodii*, the effect of hydrogen partial pressure on CO_2/H_2 fermentation was investigated. It was shown that acetate productivities increased linearly with pH_2 between 400 and 1,700 mbar with a maximal acetate productivity of 1.6 $g_{acetate}/g_{cdw}$/day and a final acetate concentration of 44 g/L after 11 days [134]. In a follow up study, rates up to 147.8 g/L/day has been demonstrated in continuous fermentations at a dilution rate of 0.35/h [135].

5.2.4 Media Formulation

During gas fermentation, acetogens consume CO and $CO_2 + H_2$ as carbon and energy sources. Beside carbon, all bacteria need other elements such as nitrogen, sulfur, phosphorus, trace minerals and metals, vitamins, and reducing agents for synthesis of cell materials and products. Several media optimization studies have been carried out for acetogens including *C. autoethanogenum* [126, 136–138], *C. ljungdahlii* [122], *C. ragsdalei* (P11) [57, 128, 139–144], *C. aceticum* [145], and *Moorella thermoacetica* [146, 147] with the aim of increasing growth and product formation and establishing a defined or least-cost media.

A study by Phillips and Gaddy on *C. ljungdahlii* showed that, by reducing the B vitamin concentration and by eliminating yeast extract, a maximum concentration of 48 and 23 g/L of ethanol could be achieved in a CSTR with and without cell recycling, respectively [122].

Beside vitamins, trace metal concentrations were found to have a significant influence on growth and product formation as many of the enzymes involved in the Wood–Ljungdahl pathway and ethanol formation require metal co-factors including rare metals such as selenium and tungsten [148, 149]. Nickel, for example, is an important co-factor for enzymes such as CO dehydrogenase and acetyl-CoA synthase [150]. The use of nickel has been shown to improve CO uptake and

ethanol production in a variety of acetogens [137]. The effect of various trace metal ions on growth and ethanol production in *C. ragsdalei* was investigated and it has been observed that the removal of Cu^{2+} from the medium and increasing concentrations of Ni^{2+}, Zn^{2+}, SeO_4^{2-}, and WO_4^{2-} had a positive effect on ethanol production [57].

As a low redox potential is required for strict anaerobes to grow, reducing agents such as titanium(III) citrate, cysteine, sodium sulfide, and sodium thioglycolate are commonly added to the fermentation medium and are shown to bring about an increase in solvent formation [127, 140, 151–153]. A study on *C. ragsdalei* showed that addition of methyl viologen promoted solventogenesis, where 1.3 g/L of ethanol was produced compared to 0.51 g/L without the addition of any reducing agent [140].

5.2.5 Inoculum

Inoculum preparation is important to ensure a quick start up and achieve maximum production rates as fast as possible and without a lag phase. For *C. ljungdahlii*, it was shown that pre-adaptation is important and the presence of gas in pre-adapted cultures led to better ethanol overall production [154] and in *C. ragsdalei* (P11) a positive effect on ethanol production was observed from heat shocking the cells prior to inoculation [155].

6 Mass Transfer and Reactor Optimization

Mass transfer is a major challenge in gas fermentation. Transferring the gaseous substrate to the reaction site in the cell is complex and involves a series of resistances at a micro scale: the resistance encountered when the gaseous substrate passes through the gas-liquid interface, during dispersion through the fermentation media, during the diffusion of the gaseous substrate through the microbial membrane, and the intracellular resistance through to the reaction site. The major mass transfer resistance for sparingly soluble gases such as CO, CO_2, and H_2 is encountered when diffusing through the gas–liquid interface [129]. This resistance can be overcome either by increasing the surface to volume ratio of gas bubbles or by reducing the resistance at the gas–liquid interface by minimizing the surface tension [131].

Surface tension can be reduced by addition of chemicals such as detergents, surfactants, solvents, or polymers [131, 156], and several studies have demonstrated that mass transfer can be enhanced by addition of functionalized nanoparticles or catalysts that can absorb and then release the CO to the fermentation broth [157, 158].

In addition, a variety of bioreactor configurations have been investigated to address these challenges. Techniques used in different reactor configurations to enhance mass transfer include optimizing pressure, fluid flow rates and patterns, the

use of microbubbles, and the use of various impeller designs to facilitate shearing and break-up of gas bubbles. The main types of reactors currently being considered for gas fermentation include Continuous Stirred Tank Reactors (CSTR), Trickle Bed Reactors (TBR), Bubble Column Reactors (BCR), Membrane Bioreactors (MBR), and Moving Bed Biofilm Reactors (MMSB), which are discussed in detail below.

One main objective of bioreactor optimization is to enhance mass transfer rates, at the same time lowering operational costs to allow the process to be scaled up to commercially viable production levels. Therefore, the performance of a reactor design should be measured based on the volumetric mass transfer coefficient per unit power input (k_La/P_g). Several studies have modeled and compared performance of these reactor types for gas fermentation [132, 159–161] and a few studies have also explored use of a combination of different reactor types [125].

Further improvements can come from cell recycling to increase the number of cells in a reactor and gas recycling to utilize gas most efficiently. Cell recycling has been shown to be effective for increasing ethanol concentrations in gas fermentations [122], but also adds cost to the process, although gas recycling can increase the gas retention time and utilization efficiency [125].

6.1 Continuous Stirred-Tank Reactors (CSTR)

The CSTR uses a rotating impeller to break up gas bubbles, thus reducing the volume of individual bubbles and increasing the overall surface area of bubbles (the gas–liquid interfacial area). CSTRs are the most extensively used reactor type in gas fermentation. Although many studies have reported higher cell concentrations and product yield with increase in impeller speed, the high input of energy per unit volume in these reactors makes them economically challenging for large scale production processes.

6.2 Trickle Bed Reactors (TBR)

Trickle bed reactors are columns packed with inert packing material and fed with gas streams and media in either concurrent or counter flow configurations. Gas flow rate, liquid recirculation rate, and the packing material size are the main factors that affect mass transfer rates in TBRs.

6.3 Bubble Column Reactors (BCR)

BCRs employ gas sparging without mechanical agitation to achieve mass transfer. Because of the comparatively low capital and running costs associated with the

operation of a BCR, these reactors are considered to be promising candidates for commercial scale operation of gas fermentation reactions. However, the conversion efficiency of the gas substrate is low in BCRs because of the short gas retention times.

6.4 Membrane Bioreactors (MBR) and Moving Bed Biofilm Reactors (MMSB)

MBRs are a class of reactors that employs membranes to facilitate the formation of a biofilm. A subclass of MBRs known as Modular Membrane Supported Bioreactors (MMSB) consists of multiple modules of hollow fibers (also known as Hollow Fiber membrane Reactors—HFR) made up of microporous or non-porous membranes. The substrate gases are introduced into the hollow compartments of the fibers and the microbial cells are attached to the outer surface of the membrane. These fibers are then immersed in growth media and contained within an outer shell. Because of their large surface area to volume ratio, MBRs have very efficient mass transfer rates, but a major disadvantage in this type of reactors is a phenomenon called pore wetting. This occurs when the media in contact with the outer surface of the hollow fibers enter into the lumen through the membrane because of a pressure drop within the fibers. This may be overcome by incorporating a liquid-impermeable layer, such as silicone coating, onto the membranes, stopping the liquid media from entering the fibers even when the inside pressure drops. Another disadvantage is that the cells first need to be immobilized.

7 Scale-Up and Commercialization

Most of the studies reported in the scientific literature were carried out on bench-top/lab-scale bioreactors which were less than 10 L in volume, with exception of a study with *C. ragsdalei* (P11) in a 100-L pilot scale fermenter fed by a gasifier at the Oklahoma State University [162]. In addition, three companies—INEOS Bio, Coskata, and LanzaTech—are operating gas fermentations at a larger scale and are working on commercialization of this new technology.

7.1 Process Integration

Several things need to be considered when scaling up a gas fermentation process. From integration with gas sources, through efficient reactor design (as discussed in Sect. 6), to integration with downstream processes as distillation or other separation

technologies and the use of process water and bulk chemicals as well as water recycling.

A wide range of readily available gas sources can be considered as feedstock for gas fermentation, such as industrial waste gases such as off-gases from steel mills (>1.4 billion metric tonnes/year) or ferroalloys that are mainly composed of CO, reformed methane (biogas or natural gas; >180 Tera m^3/year that is mainly composed of CO and H_2), or syngas (composed of varying concentrations of CO, H_2, and CO_2) from biomass (>1.3 billion metric tonnes/year in the US only) or municipal solid waste MSW (>2 billion metric tonnes/year). These often contain trace amount of impurities such as different sulfur species (H_2S, SO_2, SO_x, COS), nitrogen species (NH_3, NO_x), BTEX species (benzene, toluene, ethylbenzene, xylenes), methane, HCl, HCN, acetylene, naphthalene, phenol, light hydrocarbons, metal species (arsenic, vanadium, bromide, copper, iodide, chromium), and tar [163, 164]. Although acetogenic bacteria are generally much more tolerant to such impurities in the gases than chemical catalysts and can even utilize some of these impurities, such as certain sulfur, nitrogen, and metal species [165–167], it is important to track these and monitor the productivity of the fermentation process in response to contaminants in the gas streams. If certain impurities in the feed gas are present in too high concentrations, they have been shown to cause reduced cell growth, lower production rates, and even cell dormancy [168, 169].

Impurities such as NO_x and acetylene are known to be potent irreversible inhibitors of hydrogenase enzyme activity [170, 171]. Any inhibition of the hydrogenase activity thus results in cells obtaining electrons from CO rather than H_2, leading to reduced availability of CO as a carbon source for ethanol formation. CO itself is also known to be a competitive inhibitor of hydrogenase and it has been shown that in *B. methylotrophicum* the utilization of H_2 is inhibited until CO is exhausted [43]. CO inhibition has also been investigated for the Hyt hydrogenase of *C. autoethanogenum*; the K_i for reduction of CO_2 to formate was 0.3% CO [172].

Recent studies with *C. carboxidivorans* have shown the effects of inhibitors can be mitigated by cleaning the syngas using gas scrubbers or cyclones and a filter prior to introduction into the fermenter [169].

7.2 Commercial Projects

INEOS Bio, Coskata, and LanzaTech have all operated pilot and demonstration plants for extended periods of time and INEOS Bio and LanzaTech are currently scaling up their processes to a commercial scale.

INEOS Bio [173], a subsidiary of major chemical company INEOS (which acquired technology developed by gas fermentation pioneer James L. Gaddy of the University of Arkansas in Fayetteville in 2008), has built an 8 million gallons/year semi-commercial facility in Vero Beach, FL operated as New Plant Energy (NPE) Holding, LLC [174]. Construction of the $130 million project was completed in 2012 and, after commissioning, INEOS Bio declared mid-2013 that the

plant was online and producing ethanol [175]. The facility uses MSW and generates 6 MW of electrical power. By the end of 2014 there had been reports and a statement from INEOS about problems with impurities such as HCN that were negatively impacting operations, and the commissioning of new equipment to address this problem [176].

LanzaTech [177], a start-up founded in Auckland, New Zealand in 2005 with its global headquarters in Chicago, IL, successfully operated a 100,000-gallon/year pre-commercial plant at one of Baosteel's steel mills outside Shanghai, China in 2012. Using steel-making off-gases as substrate for the fermentation process, all productivity expectations were exceeded and all commercial milestones achieved [178]. In 2013, the company operated a second 100,000-gallon/year pre-commercial plant at a Shougang Steel mill near Beijing, China. LanzaTech's process using steel mill waste gases at this facility has been certified by the Roundtable on Sustainable Biomaterials (RSB) [179]. In April 2015, China Steel Corporation out of Taiwan approved investment in a full LanzaTech commercial project. A 50,000 metric tonnes (17 million gallons)/year facility is planned for construction in Q4 2015, with the intention to scale up to a 100,000 metric tonnes (34 million gallons)/year commercial unit thereafter [180]. In July 2015, the company announced a second commercial project in partnership with ArcelorMittal, the world's leading steel and mining company, and Primetals Technologies, a leading technology and service provider to the iron and steel industry. The 47,000-MT/year facility is to be built at ArcelorMittal's flagship steel plant in Ghent, Belgium, is anticipated to commence later in 2017, with bioethanol production expected to start 2018. The intention is to construct further plants across ArcelorMittal's operations. If scaled up to its full potential in Europe, the technology could enable the production of around 500,000 MT of bioethanol a year [181]. Although the initial product focus is to be industrial ethanol and gasoline additives, plans are for increased product diversity utilizing LanzaTech's unique microbial capability. One example the company is working on is to produce jet fuel and a first demonstration flight in partnership with Virgin Atlantic and HSBC is being prepared [182]. Together with the world's largest nylon producer Invista [183] and Korean energy and petrochemical company SK innovation [184], the company is working on new processes for the production of nylon and rubber precursor butadiene [185] and also has an agreement with major chemical company Evonik Industries for development of precursors to speciality plastics [186]. Evonik has recently announced the first successful production of PLEXIGLAS® precursor 2-hydroxyisobutyric acid from syngas [187].

Although Coskata [188], a start-up founded in 2006 in Warrenville, IL, has not yet announced any commercial project, the company has successfully operated a 40,000-gallon ethanol/year semi-commercial facility in Madison, PA over a 2-year period [189] and have recently announced that Elekeiroz, a Brazilian chemical company, has acquired technology rights on their butanol production processes [190].

7.3 Barriers to Market

Much of today's legislation was written prior to the development of gas fermentation technologies and does not provide a clear framework for fuels produced from bacterial biomass through recycling waste carbon gases, such as those generated in the process of steel making [191]. Below, an overview is provided of some of the most relevant legislative framework.

7.3.1 European Union (EU) Waste Framework Directive 2008/98/EC (WFD)

This legislation is currently being transposed into member state law, and a proposal to revise the directive is pending withdrawal by the EU commission services. The current definition of waste in article 2(a) excludes gaseous effluents emitted into the atmosphere. The narrow scope of this definition does not allow for innovative solutions such as gas fermentation for fuel production from these gas emissions to benefit from advantages of recycling mentioned in the directive. CO/CO_2 is valuable waste for CO_2 reuse industries and, by including it into the waste definition, solutions such as carbon recycling can benefit from the waste hierarchy where prevention, reuse, and recycling are top priority. CO_2 reuse technologies prevent pollution and at the same time reuse and recycling the carbon, so they fulfill key elements from the waste hierarchy.

7.3.2 Industrial Emissions Directive (IED)

The Industrial Emissions Directive (IED) has superseded the Waste Incineration Directive (WID) of 2000. It is intended to achieve a high level of protection for the environment as a whole from the harmful effects of industrial processes by applying the Best Available Techniques (BAT). Gas fermentation technologies should be recognized as such by offering an alternative to incineration of wastes, flaring of gases, or combustion for power generation at a steel mill.

7.3.3 European Union (EU) Carbon Capture and Storage Directive 2009/31/EC

To date, the CCS Directive from 2009 and the renewed strategy focus greatly on CCS, and carbon capture and utilization (CCU) technologies are becoming a reality. Therefore, any future CCS frameworks should also include and help the roll-out of CCU technologies in Europe.

A technology neutral approach is needed to provide a clear legislative framework for gas fermentation technologies in Europe today. Technologies should be

qualified by sustainability results, for example by life-cycle assessment (LCA) data and environmental impact on land resources and biodiversity such as a recent report by E4 Tech and Ecofys that compared sustainability implications of different new routes to low carbon fuels [192].

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Anaerobic Digestion

Jan Liebetrau, Heike Sträuber, Jörg Kretzschmar, Velina Denysenko, and Michael Nelles

Abstract The term anaerobic digestion usually refers to the microbial conversion of organic material to biogas, which mainly consists of methane and carbon dioxide. The technical application of the naturally-occurring process is used to provide a renewable energy carrier and – as the substrate is often waste material – to reduce the organic matter content of the substrate prior to disposal.

Applications can be found in sewage sludge treatment, the treatment of industrial and municipal solid wastes and wastewaters (including landfill gas utilization), and the conversion of agricultural residues and energy crops.

For biorefinery concepts, the anaerobic digestion (AD) process is, on the one hand, an option to treat organic residues from other production processes. Concomitant effects are the reduction of organic carbon within the treated substance, the conversion of nitrogen and sulfur components, and the production of an energy-rich gas – the biogas. On the other hand, the multistep conversion of complex organic material offers the possibility of interrupting the conversion chain and locking out intermediates for utilization as basic material within the chemical industry.

Keywords Anaerobic digestion, Biogas, Biomass, Biomethanation, Renewable energy

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1 Process Technologies: Status and Perspectives

There are numerous technologies used for the treatment of organic material. The reasons for the diversity of technologies are the different substrate characteristics and different legislative and economic conditions in the various countries.

The technologies have been developing since the beginning of the twentieth century when the process was used for the first time in the treatment of sewage and sewage sludge. However, with recent initiatives in several countries to support the development of renewable energy provision, the number of plants has been rising substantially, particularly within the solid waste treatment sector and the agricultural sector, where mainly manure and energy crops are used as substrates.

Because AD is a natural process carried out by microorganisms, the technical applications aim at the optimization of the physical and chemical conditions to obtain maximum microbial activity and consequently maximum substrate conversion rates. Besides obvious process parameters influencing the activity of microorganisms, such as temperature, pH value, the presence of inhibitory and toxic substances, the availability of nutrients and trace elements, material handling, and mass transfer between substrate and microorganism play a crucial role when selecting the proper technology. Consequently, the most important substrate characteristics for the selection of the appropriate process technology are the water content, the content of particulate matter, and the content of impurities within the digestion medium. There are several pre-treatment options (maceration, removal of disturbing material, thermal treatment, supplementation of additives) for adjusting the substrate to the requirements of the microorganisms and the technology. However, as every additional treatment step adds technical effort and thus cost, the process should be kept as simple as possible.

According to the total solids content of the digestion medium, the technologies can be classified as shown in Table 1.

Table 1 Classification of AD technologies according to the total solids content of the digestate

Total solids content	Process technology	Substrates applied
Wastewater (content of particulate matter <1%)	Systems with biomass retention: Fixed bed, Upflow anaerobic sludge blanket (UASB), Expanded granular sludge blanket (EGSB)	Sewage and industrial wastewater
Content of total solids in the digester 12–15% max.	Continuous stirred tank reactor (CSTR)	Sewage sludge, manure, energy crops, solid waste (conditioning necessary)
Content of total solids in the digester 20–30% max.	Plug flow	Solid manure, energy crops, solid waste (conditioning necessary)
Content of total solids 30–40% max, structure necessary for leach bed process necessary.	Leach-bed systems (garage style digester)	Energy crops, solid waste (stable structure for percolation necessary)

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The necessary microbial population in the continuously operated digesters is self-establishing and self-stabilizing as long as conditions such as pH, temperature, availability of trace elements, nutrients, carbon/nitrogen ratio, and content of inhibitory substances are within the optimal range (see also Sect. 3). Process start-up can be accelerated by using a culture from another biogas facility as an inoculum.

Because the solubility of methane in water is very low, the methane separates easily from the digester medium. It is normally used for electricity and heat provision. For this purpose the gas can be converted in a combined heat and power unit or a biogas boiler after conditioning. If necessary, the biogas can be converted into a substitute of natural gas by means of an upgrading process. The product of the upgrading can achieve the standards of natural gas grids. Consequently, the produced “biomethane” can be used in all applications of natural gas and is transportable and storable within the natural gas grid.

IRENA [1] states the combined 2014 installed electrical capacity of biogas plants worldwide is 12.67 GW (gigawatts). The countries with the largest contribution are Germany (4 GW), United States (2.35 GW), Italy (1.3 GW), United

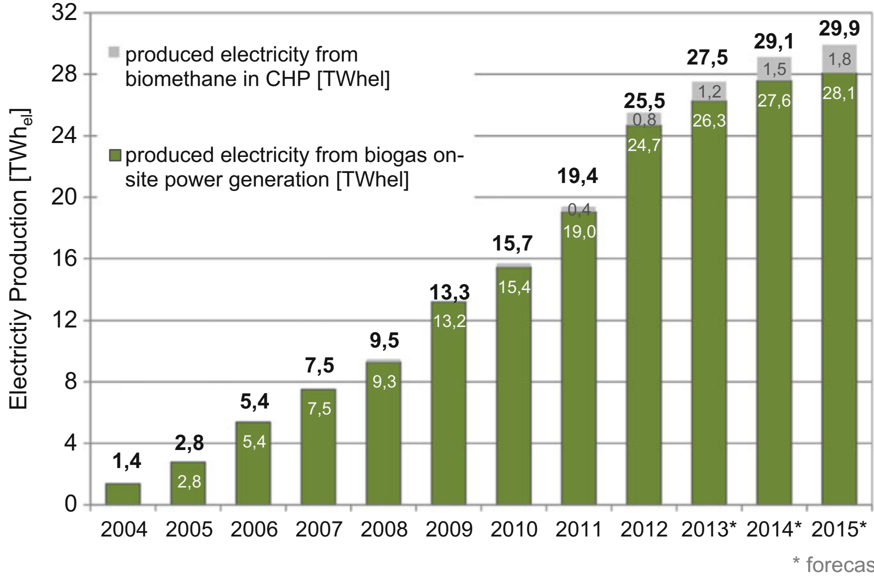

Fig. 1 Development of electricity generation from biogas (on-site conversion) and biomethane in Germany in accordance with the EEG 2009–2015 (source: DBFZ database); CHP combined heat and power

Kingdom (1.2 GW), and Czech Republic (0.38 GW). Europe is listed with an installed capacity of 8.8 GW. It should be mentioned that these numbers might differ if taken from other sources. EBA (2015), for instance, states for 2014 that there is approximately 8.3 GW installed capacity in Europe and a total number of 17,240 plants. The electricity produced is given as 63.6 TWh (terawatt hours).

Figure 1 presents the development of electricity production from biogas and biomethane in Germany since 2004.

The development of the technology in Germany has been driven by the tariffs for renewable energy resulting from the Renewable Energy Sources Act. The substrates for the 7,800 biogas plants in 2014 [2] are mainly provided from the agricultural sector. When the tariff for crop-based energy was launched for the first time, the German agricultural sector had around 1 million ha of land that were not in use. Because of the increasing production of energy crops, this is now not the case anymore. In comparison, by the end of 2014 only around 140 plants mainly based on biowaste (municipal and industrial organic wastes, excluding agricultural residues such as manure) were in operation in Germany.

The long-term guaranteed and cost oriented tariffs for electricity production allowed the plants to purchase substrates on the market for agricultural products, so-called energy crops. The most successful energy crop in Germany has been maize silage. This success is caused by the experience of the farmers with this substrate, its easy storability and preservation, and, last but not least, the already existing market for the substrate. In spite of the boom within the biogas sector and

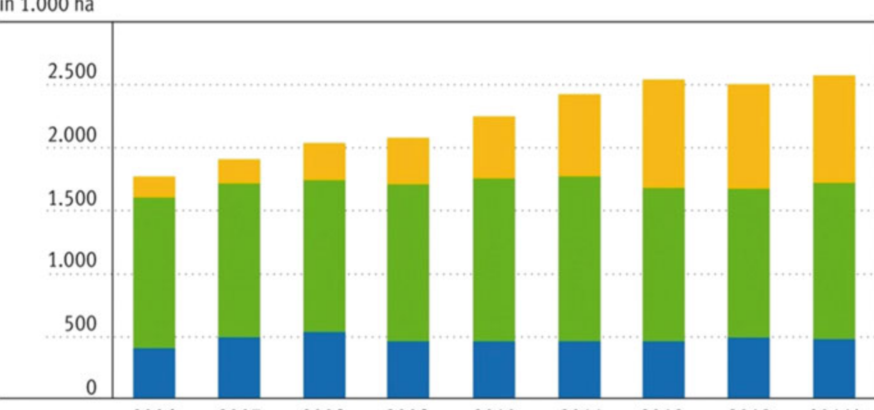

Fig. 2 Use of maize within the agricultural sector; *blue*: grain maize, *green*: maize silage (animal feed), *orange*: maize silage (biogas); * prognosis [3]

resulting demand on maize silage, two-thirds of the maize silage produced in Germany is used as fodder within the animal husbandry industry [3] (Fig. 2).

Another reason for the success of maize silage lays in the fact that there is so far no substrate as efficient as maize when looking at the economics and the energy content to be obtained per hectare. The use of alternatives as rye silage, sugar beet silage, waste, and straw has been becoming more popular during recent years because of limitations for the use of maize silage in the Renewable Energy Sources Act. In some regions the biogas industry has caused conflicts since the plants are competitors for husbandry with regard to substrates (maize silage) and land (for the distribution of manure or digestate). Besides the regions with a high density of animal husbandry, the decentralized structure of the plants allowed in most regions a reconcilable development of the industry (Figs. 3 and 4).

It should be mentioned that the utilization of residues such as straw, manure, or wastes is usually economically compromised by a more extensive technical effort (e.g. necessary pre-treatment) and a lower quality of substrate and consequently lower gas yield of the substrates. In the case of wastes, the legal requirements for plant construction and operation additionally result in greater technical effort and lead to higher overall costs. Additional requirements might include an enclosed receiving area with air collection, a pretreatment system, or a hygienization step for the waste material, as well as more detailed reporting and monitoring of the material streams.

Consequently, the utilization of residues or wastes certainly does not result in lower energy production costs.

Additionally, the often mentioned substrate alternatives such as waste or agricultural residues are not available in the same order of magnitude and underly other effects in the market, which might influence prices and availability if the demand for such residues rises. However, in any future development, the availability of

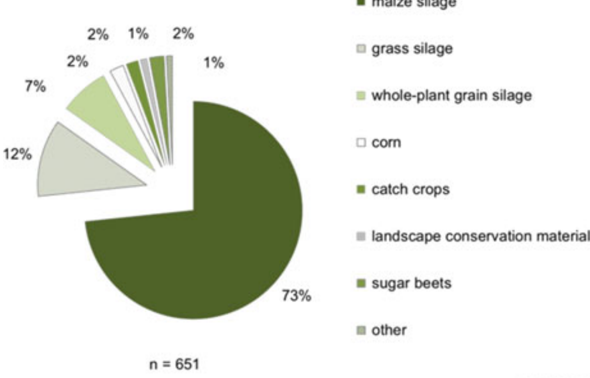

Fig. 3 Energy crops for biogas production referring to the mass content (biogas plant operators' survey of DBFZ in 2015, basic year 2014) [2]

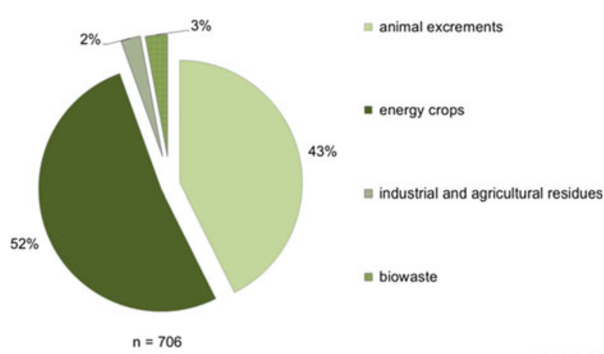

Fig. 4 Substrates for biogas production referring to the mass content (biogas plant operators' survey of DBFZ in 2015, basic year 2014) [2]

agricultural products needs to be compatible with the demand for food products and sustainability criteria. Additionally, the development of the bioeconomy and the subsequent reallocation of organic material have to be considered in future perspectives.

In the current discussion, the provision of electricity based on biogas processes is moving from a steady state operation with a constant output toward a flexible demand-oriented operation. Future energy concepts need to align the highly fluctuating input from wind power and photovoltaic plants with the demand of the consumers. Therefore, a change in the electricity market design is necessary and every producer and consumer needs to contribute to balanced production and consumption. Energy crop based biogas plants can control their output to a large extent and are a valuable element in the future energy system.

The biogas process also offers the possibility of combinations with other processes that might help to amplify the flexibility and the contribution to grid stabilization and service security. Such options include power-to-gas and power-to-heat applications as well as the upgrading of biogas to biomethane.

Future perspectives for biogas applications could include an integration into bioeconomy processes and extensive integration into local energy and material

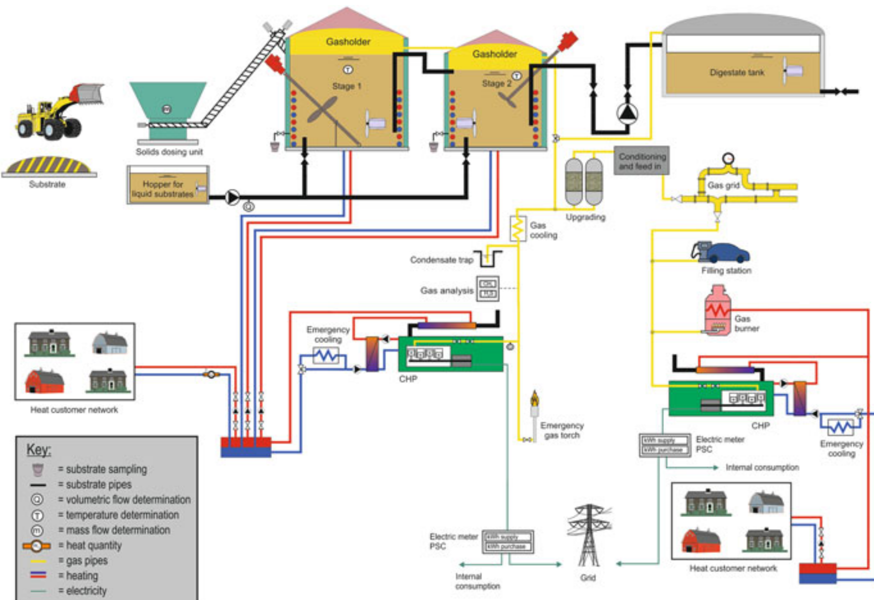

Fig. 5 Overview of technical options of an agricultural biogas plant (DBFZ)

infrastructures. In particular, the last point requires highly controllable and flexible decentral components. Perspectives concerning microbial processing are discussed in the chapter “New approaches – biological methanation and microbial chain elongation.”

2 Case Studies

Biogas facilities aim at a high degree of conversion of organic material into methane, the conversion of the methane into a product (e.g., electricity, heat, or natural gas substitute) and a proper digestate application. Depending on the substrate characteristics, the necessary pre-treatment components, the digestion technology, and the conditioning steps of the products have to be selected. Figure 5 presents the most common technical options within the agricultural sector.

The variations within the potential technologies are as various as the substrates used for AD. Consequently, every plant has a unique and adapted technical concept. This allows a reasonable adaption to the local conditions. However, this also means limited options for standardization and makes it difficult to compare and optimize plant concepts. Figure 6 gives an overview of selected technical variations that are possible within AD plants.

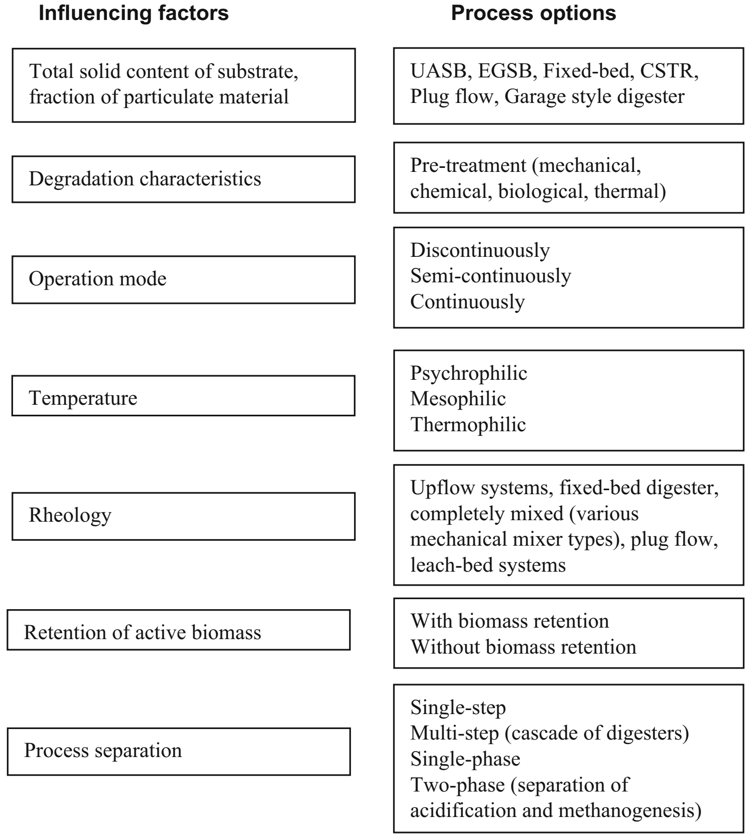

Fig. 6 Overview of technical options for components and operation of biogas plants (DBFZ)

3 Microorganisms

During the AD of organic material, biogas composed mainly of methane and carbon dioxide is produced. Minor components of biogas are water vapor, nitrogen, hydrogen, oxygen, hydrogen sulfide, and ammonia. The AD occurs in four metabolic steps, namely hydrolysis, acidogenesis, acetogenesis, and methanogenesis, catalyzed by a complex network of different microorganisms. To some extent, the first metabolic part (hydrolysis/acidogenesis) can be physically separated from the last part (acetogenesis/methanogenesis), whereas within the respective parts a separation is not possible because of metabolic interdependencies. The separation also makes it possible for alternative usages of anaerobic fermentation processes apart from biogas production. In the following, the metabolic steps are described and alternative options for applications are indicated (Fig. 7).

In the first step, the *hydrolysis*, the substrate, consisting of macromolecules such as carbohydrates and proteins as well as of fats, is cleaved in its oligo-, di-, and

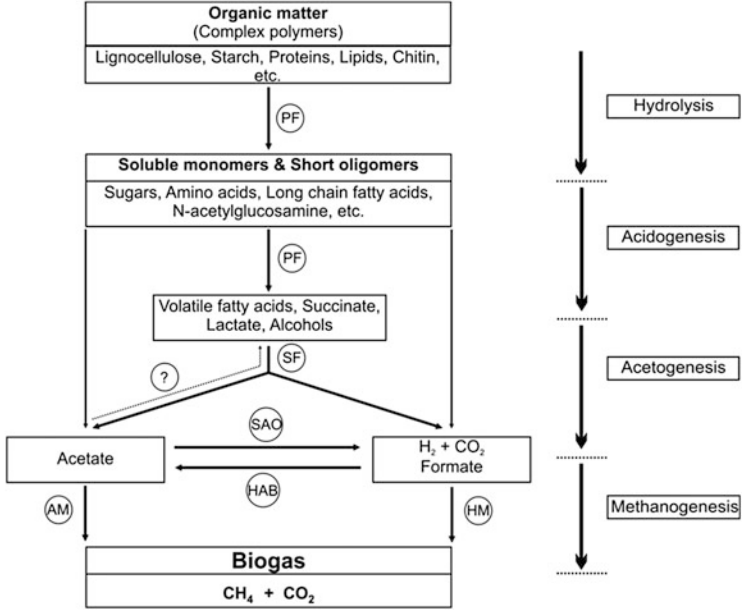

Fig. 7 Anaerobic degradation of biomass to biogas including the major intermediates and trophic groups of microorganisms. *PF* Primary fermenters, *SF* Secondary fermenters, *SAO* Syntrophic acetate oxidizing bacteria, *HAB* Homoacetogenic bacteria, *AM* Acetotrophic methanogens, *HM* Hydrogenotrophic methanogens. Microorganisms and processes involved are still not well-described [4]

monomers by the action of hydrolytic bacteria. Hydrolytic bacteria produce hydrolases, for example, amylases, lipases, proteases, cellulases, and hemicellulases, which are present as exo-enzymes on the outer surface of bacterial cells. Some bacteria excrete large hydrolytic enzyme complexes, for example, cellulosomes, into the environment [5]. The bacterial production of adhesion-proteins supports the bacteria during the hydrolysis [6]. However, as the hydrolysis is not accompanied by any energy gain for the microbial cells, the soluble hydrolytic products, for example, sugars, amino and fatty acids, or glycerol, are taken up by the bacteria and fermented in the second step. When solid substrates such as plant material are used for biogas production, the hydrolysis is the rate-limiting step [7]. In particular, the hydrolysis of cellulose and hemicellulose is comparably slow. When cellulose and hemicellulose are linked to lignin in the lignocellulose complex the hydrolysis is even slower. Lignin decreases the bioavailability of the degradable cellulose and hemicellulose. Anaerobic degradation of lignin is unknown.

The primary fermentation of hydrolysis products is named *acidogenesis* because mainly carboxylic acids are produced. In the biogas process acetic, propionic, and *n*-butyric acid are often observed as metabolites in various concentrations. However, depending on the substrate and process conditions, lactic acid, dicarboxylic (succinic acid) and aromatic acids (phenylacetic acid), and branched and

unbranched medium-chain fatty acids (*n*- and iso-valeric and caproic acid) can also be detected in smaller concentrations. Besides organic acids, alcohols (ethanol, propanol, butanol), hydrogen, and carbon dioxide are produced. The fermentation products do not always result straight from substrate degradation. For example, medium-chain fatty acids can result from ß-oxidation of long-chain fatty acids. However, they can also be obtained from carbohydrate fermentation by means of microbial chain elongation of short-chain fatty acids [8]. Thus, *n*-butyric and *n*-valeric acid can be produced from acetic and propionic acid, respectively, and *n*-caproic and *n*-enanthic acid can be produced from *n*-butyric and *n*-valeric acid, respectively. Altogether, such metabolic ways lead to a meandering of the fermentation during acidogenesis. The diversity of hydrolytic and fermenting microorganisms in AD systems is high. The bacterial community comprises several thousand different facultative and strict anaerobic bacterial genera from the classes Clostridia, Bacteroidia, Gammaproteobacteria, Actinobacteria, and Bacilli.

During the third step of AD, *acetogenesis*, the acidogenesis products are converted, and acetic acid is produced. During the degradation of alcohols and short-chain fatty acids such as propionic and *n*-butyric acid, hydrogen and carbon dioxide as well as C1-compounds are also produced. The acetogenesis can occur only at very low hydrogen partial pressure for thermodynamic reasons. Therefore, acetogenic bacteria live in the immediate vicinity of hydrogen-consuming microorganisms for interspecies hydrogen transfer. Instead of hydrogen, formate can also be transferred from one cell to another. This close relationship between microbial organisms is called syntrophy. Sulfate-reducing bacteria or methanogenic archaea can serve as syntrophic partners of acetogenic bacteria. A specific form of acetogenesis is the synthesis of acetic acid from hydrogen and carbon dioxide, which is also called homoacetogenesis. It is the reverse reaction of syntrophic acetate oxidation that plays an important role in an alternative route for methane production from acetic acid. The diversity of the syntrophic bacteria is much lower than that of the fermenters. Different representatives of *Synergistales*, *Syntrophobacterales*, *Clostridiales,* and *Thermoanaerobacteriales* were often identified in biogas systems.

In the last step of AD, the *methanogenesis*, methane is produced by the action of specialized strict anaerobic microorganisms affiliated to Euryarchaeota. Three different methanogenic pathways, namely the hydrogenotrophic, aceticlastic and methylotrophic way, can be distinguished. At the hydrogenotrophic pathway, carbon dioxide is reduced with hydrogen to methane. Thereby, the methanogens compete with other hydrogen-consuming microorganisms such as sulfate reducers for hydrogen. At the methylotrophic pathway, C1-compounds such as methanol are used as substrates. Acetic acid can be cleaved, resulting in methane and carbon dioxide at the aceticlastic methanogenesis. As an alternative route, acetic acid can be degraded by syntrophic acetate oxidizers. Particularly when protein-rich substrates are digested, acetic acid is converted in this way because of the high sensitivity of aceticlastic methanogens to high ammonia concentrations. Syntrophic acetate oxidizers oxidize the methyl as well as the carboxyl group of acetic acid to carbon dioxide and hydrogen is produced. This reaction is thermodynamically extremely

unfavorable ($\Delta G^{0\prime} = +104.6$ kJ/mol), although the reaction can proceed in combination with the hydrogenotrophic methanogenesis ($\Delta G^{0\prime} = -135.6$ kJ/mol) [9]. The total reaction is exergonic ($\Delta G^{0\prime} = -31.0$ kJ/mol). The preferred methanogenic pathway depends on the respective process conditions in the biogas reactor.

Seven different orders of methanogenic archaea are known, namely *Methanobacteriales*, *Methanococcales*, *Methanocellales*, *Methanomicrobiales*, *Methanosarcinales*, *Methanomassiliicoccales*, and *Methanopyrales*. Most methanogens can perform hydrogenotrophic and methylotrophic methanogenesis, whereas the aceticlastic way is used only from few methanogens. At high and low acetic acid concentrations *Methanosarcina* and *Methanosaeta*, both affiliated to *Methanosarcinales,* perform aceticlastic methanogenesis, respectively. Solely members of *Methanosaetaceae* are known for exclusive aceticlastic methanogenesis.

4 Products

The AD process can function as a method to condition a waste product or to obtain the energy content of an otherwise unused substrate.

The products of an AD process are first the biogas and second the digestate. The biogas usually functions as an energy source. The composition of the biogas depends on the stoichiometric composition of the substrate, the process technology and contaminants within the substrate. Table 2 illustrates the average composition of biogases from different sources in comparison to natural gas.

The biogas is mostly converted in a combined heat and power unit into electricity and heat. In Germany the electricity is remunerated according to the tariffs of the Renewable Energy Sources Act. Besides the combined heat and power (CHP), there are several other options to convert biogas or upgraded biomethane into electricity and heat. Table 3 gives an overview of the technology options.

The heat has no extra tariff within the Renewable Energy Sources Act – at least in the last two amendments of the law (2012 and 2014). However it is considered as the most important option for an additional income for the plant. Within the extensions and reconstruction measures, the addition of heat utilization facilities is the most often implemented component. In general, the existing plants are constantly under optimization and replacement procurement. Figure 8 shows the measures taken according to the survey of the DBFZ, which are mostly connected with optimization of the product utilization.

The exhaust heat from the CHP is first used to keep the process temperature of the plant stable. Depending on their size, agricultural plants need between 18% (larger than 1 MW installed capacity) and 52% (smaller than 75 kW) of the heat provided from the CHP for maintaining the biological process [2].

The excess heat can be used for external purposes. The selection of an appropriate site for the CHP, close to a consumer for the produced heat, is a crucial point for the success of heat utilization. According to the DBFZ plant database, 17% of

Table 2 Ranges of biogas compositions from AD plants, sewage treatment plants, landfill applications, and natural gas [10]

Parameter	Farm-scale AD plant	Centralized AD plant	Landfill	Sewage treatment plant	Natural gas
CH_4 (vol%)	55–60	60–70	35–65	60–65	81–89
Other hydrocarbons (vol%)	0	0	0	0	3.5–9.4
H_2 (vol%)	0	0	0–3	0	–
CO_2 (vol%)	35–40	30–40	25–45	35–40	0.67–1.00
N_2 (vol%)	<1–2	2–6	<1–17	<1–2	0.28–14.00
O_2 (vol%)	<1	0.5–1.6	<1–3	<0.05–0.70	0
H_2S (ppm)	25–30	0–2,000	30–500	<0.5–6,800	0–2.9
NH_3 (ppm)	≈100	≈100	≈5	<1–7	0
Halogenated compounds (mg/m^3)	<0.01	<0.25	0.3–225	0–2	–
Siloxanes (mg/m^3)	<0.03–<0.2	<0.08–<0.5	<0.3–36	<1–400	–
Wobbe index	24–33	24–33	20–25	25–30	44–55
Lower heating value (MJ/Nm^3)	19.7–21.5	21.5–25.1	10.7–23.3	21.5–23.3	31–40

Note: 1 kWh/Nm^3 = 3.6 MJ/Nm^3

the biogas plant operators in Germany have at least one satellite CHP for better heat utilization. The use of biomethane as fuel is the most flexible way regarding location and time of gas utilization. In Germany the incentives for renewable electricity from biomethane require a 100% utilization of excess heat. According to the database of the grid operators (BNetzA) in 2014 there have been 10,700 biogas CHP and 1,200 biomethane CHP registered.

The heat demand profile of the consumer is also of great importance. Most of the conventional heat consumers in the biogas industry are involved with heat provision for houses and barns (Fig. 9). Such processes have a seasonal profile with high demand in winter and low demand in summer, which makes it difficult to use all the excess heat which is produced constantly during the year.

Beside the heat and the electricity, the digestate is a product of the process to consider. Depending on the substrate and the process, it is either further processed to meet requirements of discharge or used as a fertilizer or soil conditioner. The quality of the digestate with regard to the requirements of the subsequent utilization steps is highly dependent on the quality of the input material. The AD process converts organic carbon into methane and carbon dioxide, reduces organic nitrogen to ammonia, and sulfur compounds to hydrogen sulfide. Some organic pollutants or toxic components can be degraded during the AD process. Recalcitrant substances and abiotic hazardous materials such as heavy metals might change their type of bond, i.e. speciation, but are otherwise unaffected by the process. There are some methods to determine the degree of degradation and stabilization of a digestate as degradation of volatile solids, organic carbon, or stability tests as the self-heating

Table 3 Overview of technical options for biogas utilization [10]

Parameter	Engine	Gas turbine	Micro turbine	Stirling engine	Fuel cell
Unit capacity (kW_{el})[a]	110–3,000	3,500–15,000	30–300	<150	300–1,500
Plant size	Small to medium	Large	Small	Small	Small
Electrical efficiency (%)	30–42	25–40	25–30	30–40	40–45
Thermal efficiency (%)[b]	40–50	30–50	30–35	35–40	30–40
Overall system efficiency (%)	70–80	70–75	55–65	65–80	75–80
Power/heat ratio production control	Not possible	Very good	Very good	Very good	Good
Biogas purification requirement	Medium	Medium	Medium	Low–medium	High
Emissions NO_x	High 500–700 mg/Nm^3	Low 25–50 mg/Nm^3 flue gas	Low	Very low	Extremely low (3 mg/Nm^3 flue gas)
Alternative fuel source	Liquid gas	Natural gas	Natural gas, kerosene, fuel oil	Natural gas, fuel oil, biomass	Natural gas
Investment costs (€/kW_{el})[c]	400–1,100	900–1,500	600–1,200	1,300–1,500	3,000–4,000
Operation and maintenance cost (€/kWh)[d]	0.01–0.02	0.005–0.010	0.008–0.015	0.003–0.005	0.003–0.010

[a]Unit capacity varies depending on manufacturer
[b]Heat recovery is estimated as a percentage of fuel input
[c]Installed costs vary with type and amount of auxiliary equipment
[d]Maintenance costs are dependent on gas quality

test for compost. Other than that, quality standards for composts and digestates originating from waste are set by the legislation (Bioabfallverordnung – biowaste ordinance) or the industry (RAL Gütezeichen Kompost Quality Label Compost). Agricultural products and manure are subject to legal requirements of several ordinances, which manage the handling, application, and marketing of these products. For sewage sludge, a separate ordinance is applicable.

In the case where the digestate is applied directly onto agricultural land or is disposed in a landfill (in the case of AD as a treatment for contaminated waste), no further treatment is required. If these options are not available, several post-treatment steps are possible to adjust the digestate to the requirements for further use of the end product.

Most common is the use of a simple solid-liquid separation of the digestate and a subsequent aerobic post-composting process of the solid fraction.

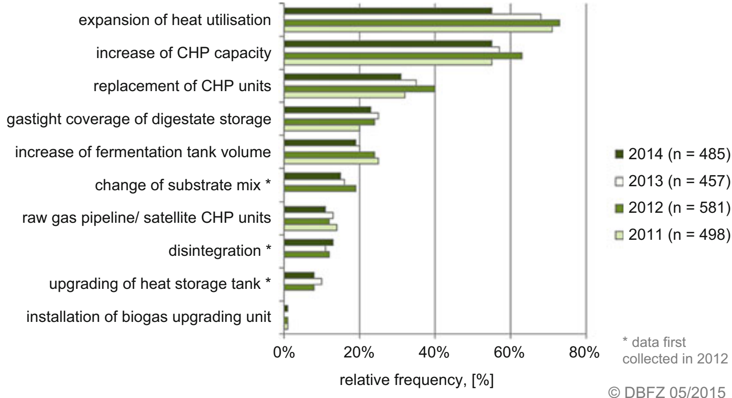

Fig. 8 Measures for plant extension during 2011–2014, (DBFZ, Survey 2011/2012–2015) [2]

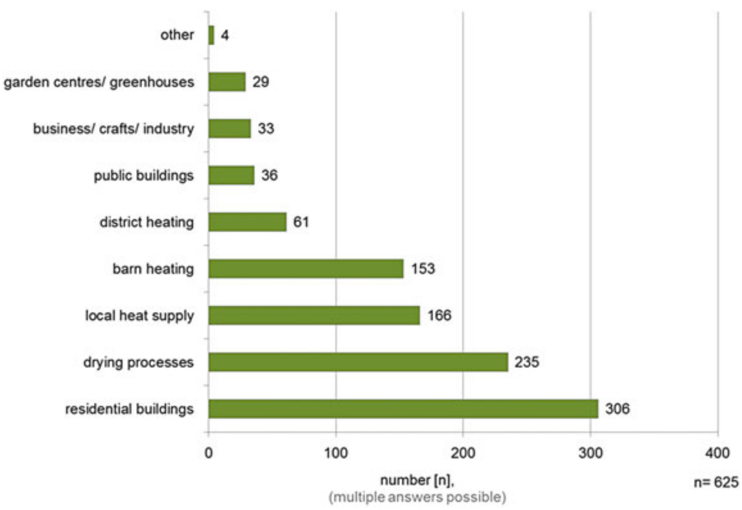

Fig. 9 Types of heat utilization, number of answers (DBFZ, Survey 2015) [2]

Separation is occasionally done with a more sophisticated multistep solid-liquid separation with decreasing pore size (ultrafiltration and reverse osmosis) to get below the thresholds for receiving stream level standards for a discharge into the next surface water system. However, this process requires extensive technical effort and consequently high costs. If post-treatment is necessary, it has a determinant effect on the technical and economic feasibility of the overall process.

However, because the AD processes remove only carbon, sulfur, and traces of nitrogen, the digestate contains all the micro nutrients from the original organic material. The concentrations of nutrients are usually too low to compete with conventional fertilizers (e.g., ammonia concentration of 1–5 g/L) but too high to

find easy discharge opportunities. The fate of digestates is therefore still too often seen as a discharge rather than an application of a valuable fertilizer. There might be change in the future if prices for fertilizers rise.

The perspective for the marketing of products of AD processes in the future might include the separation of intermediates from the process for a specific use.

5 New Approaches: Biological Methanation and Microbial Chain Elongation

The AD process mainly focuses on the biogas production for energetic purposes, exploiting a wide spectrum of organic materials. Within the concept of integrated biorefineries, the AD process is supposed to take part as an end-of-pipe-technology for energetic utilization of process residues by producing a methane-rich biogas. According to the varying composition of the substrates (e.g., share of proteins, fats, and carbohydrates), the methane content in biogas varies between 48% and 73% [11]. For storage and economic reasons, it is of great interest to maximize the methane content up to 100%. Beside conventional biogas upgrading techniques for grid injection (e.g., carbon dioxide removal by pressure swing adsorption, amine and water scrubbers, or membrane separation), there is an approach to increase the methane content of biogas by reducing the residual carbon dioxide in the biogas to methane. This can, in principle, be done either by injection of hydrogen into the fermenter, so-called biological methanation [12], or by direct provision of electrons using electrodes, so-called electromethanogenesis [13].

Either way, the processes can be used to store surplus electricity from the power grid, necessary in a future with a high contribution from renewable sources. In the case of hydrogen injection, the surplus energy is used to produce hydrogen via water electrolysis followed by biological methanation [14]. The methane produced can be stored in the gas grid. This process is called Power-to-Gas or Power-to-Methane. In practice, thermochemical methanation (the Sabatier process) is mainly discussed for Power-to-Gas approaches. The following Sect. (5.1) focuses on the biological process.

Besides increasing the methane yield, sub-steps of the AD process can be used to produce medium- and long-chain fatty acids as platform chemicals for further processing. Therefore, the microbiological process of methanation has to be optimized in such a way that products from acidogenesis can be enriched and further processed.

5.1 Biological Methanation

Biological methanation of carbon dioxide with hydrogen within the AD process is realized via the hydrogenotrophic methanogenesis step uncoupled from aceticlastic and methylotrophic methanogenesis according to the following reaction [9]:

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad \left(\Delta G^{0'} = -135.6\,\mathrm{kJ/mol}\right)$$

According to the stoichiometry of the reaction equation, hydrogen and carbon dioxide have to be provided at a ratio of 4:1 for the production of 100% methane. Concerning "Power-to-Gas" applications, renewable hydrogen can originate from electrochemical water cleavage, consuming wind and sun power, or from anaerobic fermentation processes, and carbon dioxide can be obtained, e.g., from air or biogas processes. Such a process can be performed in a stand-alone biomethanation module containing only hydrogenotrophic methanogens as biocatalysts.

Biological methanation is also thought to be combined with the degradation of organic material with the overall aim of upgrading the biogas. In such a case, pure hydrogen or hydrogen-rich gases, for example, from thermochemical gasification, are injected into the fermenter [15, 16]. Thus the methane content of biogas can be increased until natural gas quality is reached, making the upgrade processes prior to injection into the gas grid less complicated and expensive. When introducing pure hydrogen or hydrogen-rich gases into the AD process, two major facts have to be considered. First, the share of methane in the product gas depends on the amount of available carbon dioxide and the hardly soluble hydrogen in the liquid phase. Second, hydrogen can disturb the acetogenesis step as the amount of dissolved hydrogen influences the kinetics and thermodynamics of propionic and butyric acid degradation to acetate [17]. If the amount of dissolved hydrogen is too high, the acetogenesis can be interrupted and organic acids accumulate in the system until the whole process breaks down (this accounts only for the integration of biological methanation into running AD systems).

5.2 Electromethanogenesis

The term electromethanogenesis describes the ability to produce methane by the use of electroactive microorganisms that gain electrons from a cathode within a so-called microbial electrolysis cell [13, 18]. In principle, this process is similar to hydrogenotrophic methanogenesis with the exception that, instead of hydrogen gas, electrons are provided. Methane is produced from carbon dioxide, electrons, and protons according to the following reaction equation [13]:

$$CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O$$

It is part of ongoing research to identify whether the microorganisms take up electrons directly from the electrode or from intermediately produced hydrogen. Irrespective of that, this process can be integrated into the AD process as the microorganisms at the cathode were amongst others identified as methanogenic Archaea, for example, *Methanobacterium spp*., which occur naturally within the process. Anaerobic digesters with built-in electrodes can therefore perform as microbial electrolysis cells, reducing the residual carbon dioxide in the biogas with electrons from the cathode and protons from the liquid phase to methane. In laboratory scale experiments it has been shown that the carbon dioxide content in biogas could kept below 10% [19].

5.2.1 Microbial Chain Elongation

The extensive product spectrum of the acidogenesis step harbors the potential for its commercial exploitation. Thus, in addition to methane and hydrogen, chemicals can be produced from biomass by anaerobic fermentation. The organic molecules belonging to the carboxylate platform can be further converted to fuels, materials, and chemicals for the chemical and pharmaceutical industries and for the production of cosmetics and food. An example of further processing of medium-chain fatty acids is electrochemical refining to, amongst others, *n*-alkanes using Kolbe and non-Kolbe reactions at the anode. However, when systems with undefined mixed cultures and complex substrates such as plant materials are used, natural mixtures of different carboxylates and alcohols result from anaerobic fermentation. This is a significant drawback because of the challenging extraction of target substances. Applications for the production of chemicals from biomass are therefore limited to processes where side products do not interfere with the further processing of the desired platform chemicals. The main advantage of processes with mixed cultures and complex substrates is that they are comparatively cheap. There is no need for sterilization as is necessary for pure strain systems. Low-cost raw material or even waste can be used instead of processed high-value substrates such as sugars.

Additionally, technical systems that can be used for anaerobic fermentation might be adapted from biogas technology, which is well-developed because of advances in the last decade. This includes the operation of two-stage reactor systems with the separation of the first two metabolic phases, namely hydrolysis and acidogenesis, from the last two phases, namely acetogenesis and methanogenesis. In such systems, typically acidogenic processes dominate in the first-phase reactor, bearing an intrinsic optimization potential for high yields of organic acids.

To obtain anaerobic fermentation products such as carboxylate platform chemicals in considerable concentrations, their microbial degradation by acetogenic and methanogenic processes has to be suppressed. This can be achieved by the addition of chemicals such as 2-bromoethanesulfonic acid or iodoform. However, the addition of such chemicals seems not to be feasible in full scale. Appropriate process conditions such as low pH-values and low retention times are alternatives for an effective inhibition of methane production. Organic acids in higher concentrations affect their bacterial producers. This inhibition is even

stronger at low pH values because mainly the undissociated form of the products has a toxic effect. Therefore, an effective product extraction is indispensable for high yields. Furthermore, fermenting bacteria can often grow faster than acetogenic and methanogenic microorganisms. This can be exploited for a supportive inhibition of methanogenic processes. At low retention times, slow-growing microorganisms are washed out. However, in systems with biomass retention, where biofilm formation otherwise supports AD for biogas production, an undesired methanogenic population is able to develop. In such a system, low retention times have no effect in the long-term, and methanogenic processes must be controlled by other means.

6 Utilization of Residues

Depending on the process, the digestate can be either product for further use or waste for disposal. The AD process converts degradable fractions of the substrates and usually leaves the lignocellulosic fraction of the plant material. Because of its almost recalcitrant nature, the digestate cannot be used as a substrate for other biological processes or as animal feed. Digestate can be used as an organic compound for soil conditioners. The fertilizer value depends on the nutrients coming with the substrate. The occurring concentrations are usually low compared to chemical fertilizers.

There are also approaches for separating the fiber fraction and using it as a substitute for specific wood products.

If the digestate is subjected to a solid-liquid separation, the liquid fraction of the procedure requires the major effort. Usually the liquid fraction is recycled within the process as often as possible, and can be used for application on land in irrigation systems or as a conditioner in post-composting of the digestate. If such options are not applicable, either further solid-liquid separation, drying, or aerobic biological treatment can help to achieve discharge requirements. The biological treatment is not an easy task because of high concentrations of recalcitrant organics and nutrients as ammonia. In the case of external H_2S removal systems (e.g., based on activated carbon) and reactivation of the material impossible, the disposal of the material might become necessary.

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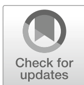
Pyrolysis Oil Biorefinery



Dietrich Meier

Abstract In biorefineries several conversion processes for biomasses may be applied to obtain maximum value from the feed materials. One viable option is the liquefaction of lignocellulosic feedstocks or residues by fast pyrolysis. The conversion technology requires rapid heating of the biomass particles along with rapid cooling of the hot vapors and aerosols. The main product, bio-oil, is obtained in yields of up to 75 wt% on a dry feed basis, together with by-product char and gas which are used within the process to provide the process heat requirements; there are no waste streams other than flue gas and ash. Bio-oils from fast pyrolysis have a great potential to be used as renewable fuel and/or a source for chemical feedstocks. Existing technical reactor designs are presented together with actual examples. Bio-oil characterization and various options for bio-oil upgrading are discussed based on the potential end-use. Existing and potential utilization alternatives for bio-oils are presented with respect to their use for heat and power generation as well as chemical and material use.

Keywords Applications, Bio-oil, Fast pyrolysis, Pyrolysis reactors, Upgrading

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1 Process Technologies: Status and Perspectives

Fast pyrolysis is one of a number of possible conversion routes that can convert biomass into higher-value-added products. As such, this technology could play a role in a biorefinery model to expand the suite of product options available from biomass.

In general, thermochemical processes convert lignocellulosic biomass into liquids, solids, and permanent gases. Pyrolysis is thermal decomposition occurring in the absence of oxygen. The biomass cell wall components cellulose, hemicellulose, and lignin are degraded through cleavage reactions. They react differently depending on the temperature level and duration of reaction, and thus determine the yields of solid, liquid, and gaseous products. The overall yields of the three main groups of products and fundamental reaction parameters are shown in Table 1. Depending on the pyrolysis conditions applied, four procedures can be distinguished: fast (flash), intermediate, slow (higher temperature for carbonization), and slow (lower temperature for torrefaction).

Slow pyrolysis has been practiced for the production of charcoal for thousands of years and is used commercially today. The other methods have been known for some 30 years and are still at the development and testing phase. There are very few commercial examples.

1.1 *Fast Pyrolysis*

1.1.1 Fundamentals

Fast pyrolysis has been under development for about 30 years. Numerous publications and review articles have been published on this topic [1–10]. The basic idea and challenge in fast pyrolysis is to guarantee a rapid heat and mass transfer to prevent further cracking and condensation reactions and thus obtain a high yield of liquid product (also called bio-oil). The essential features of a fast pyrolysis process for producing liquids are:

- Rapid heating of the biomass particles (with heating rates of more than 1,000°C/s). In this case, the pyrolysis temperature should be in the range 450–500°C. Because of the low thermal conductivity of wood (approximately 0.2 W/m/degree K), small particle sizes (generally <3 mm) are required.
- Careful monitoring and control of the reaction temperature.

Table 1 Typical conditions and yields (wt% based on dry biomass) of the main products in the biomass pyrolysis

Process	Conditions	Liquid	Solid	Gas
Fast (Flash)	~500°C, short hot gas residence time 1–3 s	60–70	10–20	10–20
Intermediate	~500°C, hot gas residence time 10–30 s	45–55	~20–30	20–30
Slow (carbonization)	~400°C, long hot gas residence time hours – days	25–35	25–35	25–35
Slow (torrefaction)	~250–290°C, solid residence time 10–60 min	5–15, when condensed	75–85	15–25

- Short residence time of the pyrolysis products in the hot reaction zone (usually <2 s).
- Favoring rapid and complete removal of coke, because the hot coke acts catalytically and favors undesirable cracking reactions of the products to form water and CO_2.
- Rapid cooling of the hot vapors to obtain high yields of bio-oil.

In addition to the operating parameters, the type of biomass (its chemical composition, for example, lignin and ash content) also plays an important role. Ash has a catalytic effect and influence on the reaction mechanism. In general, higher ash contents, for example, in straw have an unfavorable effect on the bio-oil quality, because cracking reactions are favored, resulting in the formation of a two-phase liquid consisting of aqueous and tarry fractions. Bio-oils from wood, however, can be produced as a single-phase homogeneous product.

Bio-oil yields can reach as high as 75 wt%. The by-products coke and gas can be energetically used for the provision of process energy and pre-drying of the biomass as the water content of the feedstock should be <10%. Too high a moisture content would both reduce the heating rate, as the water must evaporate, and it would co-condense and increase the water content in bio-oil, thereby lowering its calorific value and causing undesirable phase separation of the oil.

Regardless of their technical designs, fast pyrolysis processes usually include three important stages (Fig. 1):

- Biomass preparation (storage, handling, pretreatment by screening, and crushing)
- Conversion of solid biomass into liquid product (bio-oil)
- Further processing of this primary product by purification or refining into marketable products such as heat, electricity, biofuels, and chemical products

In contrast to other thermochemical conversion processes, fast pyrolysis provides various possibilities for modification to influence the oil quality (Fig. 1). This includes the selection and preparation of the raw material (ash removal by an acidic wash [11–13], separation of the biomass in biorefineries, and utilization of the lignin fraction [14–17]), as well as changing reaction parameters (use of catalysts

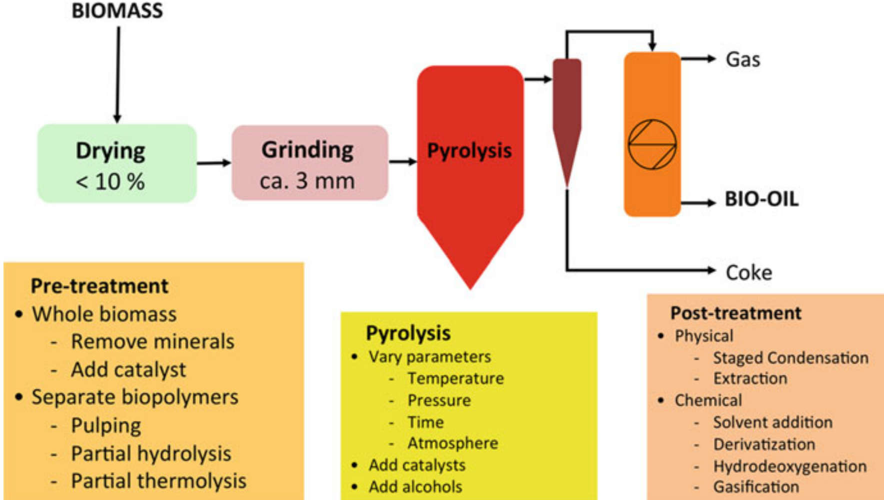

Fig. 1 Basic steps in fast pyrolysis, and ways to affect bio-oil quality through pre-treatment, pyrolysis, and post-treatment

and hydrogen) [18–20]) or treatment of bio-oil (fractional condensation, oxygen removal by hydrotreating, etc. [21–26]).

1.2 Reactor Designs

To realize the requirements of fast pyrolysis, special reactors are necessary to provide a fast and efficient heat transfer and an effective mass transport. For this purpose, reactors are frequently used with sand as heat carrier, which is fluidized either pneumatically (reactors with stationary or circulating fluidized layer) or mechanically by means of rotating elements such as screws or cones. Reactors with fluidized heat carriers require small biomass particles. There are also some other techniques (e.g., reactors with ablative effect, reactors with vacuum) which are used when larger biomass particles are used such as wood or bark chips. Figure 2 shows an overview of the most common techniques and procedures which are presented below and discussed.

1.2.1 Bubbling Fluidized Bed Reactors

Bubbling fluidized bed reactors have the advantage of already being in use at a large scale, for example, in combustion or drying processes [27, 28]. They are also relatively simple in construction and therefore can be operated easily. They have no moving parts, temperature and temperature distribution can be adjusted and

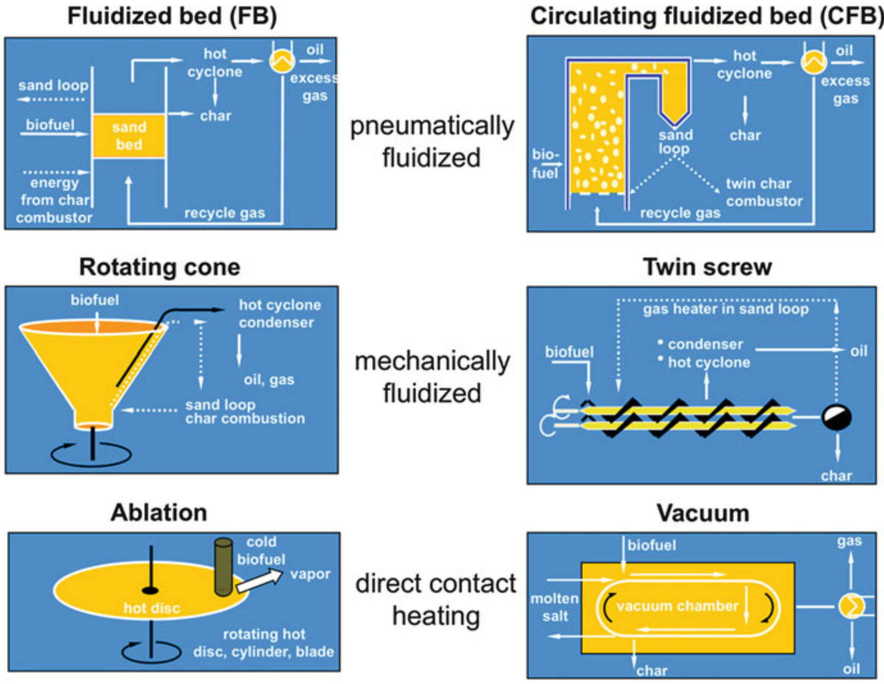

Fig. 2 Schematic designs of reactors for fast pyrolysis of biomass

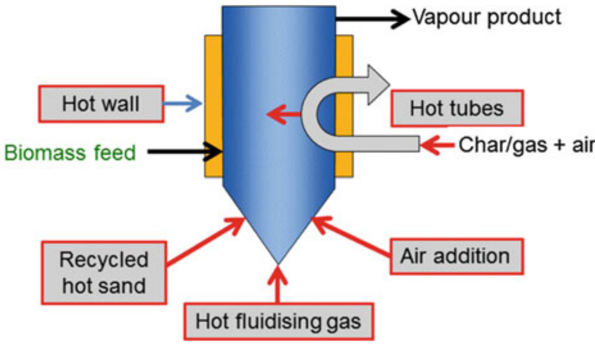

Fig. 3 Methods of heat transfer into stationary fluidized bed reactors [29]

easily controlled, and thus good heat transfer is ensured. The problem lies in the heat input, which can be realized (1) indirectly through the reactor wall, (2) by installing radiant heaters in the sand bed, (3) by supplying hot sand, or (4) using preheated gas with low intake of air-oxygen (Fig. 3).

In fast pyrolysis reactors (bubbling fluidized bed), biomass is thermally decomposed in the 450–500°C hot sand bed. As an example (Fig. 4), a typical pyrolytic decomposition of biomass in such a system is presented.

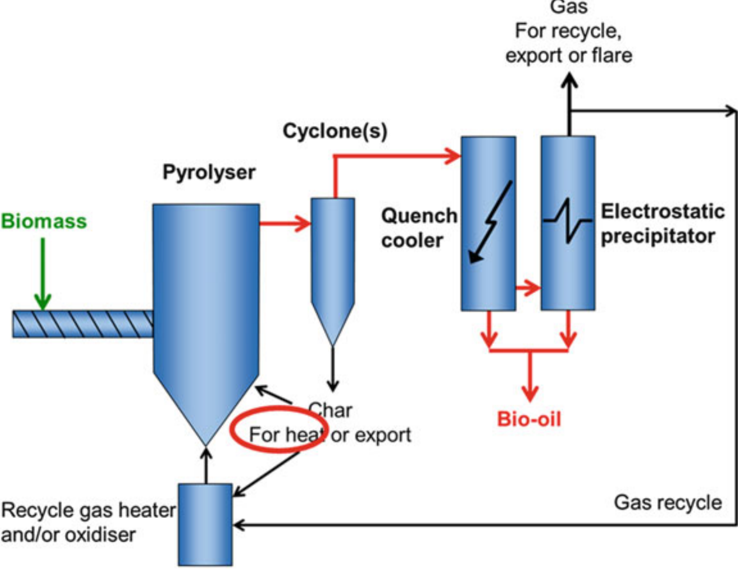

Fig. 4 Components of a complete fast pyrolysis unit with fluidized bed reactor [29]

Dried and chopped biomass is introduced laterally into the hot fluidized bed of sand (see Fig. 4) via a two-stage auger system. In the fluidized bed, the organics are thermally decomposed under exclusion of oxygen. The fluidizing gas is derived from the permanent gases from pyrolysis (gas recycle). The volatile degradation products are discharged, together with the resulting aerosols, with the fluidizing gas at the top of the reactor. Coke particles and entrained sand particles are separated in multi-cyclone systems. The hot gas flow is suddenly cooled to room temperature in jet scrubbers or quenchers. The quench liquid consists of either previously derived pyrolysis oil or a non-miscible hydrocarbon liquid. The condensed oil is collected in a container. The aerosol content of the cold gas stream is passed through one or more electrostatic precipitators, where the remaining oil droplets in the gas phase are collected. A part of the thus purified pyrolysis gas is recycled via a compressor as fluidizing gas into the reactor. As the pyrolysis process in fluidized bed reactors is largely endothermic, heat must be supplied. This can, for example, be done by burning the resulting pyrolysis coke and warming of the recycle gas. The pyrolysis gas, which mainly consists of carbon monoxide (CO), carbon dioxide (CO_2), and methane (CH_4), is also burned and used for pre-drying of the feedstock.

The achievable oil yields (including reaction water) are about 70%, based on dry biomass. The optimum temperature range is 450–500°C [1, 30]. Under optimized pyrolysis conditions the yields shown in Table 2 are achieved.

The largest demonstration plants with stationary fluidized bed reactors were operated by the company Dynamotive, Canada. Their patented Biotherm® process was realized in systems with 100 tons/day biomass throughput (West Lorne,

Table 2 Yields from pyrolytic decomposition of various types of wood in bubbling fluidized bed reactors [31]

	Poplar	Spruce	Maple
Process conditions			
Temperature (°C)	504	500	508
Water content (wt%)	5.2	7.0	5.9
Particle size (mm)	1.0	1.0	1.0
Residence (s)	0.47	0.65	0.47
Yields based on dry wood mass			
Reaction water (wt%)	9.55	11.90	9.60
Coke (wt%)	16.50	12.90	13.45
Oil (anhydrous) (wt%)	62.70	67.40	67.45
Gas (wt%)	11.25	7.80	9.50
Including CO	4.70	3.80	4.10
CO_2	5.90	3.40	4.90
H_2	0.02	0.02	0.01
CH_4	0.44	0.38	0.34
C_2H_4	0.19	0.20	0.15

Canada) and 200 tons/day (Guelph, Canada) [32]. However, because of financial difficulties, the plants have been partially dismantled. Currently, there are no important plants using bubbling fluidized bed technology.

1.2.2 Circulating Fluidized Bed Reactors

In parallel with plants with bubbling fluidized bed technologies for the pyrolytic decomposition of organic substances, circulating fluidized bed reactors (see Fig. 2) have been developed. They have maximum throughput capacities in the range 400–4,000 kg/h [3]. Circulating fluidized beds have the advantage of having extremely high heating rates and short product residence times. Circulating fluidized beds have a lot of similar positive characteristics as stationary fluidized beds. However, the residence time of the coke corresponds to that of the gases and vapors. Furthermore, the sand and coke are subject to greater abrasion, so that separation of solids from the hot gas stream is more difficult and the organic oils may contain more coke [3].

In systems based on circulating fluidized beds (see Fig. 5) the finely ground biomass particles with a particle size of less than 1–3 mm are laterally fed into the circulating fluidized sand bed.

At the same time, the resulting coke and the bed material (sand) is continuously removed from the reactor. In a first cyclone, the sand is separated from the flow of material and recycled to the reactor. The further entrained coke is separated in a second cyclone and burned to provide the heat for the process (e.g., for heating the bed material). The remaining vapors flow into the cooling system where they are condensed with conventional technology (i.e., scrubbers and quenchers) to form the liquid end product.

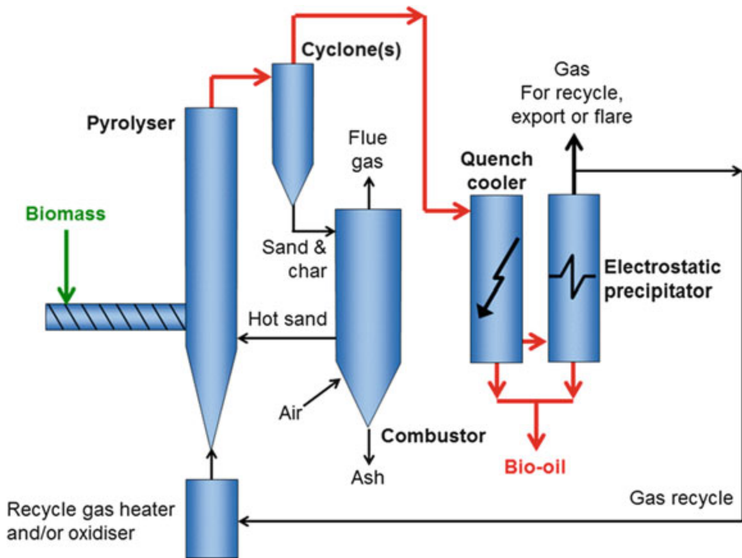

Fig. 5 Components of fast pyrolysis with a circulating fluidized bed reactor [29]

Circulating fluidized bed processes are used on a large scale in Canada and Finland. The Canadian company Ensyn Technologies, Inc. markets its process as "Rapid Thermal Processing® (RTP)." Six plants have been built to date for Red Arrow, Manitowoc, WI, USA, which sells a portion of the bio-oil as liquid smoke flavoring. Here, the complete bio-oil is not used but only the water-soluble, low molecular weight components. The remainder is burned to generate energy. In Ontario, Ensyn operates a demonstration plant with a throughput of 3,200 kg/h, and specifies the energy and mass balance as given in Fig. 6.

In 2013, Valmet in Finland (formerly Metso) put into operation a fast pyrolysis plant [30, 34, 35] (Fig. 7). It was a joint development with a consortium of research and industrial partners (VTT, Fortum, Metso) and designed to produce 50,000 tons of bio-oil per year, which can replace light fuel oil in a district heating network of the city of Joensuu. It is the equivalent of a heat supply for 10,000 households. The feature of the system is the integration of the pyrolysis unit into an existing cogeneration plant based on wood combustion in a fluidized bed boiler. This plant provides enough hot sand by which a slip stream is used for the integrated pyrolysis system. The by-products coke and permanent gases are simply recycled into the combustion chamber of the main system. Forest residue from the nearby environment is the sole feedstock.

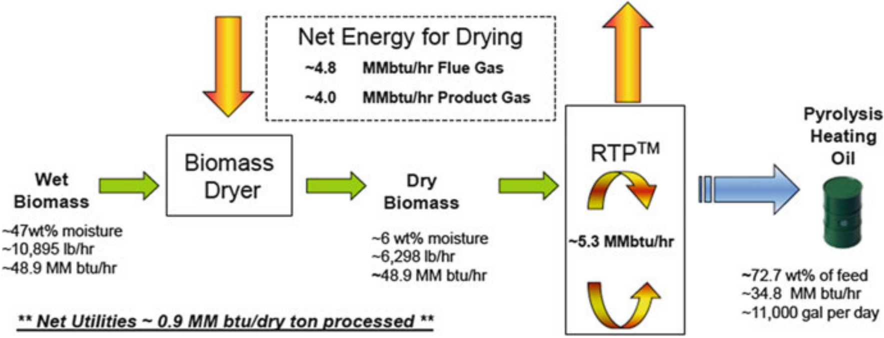

Fig. 6 Energy and mass balance of the RTP® Processes of the company Ensyn [33]

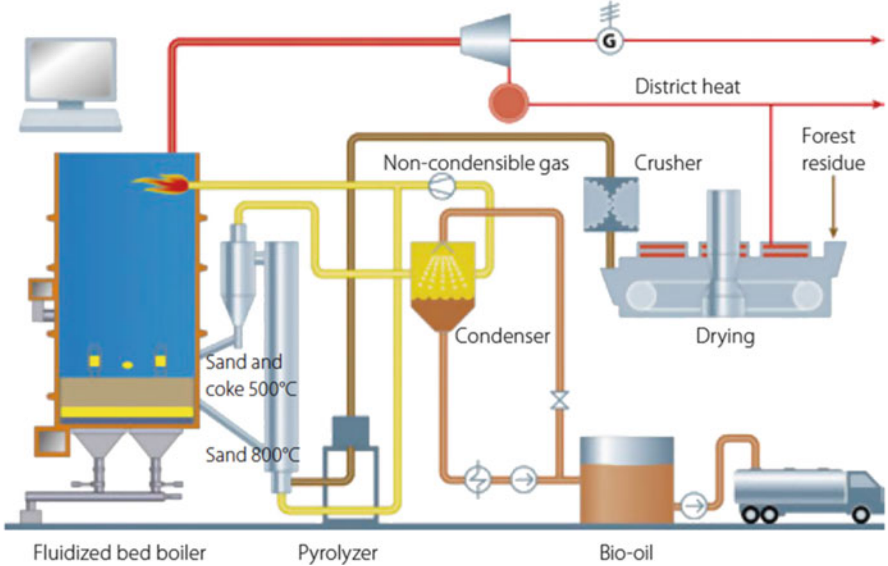

Fig. 7 Principle of integrated pyrolysis system of Valmet in Joensuu, Finland [35]

1.2.3 Rotating Cone Reactors

Instead of pneumatically fluidized heat carriers, the required heat energy can also be provided by mechanical fluidization of the hot sand with a reactor equipped with a rotating cone (Fig. 2) [36, 37]. This RCR technology (Rotating Cone Reactor) was developed at the beginning of the 1990s at the University of Twente, Enschede, The Netherlands [38, 39]. The crushed organic solids (e.g., sawdust) and preheated sand are transported in separate lines to the bottom of the rotating cone and mixed there intensively. By centrifugal force in the rotating cone, the solids are pressed against the hot inner wall and creep upward. On the way to the top, the biomass particles

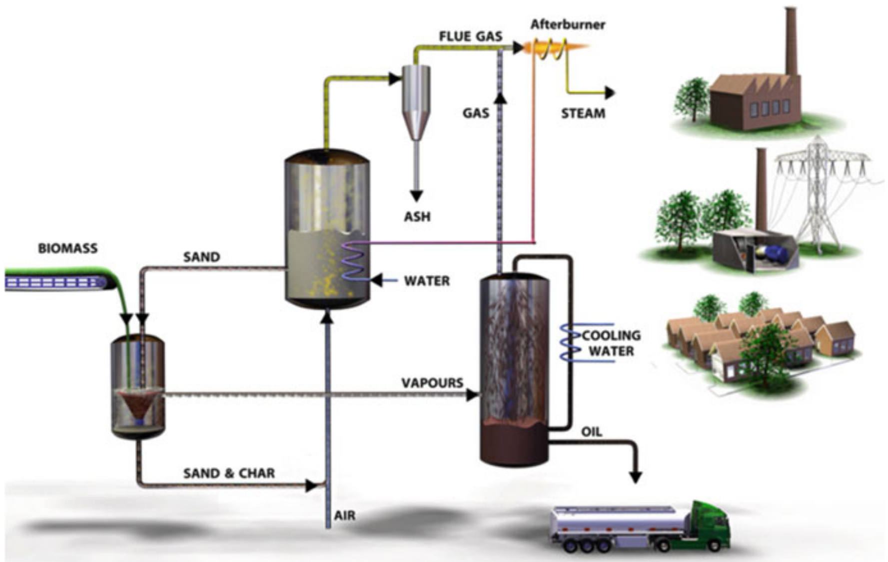

Fig. 8 Process scheme for a pyrolysis plant with rotating cone reactor (RCR) [40]

decompose on the hot surface and in the presence of the hot sand. The remaining coke – along with the hot sand – leaves the reactor at the edge of the reactor wall. The resulting volatile pyrolysis products leave the hot surface and are condensed with cold bio-oil in a corresponding cooling device. Sand and coke are discharged, the coke burned in a special burner, and the sand reheated and returned into the reactor (Fig. 8).

The technology has been further developed by the company BTG Biomass Technology Group BV, Enschede, NL. BTG itself operates a 1,700 ¯tons/year pilot plant. In May 2015, a demonstration plant (EMPYRO) with a capacity of 120 tons/day went into operation in Hengelo [30, 41]. The oil from this plant is co-combusted in a boiler from a dairy company and replaces 70% of fossil natural gas. The feedstock is fines from the handling of wood pellets, which are imported from Canada and USA via Rotterdam. The material provides ideal conditions for fast pyrolysis, as grinding is virtually omitted because the feedstock is of uniform quality and very homogeneous.

1.2.4 Twin-Screw Reactors

This type of reactor is used at the Karlsruhe Institute of Technology (KIT) at laboratory and demonstration scales and has been further developed. The pyrolysis principle can be described as follows: The dry and chopped biomass is used for fast pyrolysis at atmospheric pressure in a twin-screw mixing reactor and mixed with hot mechanically fluidized sand as heat carrier (ratio of ca. 1:10 to 1: 5). In

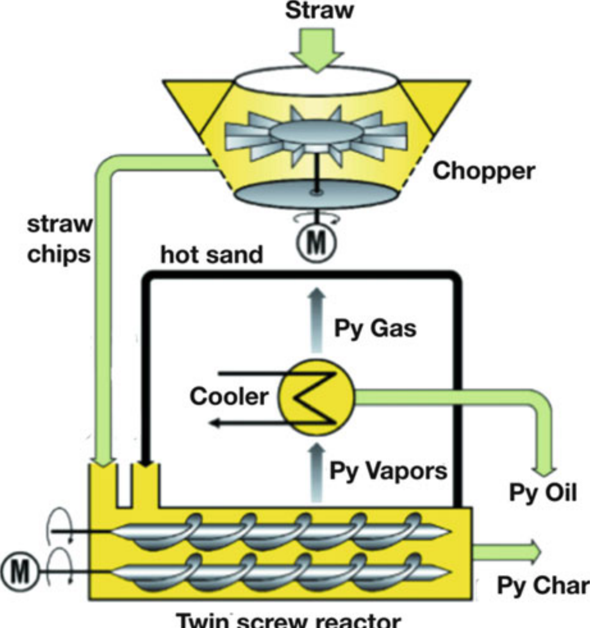

Fig. 9 Process principle of the twin-screw mixing reactor [42, 43]

mechanical fluidization, high heat losses for the circulating hot gas stream are avoided, as is the case in fluidized beds. Chopped straw and hot sand (heat carrier, 500°C) are mixed together at one point of the twin screw reactor. Heating and pyrolytic conversion of biomass particles at about 500°C occurs within less than 3 s. Pyrolysis vapors and light coke particles are blown out of the reactor (coke separation in cyclones). This produces 40–70% of an organic condensate (pyrolysis oil) and 15–40% of pyrolysis coke. The rest is a non-condensable pyrolysis gas, whose combustion heat can be used either alone or optionally together with part of the resulting coke for heating the circulating sand to reaction temperature or for drying and preheating the biomass. The process principle is described in Fig. 9 [42].

In the process demonstration unit (PDU, 10 kg/h) of KIT, numerous studies have been carried out to investigate the pyrolytic behavior of various biomasses and to explore the basics for the development and testing of the bioliq® concept described in Sect. 3.1.4.

To demonstrate the bioliq® process, the entire process chain – fast pyrolysis of wheat straw, energy densification, high pressure entrained flow gasification, gas cleaning, and fuel synthesis – was completely put into operation on the premises of KIT in 2014 [44–47]. The fast pyrolysis unit with a rate of 500 kg/h (2 MW_{th}) is being further developed and operated in cooperation with Lurgi GmbH, Frankfurt (see. Fig. 10).

It is used for the generation of an energy-dense, free-flowing “bioliq-Syncrude,” a mixture of pyrolysis oil and pyrolysis coke. First, the chopped wheat straw is converted at 500°C in a twin screw reactor to pyrolysis vapors and fine coke. The

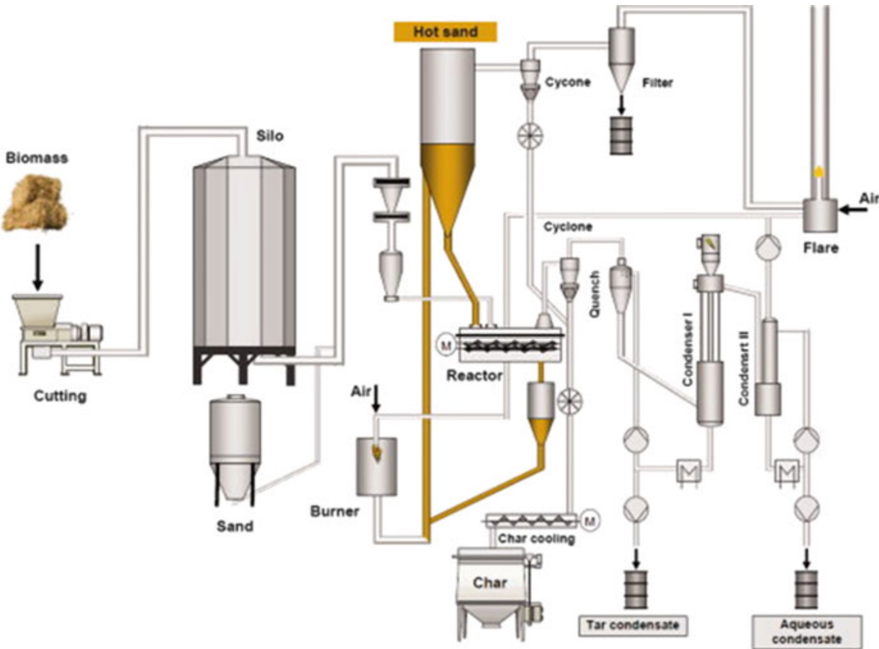

Fig. 10 Process flow diagram of the fast pyrolysis with double screw reactor [44]

latter is separated and the vapor is liquefied after cooling into an organic and an aqueous condensate.

For the rapid heating of biomass, a heat transfer circuit is used with a five- to tenfold excess of sand vs biomass. The sand is reheated in the circuit with the hot flue gases. Coke therein can be burned to meet the energy needed for heating and pyrolysis of biomass. The fine coke particles in the smoke and product gas are separated in cyclones. A quench cooler forms a heavy oil-like organic condensate. Aerosols formed thereby are held back by an electric filter before the aqueous condensate is obtained at ambient temperature. The remainder is a combustible pyrolysis gas, which consists essentially of carbon dioxide, carbon monoxide, and hydrocarbons, and may be used for flue gas generation. Under optimum process conditions their energy content (ca. 10% of the original biomass energy content) is sufficient to meet fully the energy needs for heating and pyrolysis of biomass.

The powdered pyrolysis coke and pyrolysis condensates are mixed to form a suspension (bioliq-Syncrude). The size distribution of coke particles is critical to obtain a transportable and storable mixture, which can be converted quickly and efficiently into synthesis gas in the subsequent gasification.

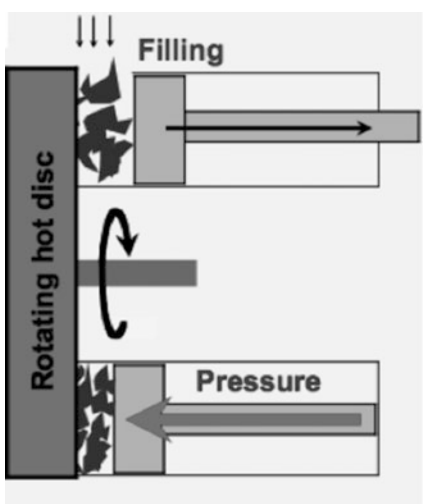

Fig. 11 Ablative pyrolysis principle of hot rotating disk (technical design of the BTO® method)

1.2.5 Ablative Reactors

"Ablation" is often used to remove material by heat. In meteorology, ablation means the slow melting of glaciers and in medicine laser ablation is used to remove cancerous tissue. Transferred to pyrolysis, it means that biomass particles are decomposed by pyrolysis through direct contact with a hot reactor surface (see Fig. 11). The scientific basis of ablative pyrolysis was studied by Lédé et al. based on the Broido–Shafizadeh pyrolysis model [48–51]. Here, the wood particles are literally brought to melting and evaporation on their contact surface by the thermal energy input together with the help of the contact pressure. Hence, in ablative pyrolysis – in contrast to fluidized bed technology – the particle size is not essential. Even wood chips can be used, because the poor thermal conductivity of the biomass means remaining portion of the particle is almost unaffected.

When pressing biomass at the hot surface, a liquid film is formed initially (similar to that between blade and ice when skating), which decomposes instantaneously. The peculiarity of the ablative procedure is that no transport gas is necessary to remove the volatile pyrolysis products because the resulting product gases take on this task. In contrast to fluidized bed reactors, however, ablative systems are mechanically more complex and therefore more difficult to scale up.

The effect of ablation can be intensified by generating additional pressing and moving forces. Such pressure forces may be established mechanically or by centrifugal force, whereas the movement forces are generated either mechanically or hydrodynamically. This principle can be implemented in different ways, for example, by using a cyclone [52–54] with a centrifugal reactor [55–57] or a rotating surface in the form of a disc [58–60] or cylinder [3, 61, 62].

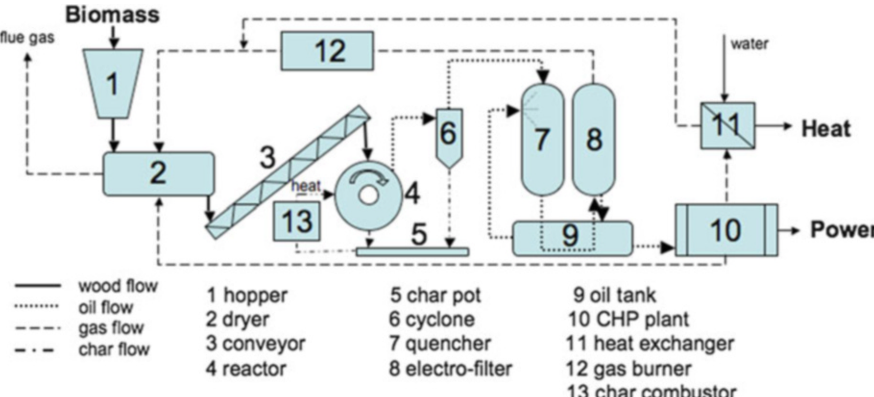

Fig. 12 Structure of the BTO process [59]

Reactor with Rotating Disk

In the following, the technical approach with a rotating disk is illustrated, which was developed and tested in the last few years by PYTEC, Hamburg at pilot scale (250 kg/h, Fig. 11). The complete BTO process is shown in Fig. 12.

In PYTEC's technical implementation of ablative pyrolysis, the hot disk is vertical. The biomass is pressed against the disk by ten hydraulically operated pistons with pressures of 30 bar [63]. The hot gases are released from fine coke and ash through a cyclone and then quenched with cold bio-oil, and aerosols are collected with an electrostatic precipitator and deposited in a knock-out pot. The pyrolysis oil should then be burned in a 12-cylinder MB-diesel engine of a CHP plant (combined heat and power). However, it never occurred in long-term experiments, so that the project was stopped.

Reactors with Horizontal Drum

The diameter of a disc reactor cannot be easily further upscaled. To circumvent this problem, a system has been approved, designed, and built by CLAAS (agricultural machineries) with a rotating drum reactor, and tested at Fraunhofer UMSICHT, Oberhausen [64]. The basic idea was previously published by a French group [51]. They reported that there were no significant differences in the embodiments of the ablative pyrolysis (drum or disc). In both cases, they found similar ablation rates.

1.2.6 Vacuum Reactors

A special form of fast pyrolysis is heating under vacuum (see Fig. 2). In this system, the feedstock (e.g., chips, pieces of bark) is introduced via an airlock into the vacuum chamber in which a pressure of about 15 kPa prevails. [65]. The reactor is equipped with a heated endless belt, which conveys the biomass slowly through the reactor and also ensures that the particles are turned around. The heat transfer to the belt on which the pyrolytic decomposition of the organic material takes place is via heating plates, which are flowed through by a molten salt of potassium nitrate, sodium nitrate, and sodium nitrite. Typically, temperatures of around 500°C are reached. The residence time of the material to be pyrolyzed within the reactor can be half an hour or more. However, the pyrolysis products can be rapidly removed from the hot reaction zone with a vacuum pump. As with the other systems, the vapors are condensed in a cooling apparatus. The remaining permanent gases and coke can be combusted and are used to heat the salt bath [66].

In Canada, such a pilot plant with a processing capacity of 3.5 tons/h of coniferous bark was operated until 2004. The liquid products should be used for the production of phenol formaldehyde resins [67–69]. Heart of the system was a 13 m long horizontal reactor. This plant of the company Pyrovac has meanwhile been dismantled.

2 Pyrolysis Oil: Characterization and Upgrading

2.1 General Characterization

Fast pyrolysis of woody biomass results in a single-phase, low viscosity liquid with a dark red to very dark brown color and a water content in the range 25–30%. The dark color is caused by the presence of micro-carbon particles. Oils, which are condensed by a hot gas filtration, appear transparent with a honey-yellow color. The fast pyrolysis oil consists mainly of a mixture of alcohols, furans, aldehydes, phenols, organic acids, oligomeric carbohydrates, and oligomeric lignin fractions. Chemically, they consist of several hundred individual components with the following functional groups: acetyl groups, carbonyl groups, esters, acetals, hemiacetals, alcohols, vinyl groups, aromatic compounds, and phenols. A typical composition of pyrolysis oil divided into GC-detectable components, polar components (HPLC detectable), oligomers (pyrolytic lignin), and water is shown in Fig. 13. The composition is dependent on the feedstock, the pyrolysis deposition system, and the storage conditions.

Pyrolysis oils are – in contrast to mineral oils – immiscible with fossil fuels and other hydrocarbons because of their high polarity. They can, however, be mixed in any ratio with lower alcohols, but have limited miscibility with water. If too much water is added (about 35%), phase separation occurs, and a heavy tar-like liquid is

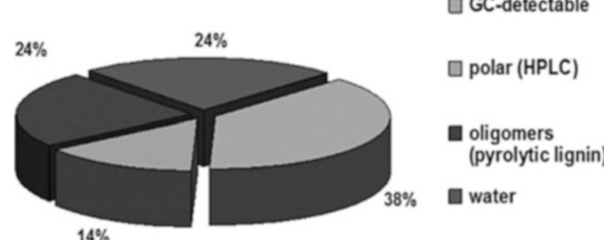

Fig. 13 Typical portions of the main component groups in fast pyrolysis oils [70]

Table 3 Typical physical and chemical properties of fast pyrolysis oils and petroleum-based fuel oils [71]

	Pyrolysis	Light fuel oil	Heavy fuel oil
Water content (wt%)	20–30	~0	~0
pH	2.0–3.5	–	–
Density (g/cm^3)	1.1–1.3	0.845	0.99–0.995
Viscosity at 40°C (centistokes)	15–35°	2.0–4.5	180–420 max
Calorific value (MJ/kg)	16–19	42.8	About 40
Ash content (%)	0.01–0.1	0.001 max	0.08 max
Flash point (°C)	40–110	60 min	65 min
Carbon content (wt%)	32–49	90	90
Hydrogen content (wt%)	6–8	10	10
Oxygen content (wt%)	44–60	0.01	12:01
Sulfur content (wt%)	<0.05	0.001 max	1.0 max
Nitrogen content (wt%)	<0.4	0.02	0.4
Solids content (wt%)	<0.5	0	0
Pour point (°C)	–9 to –36	–5 min	15 max
Calorific value (MJ/kg)	13–18	42.6	40.6

formed below the aqueous phase. The tarry phase contains mostly of lignin-derived oligomers. The water-soluble portion of the oils contains mainly pyrolysis products from cellulose and hemicellulose as well as low molecular phenols from lignin.

Table 3 shows important physico-chemical properties of pyrolysis oils. For comparison, data for light and heavy fuel oil are also listed. The water content of the pyrolysis liquids results partly from the water content in the biomass and partly from the reaction water from dehydration of carbohydrates. Too much water in the oil (>40 wt%) leads to calorific value reduction and phase separation. To control the water content in the oils, therefore, the water content of the biomass used should not be higher than 10%.

The pH of the oils is in the acidic range. This is because of the organic acids produced during the pyrolysis (e.g., formic acid, acetic acid) derived from the hemicellulose and the lignin. Therefore, special attention should be paid to materials coming into bio-oil contact.

The viscosity of the oils can vary over a wide range, depending greatly on the particular water content, the content of volatile constituents, and the storage period.

Table 4 Requirements for fast pyrolysis oils for use as fuel oil (ASTM D7544-12)

Property	Grade G	Grade D
Calorific value (MJ/kg)	15	15
Water content (wt%), max	30	30
Solids content (wt%), max	2.5	0.25
Kinematic viscosity at 40°C (mm^2/s)	125	125
Density at 20°C (kg/dm^3)	1.1–1.3	1.1–1.3
Sulfur content (wt%), max	0.05	0.05
Ash content (wt%), max	0.25	0.15
pH	Declare	Declare
Flash point (°C), min	45	45
Pour point (°C), max	–9	–9

After several months of storage, the oils prone to viscosity increase because of the reactive components which tend to polymerize. [72]. However, these effects can easily be limited by addition of small amounts of alcohols [73, 74]. In addition, stability improvements can also be achieved by removing highly volatile components [75].

The calorific values of pyrolysis liquids are calculated from the elemental analysis data. It is about 42% of the calorific value of fossil liquid fuels (Table 3).

There are few data on the toxicity of fast pyrolysis oils. They have a characteristic, slightly pungent odor, reminiscent of smokehouses. Skin and eye contact should be avoided. Exact representative toxicological investigations are still pending. Studies of environmental effects in the case of accidents were carried out and published [76]. In general, pyrolysis oils can be classified in the same way as wood smoke or other wood distillates [77]. The pyrolysis oils may contain very small amounts of polycondensed aromatics, which are to be considered as carcinogens in accordance with existing guidelines.

Bio-oils are difficult to distill because they are thermodynamically unstable and prone to polymerization reactions. Similarly, the storage temperatures should not exceed 30°C.

In recent years the increasing demand for pyrolysis oils has led to improved identification of fuel properties for potential end users [78–81]. Likewise, an American standard (ASTM D7544-12) was established for pyrolysis oils to be used as a substitute fuel oil (Table 4). A European standard for bio-oils is currently under development [82].

In the field of direct combustion or co-combustion, considerable progress has been made. Substantial amounts, about 40 tons, were used, for example, by Fortum Power and Heat in a district heating system for the city of Masala, Finland. The nozzle head of a special heavy oil burner was modified. In addition to the combustion behavior, the infrastructure and handling of pyrolysis oil was also tested. Despite outdoor temperatures down to −20°C, the system worked properly [34, 79].

2.2 Conditioning and Upgrading of Pyrolysis Oils

Depending on the intended use of the pyrolysis oil, more or less complicated processing steps are required. Distinction can be made between physical and chemical treatment methods.

2.2.1 Physical Methods

The parameters in the standard ASTM D7544-12 (see Table 4) are especially important for use as a substitute fuel in oil burners in thermal power stations. Of particular importance are the particle content and the viscosity. Particles such as sand dust (from the fluidized bed pyrolysis) as well as coal and ash particles could be entrained into the pyrolysis oil. The easiest way to avoid such contamination is the use of multi-cyclone systems with a separation range down to 10 μm. Deposition of such particles can also be realized with hot gas filters, which are directly integrated in the hot gas stream [83–85]. However, it is likely to produce oil yield losses up to 30% as the forming filter cake favors cracking of the pyrolysis oil to gas and water. Additionally or alternatively, a cold filtration of the oils after condensation is possible. However, this has not yet been carried out at larger scale.

The viscosity of the pyrolysis oil can easily be reduced by adding small amounts of lower alcohols. However, this may reduce the flashpoint [86].

Another possibility is the fractionation of the hot pyrolysis gases by staged condensation. Here, several coolers and electric filters in series are operated at different temperatures, so that fractions with different product concentrations can be obtained. For example, volatile organic acids and water can be separated together. Staged condensation is usually applied to enrich interesting chemical raw materials [22, 87, 88].

In addition to the staged condensation, undesired volatiles such as water and acetic acid can also be greatly reduced by simply increasing the exit gas temperature to about 50°C. The losses and increased viscosity can be compensated by addition of alcohols (e.g., 1-propanol) [86].

Because pyrolysis oils are immiscible with fossil liquid fuels because of their high oxygen content and the associated high polarity, measures are studied to use them with addition of suitable emulsifiers for application as fuel in burners and diesel engines [89–92].

Recently, the miscibility of pyrolysis oil and biodiesel with addition of various alcohols (ethanol, 1-butanol, and 1-propanol) was examined. Addition of 1-butanol gave a single-phase oil with the largest portion of pyrolysis liquid (about 50%) in the mixture [93].

2.2.2 Chemical Methods

To mitigate the acidic nature of the oils, attempts have been made to esterify the acids with alcohols. By injection of alcohol into the hot pyrolysis-gas stream, a significant reduction of the acid number (Total Acid Number, TAN) could be achieved [94]. In a two-stage process, pyrolysis oil can be oxidized first by treatment with oxidizing agents (ozone, hydrogen peroxide). Thereafter, esterification can be achieved by integrating more alcohol in the product, so that the fuel properties with respect to the pH and the calorific value can be improved [95, 96]. Esterification using catalysts (base, acid ion exchange resins) or solid acids is also possible and has been successfully described [97–102].

Another option is to separate the more water-soluble substances by hot water treatment under pressure (high pressure thermal treatment, HPTT) at about 250°C. This leads to the formation of an aqueous phase and a tarry phase with less oxygen. This fraction is more suitable for further upgrading methods such as hydrogen treatment [103].

2.2.3 Catalytic Cracking

The most common processes for upgrading pyrolysis oils employ catalysts. Distinction is made between catalytic cracking and catalytic hydrotreatment. Both options come from petroleum refining technology and have been comprehensively discussed [104].

Catalytic cracking is a standard method of oil refineries. It is used in reactors with fluidized catalyst (usually zeolites) and applied in the presence of water vapor and at temperatures of 500–550°C (Fluid Catalytic Cracking, FCC) to split high-boiling gas oil into olefins, heavy oils, gasoline, and diesel fractions. In the catalytic fast pyrolysis of biomass (CFP™) – depending on the location of the catalyst – a distinction between "in-situ" and "ex situ" catalysis is made [3, 105, 106]. "In-situ" means the placement of the catalyst in the reactor, that is, the heat carrier sand is replaced by catalyst material. The "ex situ" catalytic pyrolysis approach only affects the catalytic upgrading of the raw, hot pyrolysis gases outside the fast pyrolysis reactor (Fig. 14).

In general, zeolites are used in both process variants, which ideally remove oxygen as CO_2 and water. The major reactions are C–C cleavage, hydrogen transfer, isomerization, dehydration, decarboxylation, and decarbonylation [108] (see Fig. 17). Among the zeolites, the microporous ZSM-5 has been used most often, but materials such as mesopore MCM-41 and SBA-15, as well as metal oxides or inorganic carrier materials with copper, nickel, palladium, and platinum impregnation, have also been studied [109]. The use of ZSM-5 can be attributed to the successful use of the material in the mobile MTG process (methanol-to-gasoline) where methanol reacts over H-ZSM-5 to give chain-like hydrocarbons. In the early 1980s the conversion of oxygen-containing pyrolysis products was reported

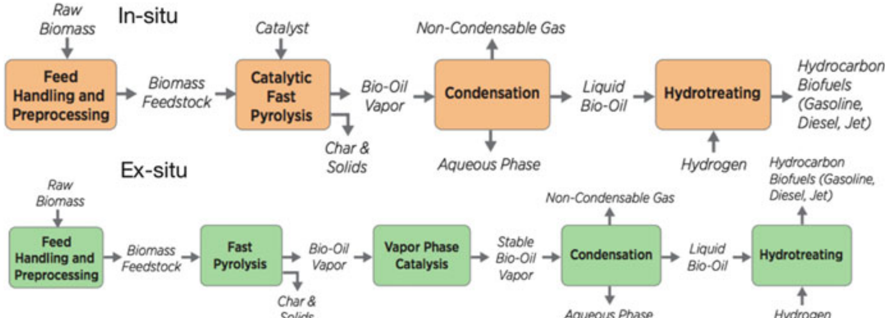

Fig. 14 In-situ and ex-situ alternatives for catalytic cracking of fast pyrolysis [107]

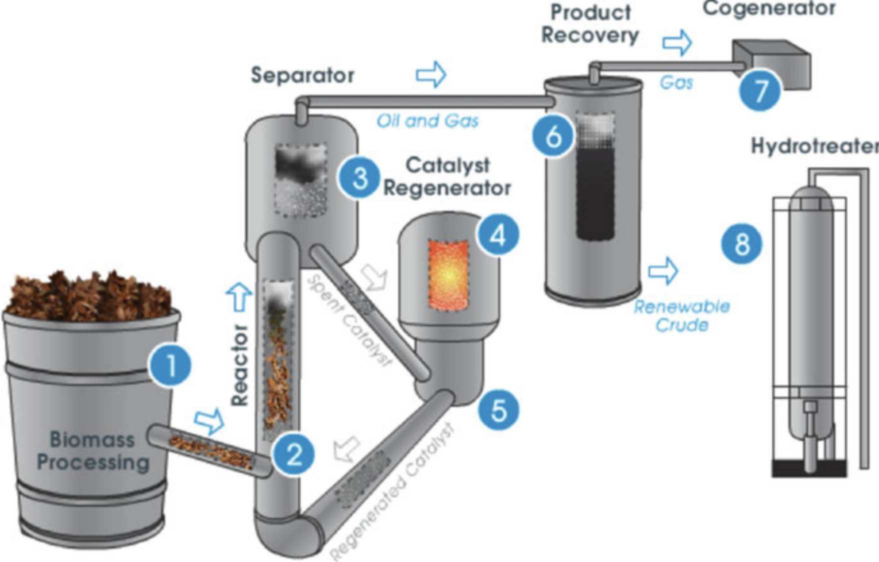

Fig. 15 Example of an "in-situ" catalytic fast pyrolysis process of KIOR, USA [116]

[110, 111]. Although the C:O ratio of methanol and pyrolysis oil is similar (about 1:1), pyrolysis oil lacks the necessary amount of hydrogen. Hence, rather alkylated benzenes and other aromatics are formed [112] as well as unwanted coke [113, 114].

Some companies in the United States such as Envergent [115], Kior [116, 117] (see Fig. 15), and RTI [117] have already developed processes using the in situ approach in the pilot and demonstration scales. The specific production of aromatics such as benzene, toluene, and xylene (BTX) is under development by the company Anellotech, USA [118]. They operate the Biomass-to-Aromatics (BTA) process, which is based on modifications of the ZSM-5 catalyst [119–125]. Recently it was possible, for example, by reducing the outer pore diameter of a HZSM-5

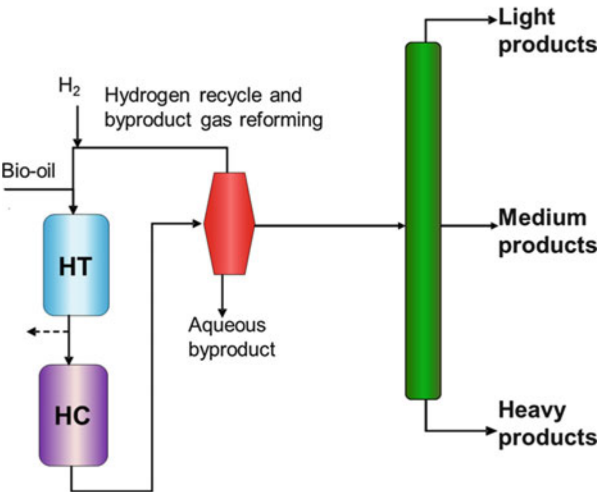

Fig. 16 System diagram for two-stage hydrotreating for hydrotreatment (HT) and hydrocracking (HC) [128]

catalyst and doping with gallium, to increase selectivity for *p*-xylene from 33% to 95% [126]. However, a lot of research work is still necessary to improve the selectivity and yield of desired products in the CFP process.

2.2.4 Hydrotreatment

Treatment with hydrogen is another option to convert raw pyrolysis oils into products that are used both in conventional refineries because they can be mixed with fossil oil and directly as “drop-in” fuel. Excellent reviews are available on the historical development of the hydrogen treatment of pyrolysis oils [26, 127]. The main rationale for the use of hydrogen is the elimination of oxygen through water formation and the hydrogenation of unsaturated bonds (hydrodeoxygenation, HDO). The reactions are usually carried out in two stages. First, a hydrogen treatment (hydrotreatment) is carried out for the hydrogenation of double bonds, whereby the thermal stability is increased. Here, highly active noble metal catalysts and relatively mild temperatures are usually applied (ca. 200°C) at 200 bar hydrogen pressure. The next stage involves hydrocracking to crack the previously stabilized intermediate products under more severe conditions (300–450°C) and to hydrogenate further so that more oxygen is removed in the form of water (see Fig. 16). Here noble metal catalysts (Pt, Pd, Ru) are often used on carbon carriers because of their good chemical stability, but also noble metals on ZrO_2 and NiMo and CoMo on alumina or silica are used. Because of the high oxygen content of the pyrolysis products and the plurality of possible reactions, long residence times are required. Typical liquid hourly space velocities (LHSV) are 0.1–1.5. The hydrogen consumption is in the region of 1–10 m^3 per liter of bio-oil [25].

Figure 17 summarizes the main cleavage reactions occurring in the catalytic upgrading of pyrolysis oils.

Fig. 17 Major reactions of pyrolysis oil components in hydrodeoxygenation (HDO) [129]

Table 5 Characteristics of pyrolysis, catalytic pyrolysis oil and refined petroleum [129]

	Pyrolysis oil	Catalytic cracking	Catalytic HDO	Petroleum
$Yield_{Py\text{-}oil}$	100	12–14	21–65	–
$Yield_{water\ phase}$		24–28	13–49	–
$Yield_{gas}$		6–13	3–15	–
$Yield_{coke}$		26–39	4–26	–
Oil properties				
Water (wt%)	15–30	–	1.5	0.1
pH	2.8–3.8	–	5.8	–
Density (kg/L)	1.05–1.25	–	0.76–1.2	0.86
Viscosity (cP)	40–100	–	1–5	180
Energy (MJ/kg)	16–19	21–39	42–45	44
C (wt%)	55–65	61–79	85–89	83–86
H (wt%)	5–7	2–8	10–14	11–14
O (wt%)	28–40	13–24	<5	<1
N (wt%)	<0.4	–	–	<1
S (wt%)	<0.05	–	<0.005	<4
Ash (wt%)	<0.2	–	–	0.1
H/C	0.9–1.5	0.3–1.8	1.3–2.0	1.5–2.0
O/C	0.3–0.5	0.1–0.3	<0.1	~0

Table 5 shows fundamental differences of yields and physico-chemical properties of fuels, which are created with the help of the aforementioned variants “catalytic cracking” and “catalytic deoxygenation” (HDO) as compared to

untreated pyrolysis oil and fossil crude oil. Note the low yields after the cleavage reactions, which can be explained by the removal of oxygen and increased coke formation.

To reduce the cost of hydrogen, multistage processes are being developed, such as UOP (USA), where, in a first stage, the pyrolysis oil is cleaned from metals by ion exchange resins, followed by distillation to lower the water content to $<15\%$. After that, esterification follows to lower the acidity, with subsequent hydrogen treatment for partial deoxygenation with a neutral catalyst. There follows a second deoxygenation step and the recycling of previously obtained intermediates from the first and second stages of the hydrotreater [130, 131]. Another refinement process (Biomass Into gasoil, BINGO) is described by IFP (France), where raw pyrolysis oil is mixed with other hydrocarbons and partially deoxygenated intermediates and fed to the hydrocracker [127, 132].

3 Pyrolysis Oil Applications

An overview of the possible utilization lines of pyrolysis oil is given in Fig. 18.

Thus, one can distinguish between an energetic and a chemical or material use. Against this background, the main uses of pyrolysis oils are discussed below.

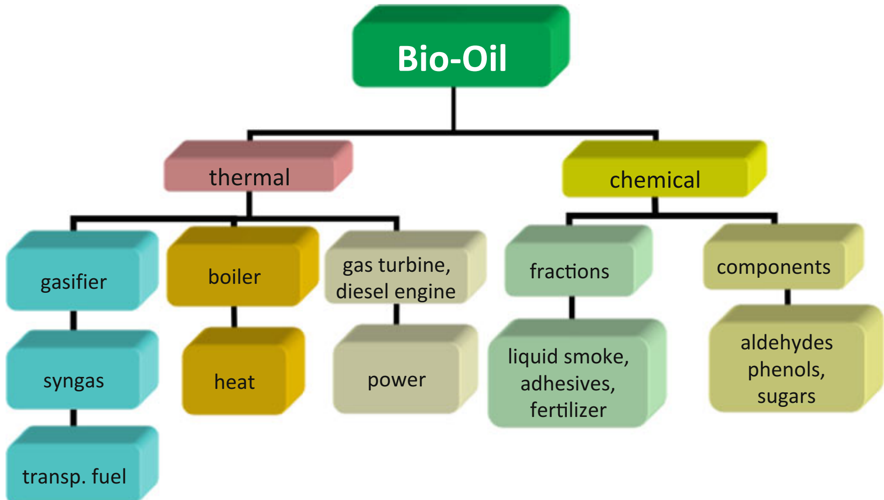

Fig. 18 Alternative uses for pyrolysis oil from biomass

3.1 Heat and Power

A thermal or energy recovery from pyrolysis oils is possible through a number of different techniques and procedures.

3.1.1 Combustion and Co-firing

Combustion of pyrolysis oil is considered to be the easiest application for this specific fuel. Because of its properties, some modifications are required on the combustion system and operating conditions. Co-combustion with fossil fuels is a simple way to introduce this fuel in the energy market. Also, 100% pyrolysis oil combustion has been demonstrated. In contrast to solid biomass, pyrolysis oil can also be co-fired with natural gas [80].

Pyrolysis oils resemble heavy fuel oil in a way (see Table 3), but because of their relatively high water content, ignition is delayed. However, they can be burned in a similar way to heavy fuel oils when appropriate modifications of spray nozzles and combustion parameters are selected. The oil grades are now regulated by an ASTM standard (see Table 4) and a European standards is in course of preparation. In the literature there are numerous references to the combustion characteristics, quality requirements, and emissions [78, 79, 133–135]. The most important criterion apart from the calorific value is the oil viscosity, as it affects atomization and therefore the droplet size [79]. It is advantageous for combustion when the combustion chamber is preheated to 600–800°C using fossil fuels. One major problem is the long-term stability and acidity of the pyrolysis liquids. [136].

Numerous combustion tests, especially in Finland and the Netherlands, have shown that after adaption of burner and combustion chamber, combustion is possible without problems. For example, at Stork (Hengelo, Netherlands) pine pyrolysis oil and heavy fuel oil were combusted in a low NO_x-double register burner for gas and oils. The measured emissions are shown in Table 6.

The co-combustion of pyrolysis oil in gas and oil burners is currently being tested in demonstration scale. Fortum operates in its cogeneration plant in Joensuu, Finland, a fast pyrolysis unit of Valmet (formerly Metso) with a circulating fluidized bed (30 MW) (see Fig. 2). The system was developed in close cooperation

Table 6 Comparison of emissions from the combustion of heavy fuel oil and pyrolysis oil of pine oil [137]

Oil	Heat total (MW)	Heat in oil (%)	Feed rate (kg/h)	O_2 (vol %)	CO (ppm vol)	NO_x (mg/m_0^3 @3%O_2)	Dust (mg/m_0^3 @3%O_2)
Heavy fuel oil	4.7	100	411	4.0	<5	550	30
Pyrolysis oil	2.6	76	606	3.0	<50	133	13–20

with the Finnish Research Centre (VTT) and Metso (now Valmet). The raw material is exclusively wood residues from the environment.

Another demonstration project on "Energy and Materials from Pyrolysis" has been established in Hengelo, Netherlands [41]. As part of the project EMPYRO (see Fig. 8) a 44,000¯-tons/year plant has been built based on rotating cone reactor (RCR) technology.

3.1.2 Diesel Engines

Diesel engines can be used in stationary applications to provide heat and electricity. An interesting feature of these engines is that high electrical efficiency (>40%) can be obtained even at relatively low capacities. So far this application has not been demonstrated [138].

The direct use of pyrolysis oils in stationary diesel engines for power generation is possible in principle; However, long-term tests were not successful. In general, the high viscosity, the slow burn rate, the lack of lubricity, and low pH create the biggest problems [139–141].

3.1.3 Gas Turbines

Gas turbines can be used to produce power or a combination of power and heat. Typically, natural gas is used as a fuel, but liquid fuels can also be used. To enable fueling of pyrolysis oil, modifications are needed to the fuel supply system, and modifications to the burner chamber might be required to overcome lower combustion speed [142].

Pyrolysis oil can in principle be used in gas turbines [143]. In the experiments realized so far, however, it has become clear that the fine coal and ash content in the oil is problematic as it significantly reduces the lifetime of the turbine. Orenda Aerospace in Canada conducted extensive experiments with a GT2500 turbine Mashproekt (2.5 Megawatt electric (MWe)) from Ukraine. Numerous optimization steps have been undertaken. Changes were made in the atomization stage to reduce the droplet size. The delivery system was modified to take account of the higher viscosity and corrosiveness. Likewise, burner and nozzle were adjusted so that larger amounts of fuel could be injected [140]. A complete gas turbine package including a heat recovery unit and fuel pretreatment skid was delivered and installed at the Dynamotive demonstration site in West Lorne. Regretfully, because of the lack of sufficient pyrolysis oil, the unit has hardly been operated [142]. The combustion of pyrolysis oil has been tested. It was found that at 70–100% load it is possible to burn 100% pyrolysis oil without the need for mixing it with ethanol. Based on this research, OPRA has been able to design a new combustor for burning pyrolysis oil and other low-calorific fuels [142].

3.1.4 Gasifiers

Solid biomass has a low energy density, so the transport of large volumes of plant material, as is the case for the production of synthetic fuels, can be uneconomic. Therefore, a two-step approach (bioliq®) is currently being pursued for the conversion of biomass into synthetic fuels in Germany. It is based on the concept of feeding several remote fast pyrolysis units with straw, which has a low volumetric energy density ($\approx$2 GJ/m^3). Within a radius of 30 km, straw can be economically transported to local fast pyrolysis units (100 MW$_{th}$ each). The products bio-oil and pyrolysis coke are mixed to generate a so-called "bio-slurry" with a ten times higher energy density. Hence, it could be economically transported over long distances ($>$200 km) by rail or barge to a large-scale pressurized entrained-flow gasification plant (1–5 GW$_{th}$). There, the slurries are processed at pressures $>$3 MPa with industrial oxygen ($\lambda \approx 0.3$) to a tar-free raw synthesis gas low in methane. After gas cleaning and conditioning, synthesis of DME and methanol follow [44, 144].

3.2 Chemical and Material Use

In addition to the use of pyrolysis oil as an energy source, it can also be used as a chemical feedstock and as a starting point for a whole series of materials. In the following examples, major options are briefly presented.

3.2.1 Use with Fractionation

Fractional Condensation This method is useful for enriching certain fractions during the pyrolysis process by condensing the hot vapors at different temperatures by use of condensers and electrostatic precipitators (ESP) in series. Depending on the temperature, acids, phenols, and sugars can be concentrated and further processed and purified [23, 145, 146]. The higher molecular fraction collected in an ESP showed superior insecticidal properties [147].

Addition of Water By addition of water, pyrolysis oil can simply be separated into an aqueous phase and an organic phase. The water-soluble phase is used for the production of a liquid smoke aroma for the food industry. It serves for preservation, flavoring, and coloring in the treatment of meat, sausages, and cheese to replace the time-consuming and ineffective traditional smoking procedure. In addition, smoke flavorings are increasingly used in soups, sauces, and snacks. Within the EU such liquid smoke flavorings need to be authorized by the European Food Safety Authority (EFSA) [148, 149].

Further options are based on the isolation of levoglucosan (LG) (i.e., 1,6-anhydro-beta-glucopyranose) which resembles, in general, the main product of cellulose pyrolysis. Levoglucosan could serve as chiral synthon to control

stereo-selective reactions during synthesis. Other applications are, for example, based on the use as educt for the synthesis of surfactants, biodegradable polymers, and resins [150–152]. Additionally, a pretreatment of biomass with mineral or organic acids as well as the use of catalysts in the pyrolyzer with zeolites significantly increase the yield of LG [12, 153, 154]. Acid washing removes ashes, thus favoring the formation of anhydrosugars and minimizing ring opening of the glucose molecule to form low molecular weight species such as glycolaldehyde, hydroxypropanone, etc.

The addition of water also results in the separation of so-called "pyrolytic lignin" [155–158]. This powder-like lignin-derived material could be converted to aromatics [159] or used to replace phenol in phenol formaldehyde resins [40] as it contains stable radicals [160]. Furthermore, hydrotreatment has been described recently to produce refinery intermediates alkylphenolics and aromatics [161, 162].

Change of pH By changing the pH of pyrolysis liquids, neutral and phenolic components can be fractionated, which can be used for the production of phenolic resins for the formulation of adhesives in the wood industry for panel production [163–165].

3.2.2 Use Without Fractionation

Unchanged, complete pyrolysis oil can partially replace phenol and formaldehyde as a binder for particleboards, especially when bark is pyrolyzed, which provides a phenol-containing pyrolysis oil because of its polyphenolic structure. The degree of substitution of fossil phenol can be 30–40%, saving formaldehyde up to 30% [67, 166–169]. Up to now, no further industrial use of this route has been reported.

3.2.3 Extraction of Chemicals

Several attempts have been made to use extraction techniques for the separation of chemical groups. Liquid-liquid extraction with solvents [170] resulted in a fractionation of sugars and phenolics. The successful separation of acetic acid and glycolaldehyde has also been reported [171]. In addition, advanced extraction methods such as gas extraction with supercritical CO_2 have been studied. Enrichment of furanoids, pyranoids, benzenoids, acids, aldehydes, ketones, furans, and aromatic compounds was observed. However, the extraction of esters, alcohols, and aldehydes was ineffective [172–174].

4 Conclusions

Biomass pyrolysis is the thermal depolymerization of biomass at modest temperatures in the absence of added oxygen. The spectrum of products from biomass pyrolysis depends on the process temperature, pressure, and residence time of the liberated pyrolysis vapors [123]. A biorefinery based on pyrolysis oil is designed much as a traditional refinery. First, biomass is converted into pyrolysis oil which can be a de-centralized process. Second, pyrolysis oil from different installations is collected at the biorefinery where it is divided into different fractions. Each fraction can be upgraded with a different technology to derive finally the optimal combination of high value and low value products from the pyrolysis oil. The major high-value compounds which are foreseen are phenols, organic acids, furfural, HMF, and levoglucosan [175–177]. The major advantage of a pyrolysis biorefinery is the possibility of decentralized production of the oil in regions where abundant biomass is readily available, making it possible to keep the minerals in the country of origin and creating the possibility of cost-effective transport of the resulting liquids. The basis for creating high-value compounds is the cost-effective fractionation of the pyrolysis oil. Fractionation results in various qualities of oil needed for further upgrading into fine chemicals, petrochemicals, automotive fuels, and energy [178].

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Products Components: Alcohols

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Abstract Alcohols ($C_nH_{n+2}OH$) are classified into primary, secondary, and tertiary alcohols, which can be branched or unbranched. They can also feature more than one OH-group (two OH-groups = diol; three OH-groups = triol). Presently, except for ethanol and sugar alcohols, they are mainly produced from fossil-based resources, such as petroleum, gas, and coal. Methanol and ethanol have the highest annual production volume accounting for 53 and 91 million tons/year, respectively. Most alcohols are used as fuels (e.g., ethanol), solvents (e.g., butanol), and chemical intermediates.

This chapter gives an overview of recent research on the production of short-chain unbranched alcohols (C1–C5), focusing in particular on propanediols (1,2- and 1,3-propanediol), butanols, and butanediols (1,4- and 2,3-butanediol). It also provides a short summary on biobased higher alcohols (>C5) including branched alcohols.

Keywords Biobased alcohol, Butanediol, Butanol, Ethanol, Higher alcohols, Methanol, Propanediol, Propanol

Contents

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1 Methanol

Methanol (CH_3OH) is a colorless and polar liquid with a global annual consumption of 70 million tons in 2015 [1]. It is one of the most important bulk chemicals and is mainly used as a starting material or solvent for synthesis [2]. The major products produced from methanol are formaldehyde, methyl tertiary-butyl ether/tertiary-amylmethylether (MTBE/TAME), dimethyl ether (DME), gasoline, and acetic acid. Approximately one-third of global methanol production (MTBE/TAME/gasoline/DME) is consumed in the fuel sector (Fig. 1).

Industrial methanol production is based on synthesis gas ($CO + H_2$) which can be manufactured from any carbon-containing source, such as fossil raw materials, CO_2, or biomass. Currently, large-scale production of synthesis gas is dominated by the conversion of fossil resources, mainly natural gas and coal [2, 4]. At present about 200,000 tons of methanol are derived from biomass feedstock [5]. In principle this bio-methanol can be produced in two different ways (Fig. 2). Gasification of "dry" biomass (e.g., waste wood) results in a gas mixture consisting of CO, CO_2, H_2, H_2O, CH_4, higher hydrocarbons, and aromatic compounds. However, the fermentation of "wet" biomass yields a biogas rich in methane. To be suitable for methanol synthesis, both gases initially require a complex purification step. Additionally, the methane-rich biogas has to be converted to synthesis gas by steam

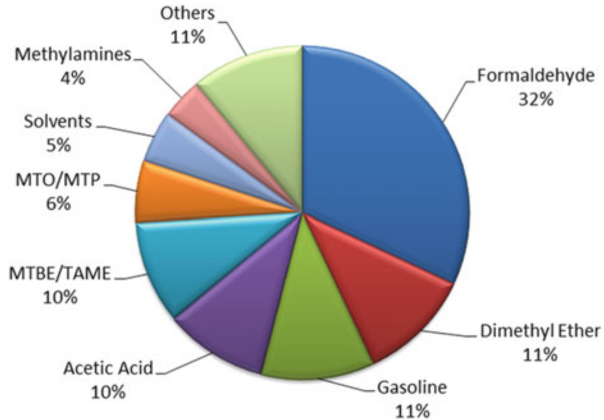

Fig. 1 Methanol demand 2011 by end use [3]

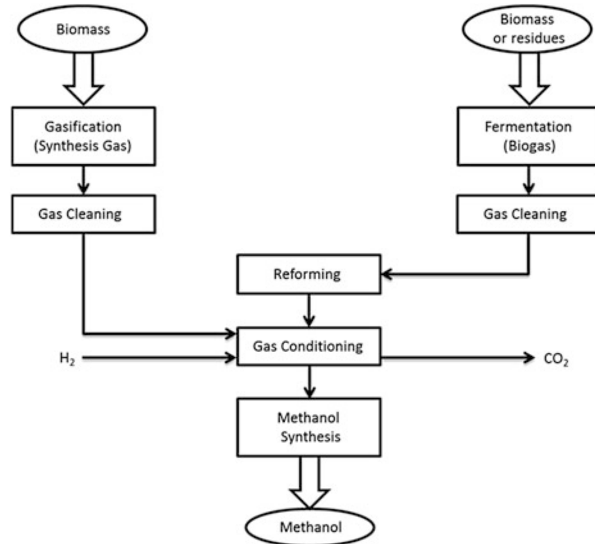

Fig. 2 Simplified scheme for methanol synthesis from biomass feedstock. Adapted from Bertau [4]

reforming. For the production of methanol a H_2:CO ratio slightly above 2 is desired and therefore the gas composition needs to be conditioned (e.g., by adding H_2 and removing CO_2) before the synthesis gas can selectively react over a heterogeneous catalyst to produce methanol [4]. The industrial methanol synthesis is conducted at 200–300 °C and 5–10 MPa with a catalyst based on Cu-ZnO-Al_2O_3. A high selectivity greater than 99% is achieved [2].

Methanol production from wood via gasification and syngas presents the most cost-efficient method from all potential regenerative raw materials. It is superior to every other process in terms of total calorific efficiency, cost of cultivation and harvesting, and net yield of methanol per hectare. Wood is planted every 10–30 years, can be harvested and used throughout the entire year, and does not cause any environmental problems [4].

The biotechnological routes to methanol have been recently reviewed by Straathof [6], Ge [7], and Hwang [8]. They discussed the oxidation of methane to methanol by methylotrophs (e.g., *Methylosinus trichosporium*) and ammonia-oxidizing bacteria (e.g., *Nitrosomonas* spp.), both using the enzyme methane monooxygenase. This route is widely known as part of the global carbon cycle. However, the maximum achievable methanol concentrations of 1.1 g/L [9] are far from economical production. Nevertheless, some patents are pending [10, 11].

Methanol is also a by-product of natural ethanol production by various yeasts in wine making. However, in these cases methanol is not desired for health and flavor reasons [12]. Aside from ethanol, isopropanol, *n*-butanol, and 2,3-butanediol, it can also be produced during syngas fermentation [13]. Nevertheless, high titers can only be attained if product inhibition is overcome [14] and acetate production is reduced. Tyurin and Kiriukhin [15] engineered an acetogenic *Clostridium* to tolerate 95 g/L methanol and achieved more than 70 g/L methanol from a 20% CO_2/80%

H_2 gas blend in continuous fermentation. The CO_2 could be provided from waste gas and the hydrogen from water via photovoltaic-driven electrolysis.

2 Ethanol

Almost all ethanol worldwide is produced by yeast in a fermentation process [16]. The process is characterized by high selectivity, low accumulation of by-products, high ethanol yield, high productivity, and high tolerance toward both increased ethanol and substrate concentrations [17]. Ethanol is produced by direct fermentation of glucose or sucrose which are obtained either through hydrolysis of starch (glucose) or extraction of sugar crops (sucrose). Nearly 40% of bio-ethanol is based on sugar crops, such as sugarcane and sugar beet, and 60% corresponds to starch crops [18]. Industrial ethanol production is usually performed in three steps: (1) provision of a solution of fermentable sugars, (2) fermentation, and (3) ethanol separation and purification, in general by distillation-rectification-dehydration [19]. The main difference between ethanol production from sugar crops, from starch crops, and from lignocellulosic biomass is the step to obtain the fermentable sugars. In contrast to sugar crops, whose sugars can be directly extracted, starch crops and lignocellulosic biomass require an additional pre-treatment to acquire a fermentable sugar solution [18]. The pre-treatment for starch crops is an easy and inexpensive process but for lignocellulosic biomass difficult and costly because of its complex structure. Thus far, the high cost of the pre-treatment is still an economical bottleneck in the production of cellulosic ethanol [20, 21].

Industrial ethanol production from lignocellulosic materials with *Saccharomyces cerevisiae* has two drawbacks which significantly decrease ethanol yield and productivity: (1) the presence of fermentation inhibitors (e.g., furfural, phenolic compounds) produced in the pre-treatment and (2) the inability of the yeast to utilize pentoses (xylose, arabinose). As a consequence, recent research is concerned with better lignocellulose utilization by engineering bacteria (e.g., *Zymomonas mobilis*) and yeasts to express genes which convert pentoses into ethanol [22, 23]. Furthermore, industry and academia are always searching for cheaper substrate and medium components to reduce process costs. The use of algal biomass for ethanol production has recently been reviewed by Li [24]. A potential future substrate for ethanol production could be syngas [13]. Presently there are already three companies (Coskata, INEOS Bio, and LanzaTech) that strongly promote gas-fermentation technologies to produce ethanol, all running demonstration plants. For example, the New Zealand Company LanzaTech has applied various patents using proprietary microbes and technologies. They are running pilot and demonstration plants (up to 300 tons/year), mainly in the USA and China. Two commercial production sites (30,000–90,000 tons/year) are expected to come on stream in 2016 [25].

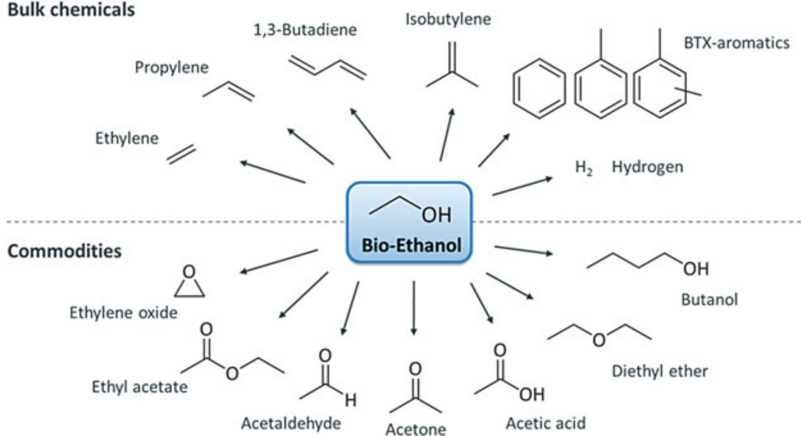

Fig. 3 Versatility of bio-ethanol as a platform chemical [39]

World bio-ethanol production has grown to 91 million tons (116 billion L) in 2013 with the USA (57%) and Brazil (27%) as the two major producers [26]. Ethanol is mainly used as a biofuel which is blended into fossil fuel-based petrol to promote energy independence and mitigate negative environmental impacts of fossil fuels [27]. However, bio-ethanol was also recently identified as one of the potential top biobased raw materials for the chemical industry because of its broad potential to become a renewable and versatile platform molecule and established high volume production [28].

The advantage of bio-ethanol compared to other biomass feedstock (e.g., lignin, cellulose, fatty acids) is the capability of being directly converted into “drop-in” chemicals [29]. For that reason, bio-ethanol can be used to produce some of the same building block chemicals that are currently obtained from petroleum, such as ethylene (ethene): 146 million tons/year [30], propylene (propene): 78 million tons/year [31], isobutylene (2-methylpropene): 10 million tons/year [32], 1,3-butadiene: 11.5 million tons/year [33], and BTX aromatics: 95 million tons/year [34] and for the production of oxygenated chemicals, such as acetaldehyde: 1.3 million tons/year [35], acetic acid: 10.4 million tons/year, acetone: 6.7 million tons/year [36], ethyl acetate: 3.1 million tons/year [36], and 1-butanol: 3.8 million tons/year [37]. Furthermore, hydrogen: 55 million tons/year [38] is also available from ethanol by steam reforming (Fig. 3).

Bio-ethanol can be converted to the various bulk and commodity chemicals either by chemo-catalytic or fermentation processes. Currently ethylene, ethyl acetate, acetaldehyde, and acetic acid are already industrially manufactured to some extent from ethanol. Thereby, only acetic acid is obtained through fermentation because food purity laws state that for the production of vinegar only acetic acid of biological origin can be used [40, 41]. Table 1 gives an overview of chemicals available from ethanol by chemo-catalytic processes.

Table 1 Chemicals available from ethanol by chemo-catalytic processes

Reaction	Catalyst	Selectivity (%)	Yield (%)	Status	Reference
$CH_3CH_2OH \rightarrow CH_2 = CH_2 + H_2O$	Al_2O_3-based	≥90 Ethylene	≥93	Commercial	[27, 42–44]
$2CH_3CH_2OH \rightarrow CH_2 = CHCH_3 + CO_2 + 3H_2$	Sc/In_2O_3	60 Propylene	60	Laboratory	[45–49]
$3CH_3CH_2OH + H_2O \rightarrow i\text{-}C_4H_8 + 2CO_2 + 6H_2$	$Zn_xZr_yO_z$	55 Isobutylene	55	Laboratory	[50, 51]
$2CH_3CH_2OH \rightarrow CH_2 = CHCH = CH_2 + H_2O + H_2$	$MgO\text{-}SiO_2$	70 1,3-Butadiene	29	Formerly commercial	[41, 52, 53]
$CH_3CH_2OH \rightarrow CH_2 = CH_2 \rightarrow BTX$	Ga-ZSM-5	74 BTX	74	Laboratory	[29, 39]
$CH_3CH_2OH \rightarrow CH_3CHO + H_2$	Cu-cat.	90 Acetaldehyde	90	Commercial	[54]
$CH_3CH_2OH + O_2 \rightarrow CH_3COOH + H_2O$	$MoVNbO_x$	95 Acetic acid	95	Laboratory	[55]
$2CH_3CH_2OH \rightarrow CH_3COCH_3 + CO_2 + 4H_2$	$Cu\text{-}La_2Zr_2O_7$	72 Acetone	72	Laboratory	[56]
$2CH_3CH_2OH \rightarrow CH_3COOCH_2CH_3 + 2H_2$	Cu-cat.	95 Ethyl acetate	95	Commercial	[57, 58]
$2CH_3CH_2OH \rightarrow CH_3CH_2CH_2CH_2OH + H_2O$	Ni/Al_2O_3	80 Butanol	20	Laboratory	[59]
$CH_3CH_2OH + 3H_2O \rightarrow 2CO_2 + 6H_2$	Ni/MgO	95 Hydrogen	95	Laboratory	[60]

Table 2 Current plants for the production of ethylene from bio-ethanol

Location	Company	Start-up year	Bio-ethylene capacity (tons/year)	Final product	Capacity (tons/year)	Reference
India	India glycols limited	1989	Unknown	MEG	175,000	[5]
Brazil	Braskem	2010	200,000	PE	200,000	
Taiwan	Greencol Taiwan Corporation	2011	100,000	MEG EO	75,000 40,000	[64]

3 Mono-ethylene Glycol

Mono-ethylene glycol (MEG) is industrially manufactured via thermal hydrolysis of ethylene and used mainly as an antifreeze agent and as a raw material for the manufacture of polyester. Furthermore, it can be directly produced from carbohydrates such as D-xylose [6]. Low yields (0.29 g/g, based on D-xylose) and MEG concentrations (12 g/L) utilizing GMO *Escherichia coli* at a productivity of 0.24 g/L/h have been reported [61].

Another process route to bio-MEG starts with bio-ethanol which is initially dehydrated to bio-ethylene (drop-in strategy). Thus the bio-ethylene can be converted to bio-MEG utilizing existing process technology [5]. In 2009 Coca-Cola announced their PlantBottle™ packaging initiative with the aim of producing PET bottles from renewable resources [62]. Initially, Coca-Cola is substituting fossil-based MEG with bio-MEG which has resulted in the production of 620,000 tons of bio-PET (14 wt% bio-MEG) in 2011 [63]. Because of the growing demand, bio-PET capacity is expected to increase to 5 million tons/year in 2020 [63]. Table 2 presents an overview of current plants for the production of MEG, EO, and PE.

4 1-Propanol

Industrially, 1-propanol is produced by catalytic hydrogenation of propanal which is derived from the hydroformylation of ethylene. It is mainly used as a solvent, antimicrobial agent, and chemical intermediate [65]. 1-Propanol can also be detected as a product in some *Clostridia* from threonine catabolism, in beer fermentation by yeast, and in propionic acid fermentation by *Propionibacterium acidipropionici* and *Propionibacterium freudenreichii* ssp. *shermanii*. Because of low yields and titers, not a single wild type microorganism can produce 1-propanol in significant quantities for industrial applications [66]. Current research therefore focuses on the microbial engineering of different hosts, such as *E. coli* [67], *S. Cerevisiae*, [68] and *Propionibacteria* [66] to improve 1-propanol production.

The best results have been reported for a recombinant *E. coli* with titers up to 10.8 g/L in fed-batch experiments. Another option to produce bio-propanol could be the drop-in replacement of fossil-based ethylene with bio-ethylene derived from bio-ethanol.

5 Isopropanol

Isopropanol is manufactured industrially in two commercial processes: (1) indirect hydration of propylene with H_2SO_4 via a mixture of mono- and diisopropylsulfate esters and (2) direct hydration of propylene over an acidic heterogeneous catalyst. It is used primarily as a solvent in inks and surfactants, as a chemical intermediate, and as a cleaning fluid [65]. Several microorganisms have been reported for isopropanol production, including wild-type *Clostridium beijerinckii*, engineered *E. coli*, and engineered *Clostridium acetobutylicum* [69]. Isopropanol is produced by reduction using an alcohol dehydrogenase from acetone, which is produced in an ABE-fermentation. The best results have been reported for a recombinant *E. coli* with a titer of 40.1 g/L at a yield of 0.24 g/g [70]. Furthermore, the fossil-based substrate propylene for the industrial production could also be substituted by bio-ethanol-derived bio-propylene to obtain bio-isopropanol.

6 Propanediols (1,2-PDO, 1,3-PDO)

Glycerol (1,2,3-propanetriol) is the most important feedstock for the production of propanediols. It is a natural chemical linker molecule in most animal and vegetable oils and fats and is used in various industries, notably cosmetics, pharmaceuticals, and food [71]. In the past, glycerol was chemically produced from propylene via the epichlorohydrin route, a method which became obsolete because of the rapid development of biodiesel production by the transesterification of vegetable oils or animal fats [71]. For every 9 tons of biodiesel formed, 1 ton of glycerol is obtained [72]. More than 23 million tons of biodiesel were produced in 2013 and, as a result, 2.3 million tons of glycerol came to the market [73]. The production volume far exceeds the glycerol demand and the surplus is presently used mainly for energy production and animal feed [71]. As early as 2004 the US Department of Energy identified glycerol as one of the top 12 building block chemicals that can be derived from biomass and converted to valuable biobased chemicals or fuels [74]. By chemical transformations, such as hydrogenolysis, dehydration, oxidation, and carboxylation, high-value chemicals can be synthesized [75]. In particular, the selective catalytic hydrogenolysis to 1,2-propanediol and 1,3-propanediol has attracted considerable attention in recent years [71, 72, 75, 76].

With a production volume of 2.18 million tons/year [77], 1,2-PDO is extensively used as a monomer for the production of polyester resins. Moreover, it is used as

anti-freezing agent, additive in nutrition products, solvent for coloring, wetting agent in tobaccos, and component of hydraulic fluids [78]. The industrial production is based almost entirely on the selective hydrolysis of propylene oxide [79].

1,3-Propanediol (production volume: 45,000 tons/year [80] is a diol in the production of polyesters, polycarbonates, and polyurethanes. It can be copolymerized with terephthalic acid to the polyesters SORONA® (DuPont) and CORTERRA® (Shell), which are used in the manufacture of carpet and textile fibers, exhibiting unique properties in terms of chemical resistance, light stability, elastic recovery, and dyeability. Currently it is produced chemically from ethylene oxide (Shell process) or acrolein (DuPont process) [72, 81, 82] and is biotechnically based on cornstarch by a patented process of DuPont/Tate & Lyle (see below).

In a future biorefinery, biobased propanediols could be obtained by chemocatalytic as well as biotechnological processes. Glycerol hydrogenolysis is a catalytic chemical reaction that breaks a carbon–carbon or carbon–oxygen bond followed immediately by addition of a hydrogen atom [83]. Besides 1,2- and 1,3-PDO, ethylene glycol can also be obtained through hydrogenolysis of glycerol. Many different catalytic systems have been described as active catalysts in the production of 1,2- and 1,3-PDO from glycerol. They can be divided into non-noble-metal, noble-metal, and modified (acid, base, or metal oxide) noble-metal catalysts (Fig. 4). Only with noble-metal catalysts modified with a metal oxide is 1,3-PDO obtained as the main product with selectivities up to 66% ($Pt/WO_x/AlOOH$) [76, 80, 84]. All other catalyst types predominately form 1,2-PDO with high selectivities (>80%) [71, 76, 85]. In 2008 glycerol hydrogenolysis to 1,2-PDO was adopted commercially in a 30,000-tons/year plant by Senergy Chemical, in 2009 at a 6,500-tons/year plant (2009) by a joint venture of Davy Process Technology and Ashland-Cargill, and in 2012 at a 20,000-tons/year plant by Oleon. It is a two-step process with an acetol (hydroxyacetone) intermediate. In the first step, glycerol is converted in the presence of a copper catalyst to acetol which is then further hydrogenated to 1,2-PDO using a similar catalyst. 1,2-PDO selectivities of >95% and a 99% glycerol conversion have been reported [86].

Furthermore, 1,2- and 1,3-PDO can be obtained by biotechnological processes from different feedstock, such as glycerol and desoxy sugars. The fermentative production of 1,2-PDO can be carried out by two metabolic routes: (1) via lactaldehyde from desoxy sugars (rhamnose, fucose) and (2) via dihydroxyacetonephosphate (DHAP) from arbitrary substrates. The microorganisms used are *Clostridia*, *Enterobacteriaceae*, or yeasts [87]. The research on fermentative production of 1,2-PDO was started by Cameron and co-workers in the late 1980s. A natural 1,2-PDO producer *Thermoanaerobacterium thermosaccharolyticum* reached 1,2-PDO concentrations of up to 9 g/L from glucose [88, 89]. However, a yield of only 50% of the theoretical value required strain optimization. Thus Cameron started extensive research on the engineering of 1,2-PDO-production pathways in microorganisms (e.g., *E. coli*). Based on this work, Cargill and Ashland projected in 2006 the 1,2-PDO production from glycerol [87]. In addition, ongoing work to develop a production process for 1,2-PDO is

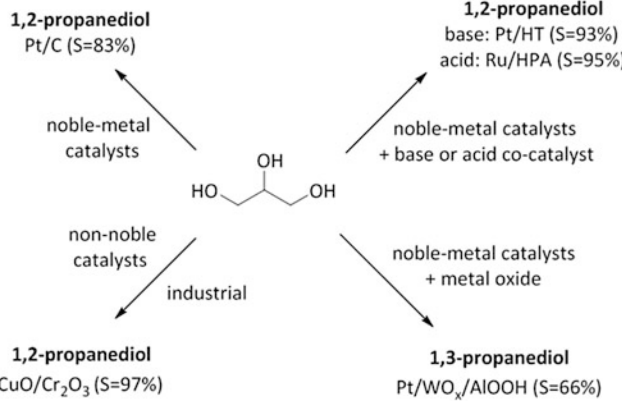

Fig. 4 Selective catalysts for the production of 1,2- and 1,3-propanediol from glycerol [76, 84]

being carried out at the French company Metabolic Explorer (MetEx) [90]. Currently, a titer of 5 g/L can be obtained from sugars with a recombinant *E. coli*.

The microbial conversion of glycerol to 1,3-PDO was first described by August Freund in 1881 [91]. Since then, a multitude of microorganisms naturally capable of 1,3-PDO production have been isolated and characterized. Known 1,3-PDO producers are *Enterobacteriaceae* from the genera *Klebsiella* [92, 93], *Citrobacter* [94], *Enterobacter* [95], *Clostridia* [96], and *Lactobacteriaceae* [97]. Among the wild-type producers *Klebsiella pneumoniae* and *Clostridium butyricum* are currently considered the most promising for application in an industrial process because of high yields and productivities [98]. Both strains can achieve product concentrations of 80–100 g/L, as summarized by Willke and Vorlop [99] and Wilkens [100]. Although *K. pneumoniae* as a facultative anaerobe is easier to handle, it generally has lower yields than *C. butyricum*. Selected results of microbial production of 1,3-PDO are shown in Table 3. It should be mentioned that, based on the European Directive 2000/54/EC, most microorganisms except some *Lactobacteriaceae and Clostridia* are potential pathogens and classified as harmful [101].

The above-mentioned DuPont/Tate & Lyle process uses a recombinant *E. coli*, in which pathways from yeasts (glucose to glycerol) and *Klebsiella* (glycerol to 1,3-PD) are combined [102]. The titer reached more than 130 g/L. The commercial production of 1,3-PD at Tate & Lyle started in 2006. The production plant was expanded in 2011 by one-third to a capacity of 65,000 tons/year. The product is sold under the brand Susterra®. In 2014 another brand of 1,3-PD (Zemea®) received USP-FCC approval for the US market [103] and may now be used in cosmetics, pharmaceuticals, and food products [104].

Chen et al. also constructed a pathway in *E. coli* to produce 1,3-PDO directly from glucose. They expressed the new "de novo" pathway starting from homoserine, a universal precursor in protein metabolism. After deamination to 4-hydroxy-2-ketobutyrate and decarboxylation to 3-hydroxypropionaldehyde, a

Table 3 Selected results of microbial production of 1,3-PDO

Strains	Substrates	Fermentation mode	1,3-PDO (g/L)	Productivity (g/L/h)	Yield (g/g)	Reference
Klebsiella pneumonia[a]	Glycerol (pure)	Fed-batch	74.6	–	0.61	[109]
Klebsiella pneumonia[a]	Glycerol (crude)	Fed-batch	80.2	1.15	0.54	[110]
Klebsiella oxytoca[a]	Glycerol (pure) + sucrose	Fed-batch	83.6	1.61	0.62	[92]
Clostridium butyricum	Glycerol (pure)	Fed-batch	93.7	3.3	0.63	[100]
	Glycerol (crude)	Fed-batch	76.2	2.3	0.62	
Clostridium diolis	Glycerol (pure)	Fed-batch	78.5	2.8	0.64	[111]
Clostridium spec. IK124	Glycerol (pure)	Fed-batch	87.7	1.9	0.54	[112]
Clostridium spec: IK123[a]	Glycerol (pure)	Fed-batch	101	1.9	0.56	[113]
E. coli GMO	Glycerol (pure)	Fed-batch	104	2.6	0.9[b]	[114]
E. coli GMO	Glucose	Fed-batch	130	–	–	[102]
Lactobacillus reuteri	Glucose + Glycerol	Fed-batch	65.3[c]	1.2	0.67[d]	[115]

[a]Risk class: 2 [101]
[b]Two-step process with glucose as growth medium. Yield only related to production phase, glucose not regarded
[c]Plus lactate at 106.5 g/L
[d]Relating to glycerol

final reduction step yields 1,3-PDO [105]. At the same time, three patent applications covering an identical approach from a US and French company were proposed [106, 107] and issued [108]. However the realized product titers of 10–100 mg/L are very low and extensive research is necessary to obtain industrial relevant values.

7 *n*-Butanol

n-Butanol is a versatile four-carbon alcohol that is industrially produced mainly via the oxo-synthesis from petroleum derived propylene. It has an established history as a chemical and solvent, particularly for use in paints, coatings, printing inks, adhesives, sealants, textiles, and plastics [116, 117]. *n*-Butanol can also be used as a fuel and has some advantages over ethanol, such as a higher energy density (29.2 MJ/L vs 19.6 MJ/L), lower corrosiveness, and being more suitable for

Fig. 5 Proposed reaction pathways for the Guerbet coupling of ethanol to *n*-butanol [121, 123, 124]

distribution through existing pipelines [41]. In 2013 the annual global market for *n*-butanol exceeded 3.6 million tons/year and was valued over 6 billion dollars [117].

In the oxo-synthesis, propylene is initially converted over a homogeneous catalyst to butyraldehyde via a hydroformylation with carbon monoxide and subsequently hydrogenated over a heterogeneous catalyst to *n*-butanol. The biphasic Ruhrchemie/Rhône-Poulenc process yields more than 98% propylene conversion per pass and *n*-butanol overall selectivities of ≥94% have been reported using an $RhH(CO)(tppts)_3$ catalyst [116, 118]. *n*-Butanol is also available through the Guerbet reaction from bio-ethanol [119, 120]. The reaction involves the coupling of two ethanol molecules and can take place without a catalyst, but it is strongly catalyzed by hydrogen-transfer catalysts [120]. Over heterogeneous catalysts, two different reaction routes for ethanol self-condensation have been proposed (Fig. 5, Routes A and B). Two features of the catalyst are required for high activity and selectivity in the Guerbet reaction. The first is a basic component in the form of an alkali metal hydroxide or salt dissolved in the reaction medium (homogeneous catalyst) or a solid base (heterogeneous catalyst). The second is the ability to facilitate the dehydrogenation of the alcohol at reaction temperature. Typical dehydrogenating agents are metals, such as Pt, Ni, and Cu. Some non-metals (e.g., MgO) can also catalyze the dehydrogenation at sufficiently high temperatures [121, 122].

In Table 4, selected active heterogeneous and homogeneous catalysts in the Guerbet reaction of ethanol to *n*-butanol are depicted, together with reaction conditions and catalytic results. It should be pointed out that homogeneous catalyst systems require, in addition to an organometallic complex, a stoichiometric amount of sodium ethoxide to convert ethanol to *n*-butanol [125].

Acetone-butanol-ethanol fermentation (ABE) is one of the oldest bioprocesses and many reviews provide an extensive overview of this fermentation process [135–137]. It was commercialized at the beginning of the last century with large production plants being built, first in England and later in Germany, to produce *n*-butanol for the chemical industry. The patented Weizmann process utilizes mainly molasses or corn mash as feedstock. Typical of ABE fermentation is an ABE ratio of 3:6:1. The most used microorganism is *C. acetobutylicum*, but other *Clostridia*

Table 4 Selected active catalysts in the Guerbet reaction of ethanol to *n*-butanol

Catalyst	T (°C)	P (bar)	Conversion (%)	Selectivity (%)	Yield (%)	Reactor mode	Reference
Heterogeneous							
20.7% Ni/Al_2O_3	250	83	25 (72 h)	80	20	Batch	[59]
5% Pd/MgAlO	260	n. a.	17 (5 h)	81	14	Batch	[126]
5% Cu/MgAlO	260	n. a.	9 (5 h)	80	7	Batch	[127]
1% Pd/MgAlO	200	32	12 (5 h)	96	12	Batch	[128]
Ca-HAP	300	1	20[a]	70	14	Continuous	[129]
Sr-HAP	300	1	11[a]	86	9	Continuous	[130]
MgO	450	1	56[a]	64	36	Continuous	[123]
Pd/MgO	250	34	41[a]	77	32	Continuous	[131]
1,8% Cu/Al_2O_3	240	70	12–15[a]	65	8–10	Continuous	[132]
8% Ni/Al_2O_3	250	176	35[a]	62	22	Continuous	[133]
Homogeneous							
Ir-complex + EtONa	120	n.a.	18 (15 h)	67	12	Batch	[125]
Ru-complex + EtONa	150	n.a.	22 (4 h)	94	20	Batch	[134]

n.a.: not available
[a]Steady-state

spp. or engineered *E. coli* are also used. Because of rising fossil oil production after the Second World War, the energy-demanding ABE process was too expensive in comparison to the petroleum-based oxo-synthesis and production was stopped [136, 137]. Since 2006, the rapid rise in oil price as well as the *n*-butanol price has caused a renewed demand for ABE fermentation technology in China. For this reason, 11 new or restored ABE fermentation plants based on corn came on stream before June 2008. Five new plants were also in the planning stage. The overall solvent production from all these ABE plants was estimated to be 1 million tons. At present only one ABE plant is running with a capacity of 40,000 tons/year using a mixture of corn stover and corn cob as feedstock [138].

The economy of the ABE process depends strongly on the feedstock as well as the energy supply because only low product titers and yields can be achieved. This is attributed to the high toxicity of *n*-butanol which limits the butanol titer to 14–16 g/L. Slightly higher values up to 20 g/L are achievable but only with expensive process technologies (see below). The yields from glucose normally reach 0.3–0.44 g/L. The productivities with suspended cells do not exceed 0.47 g/L/h [137]. Moreover, the high boiling point (117 °C) requires energy-intensive and relatively expensive *n*-butanol recovery. However, one advantage of the process is the ability to utilize most of the sugars, including pentoses, and waste and hemicellulosic residues. Many research groups are working on recombinant micro-organisms able to tolerate higher *n*-butanol concentrations and on more efficient downstream processes to lower the toxicity of the process [138–140].

Currently there are also two biotechnological companies which want to provide the market with bio-butanol in the near future using new technologies. Cobalt Technologies from Canada is using a new patented pre-treatment technology for the substrate and a classical mutated strain (patented) in a continuous process with immobilized cells [141]. On the other hand, the company Green Biologics uses a *Clostridium* GMO and agricultural residues, waste, and cellulosic feedstock. They are expected to start commercial production in a retrofitted ethanol plant in 2016 [142].

A potential future feedstock for the fermentative production of *n*-butanol production could be syngas. Syngas fermentation is becoming increasingly attractive in both academic and industrial fields, and the fermentation of syngas to ethanol is already conducted in two demonstration plants (300 tons/year). Nevertheless, a low *n*-butanol yield in the fermentation from syngas poses a technical barrier for industrial production [138, 143].

8 Isobutanol (2-Methylpropanol)

Isobutanol is manufactured industrially mainly through propylene hydroformylation (oxo-synthesis) with subsequent hydrogenation of the aldehydes formed. It is used among other things as a solvent for ink and paints and for the production of esters (e.g., butyl acetate). Isobutanol can be dehydrated with acid catalysts to produce the important platform chemical isobutylene in quantitative yields (annual production volume of fossil-based isobutylene is 10 million tons) [32]. Isobutylene is used almost exclusively for the industrial production of butyl rubber (polyisobutylene) and the fuel additives MTBE and ETBE [144].

Atsumi [145] developed a microbial route (2-keto acid-pathway) in *E. coli*, and produced 22 g/L isobutanol in fully aerobic shake flask experiments (Fig. 6) although, because of NAD(P)H imbalance, at a yield of only 0.35 g/g. Bastian et al. have overcome the imbalance by introducing NADH-dependent enzyme variants and the application of directed evolution, receiving 13.4 g/L isobutanol at the theoretical yield of 100% (0.41 g/g). The NADH imbalance was resolved by over-expression of a transhydrogenase [146].

A relatively new approach is published by a Russian group under the leadership of Tyurin [147], who developed a process based on a UVC-mutated acetogenic *Clostridium* GMO from CO_2 and H_2 (Fig. 6, right). They were the first to report a continuous process which yields more than 50 g/L isobutanol in the culture broth.

The leading company in the production of bio-isobutanol by means of fermentation is Gevo. Their proprietary technology enables the production of bio-isobutanol via an integrated fermentation/separation process. In May 2012, Gevo announced the start of the world's first commercial biobased isobutanol production plant in Luverne, Minnesota, a 54,500-tons/year facility [148]. Except for patents, Gevo has not yet published any process data. One patent describes the route via acetone with a maximal titer of 16 g/L isobutanol at 0.4 g/L/h

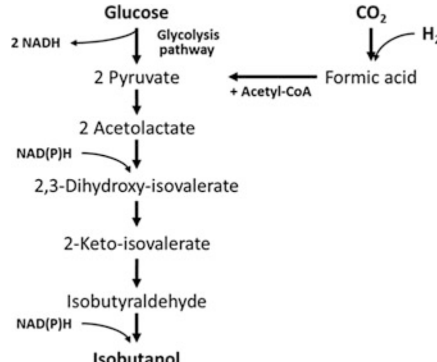

Fig. 6 Engineered microbial route from glucose to isobutanol (2-keto acid pathway), simplified. Introduction of CO_2 and H_2 via acetyl-CoA (engineered *Clostridium* sp.) [147]

[149]. Another patent describes a new process with *S. cerevisiae* (GMO) under aerobic conditions via acetolactate. Here the maximum titer is 12.6 g/L at 71% yield from glucose [150].

Another global commercial isobutanol producer is Butamax, a joint venture of BP and DuPont. They developed a route starting from isobutyraldehyde via acetolactate. This route was expressed in bacteria; however, the titer and yields are rather low. A second approach, patented by Bhalla, utilizes engineered yeasts and oleyl alcohol as extractant, providing up to 55 g/L isobutanol at a yield of 0.3 g/g [151]. This process is planned to run at a retrofitted corn ethanol plant in Minnesota, USA in 2016 [152].

9 2-Butanol

2-Butanol is produced industrially by the acid-catalyzed addition of water to 1-butene or 2-butene with an annual production volume exceeding 550,000 tons/year. Practically all 2-butanol is dehydrogenated to 2-butanone (MEK), an important solvent used for paints and adhesives [116, 153]. It is also available through the fermentation of 2,3-butanediol via the intermediate 2-butanone [154].

The production of 2-butanol from 2,3-butanediol is particularly widespread among *L. brevis* strains. However only titers less than 1 g/L can be achieved [155].

High-level production of 2-butanol has not yet been tested for bacterial systems and only recently for engineered *S. cerevisiae*. Genetically modified yeasts can produce 4 mg/L of 2-butanol from *meso*-2,3-butanediol [156].

10 Butanediols

Butanediols (BDOs) exist in four stable isomers: 1,2-BDO, 1,3-BDO, 2,3-BDO, and 1,4-BDO. Among these isomers, 2,3-BDO, a potential biobased feedstock for the production of MEK and 1,3-butadiene, and 1,4-BDO, with an annual production volume of 1,3 million tons, are the two most important commodities [157, 158]. On the other hand, 1,2-BDO and 1,3-BDO only play a secondary role and the interest of both industry and academia is limited. Industrially, 1,2-BDO is used to produce polyester resins and plasticizers and the main use of 1,3-BDO is as a solvent for food flavoring agents and as a co-monomer in certain polyurethane and polyester resins. Furthermore, biobased 1,2-BDO can be obtained as a by-product in the hydrogenolysis of starches and sugars [157–159].

11 2,3-Butanediol

2,3-Butanediol (2,3-BDO) is useful as raw material for pesticides, pharmaceuticals, plasticizers, fragrances, moisturizing agents, and others. It can exist in three isomeric forms: D-(−)-2,3-BDO, L-(+)-2,3-BDO, and meso-2,3-BDO. Because of its low freezing point of −60 °C, 2,3-BDO may be used as an antifreeze agent and 2,3-BDO or derivatives of 2,3-BDO are used in plastics and solvent production. Dehydrogenation of 2,3-BDO yields diacetyl, a highly-valued flavoring agent in food products, which gives a buttery taste [160] and can serve as a bacteriostatic food additive, inhibiting growth of some microorganisms. 2,3-BDO can be dehydrated to 1,3-butadiene, a multimillion ton bulk chemical, mainly used for the production of synthetic rubber, or to methyl ethyl ketone (MEK, butan-2-one), used as a solvent for resins and lacquers or as a fuel additive. Because of its high octane number, 2,3-BDO can serve as an "octane booster" for petrol. Esters of 2,3-BDO and maleic acid are used for polyurethane maleamides for cardiovascular applications [161].

Other products of 2,3-BDO esterification are used mainly for pharmaceuticals and cosmetics [162].

Harden and Walpole have shown that microorganisms are able to produce 2,3-BDO [163]. Fulmer et al. proposed the industrial production of 2,3-BDO with microorganisms [164]. All investigations and pilot plants were stopped after World War II, as favorable petroleum-based production routes were available [162, 165]. The petrochemical production of 2,3-BDO can be carried using the chlorohydrin route. Therefore the C_4-raffinate from steam cracking of petroleum refining is used as starting material. After the elimination of isobutene the 1- (56 wt %) and 2-butenes (44 wt%) react with hypochlorous acid to form chlorohydrins. These are epoxidized and the resulting butene oxides are hydrolyzed to 1,2-butanediol and 2,3-butanediol. Methyl ethyl ketone is also formed as a

Table 5 Overview of 2,3-BDO production using different microorganisms and substrates

Strains	Risk class	Substrates	Methods	2,3-Butanediol (g/L)	Productivity (g/L/h)	Yield (g/g)	References
Klebsiella pneumoniae	2	Glucose	Fed-batch	150.0[a]	4.21	0.43	[176]
Klebsiella pneumoniae	2	Glycerol	Fed-batch	70.0	0.47	0.39	[177]
Klebsiella oxytoca	2	Glucose	Fed-batch	130.0	1.64	0.48	[169]
Serratia marcescens	1/2[b]	Sucrose	Fed-batch	152.0	2.67	0.41	[178]
Paenibacillus polymyxa	1	Sucrose	Fed-batch	111.0	2.09	–	[179]
Bacillus licheniformis	1	Glucose	Fed-batch	144.7	1.14	0.4	[180]
Bacillus licheniformis	1	Glucose	Fed-batch	115.7	2.4	0.47	[181]
Bacillus amyloliquefaciens	1	Glucose	Fed-batch	92.3[c]	1.19	0.42	[182]
Raoultella planticola	1	Glycerol	Batch	30.7	–	0.5	[183]

[a]10 g/L acetoin
[b]In Germany
[c]22 g/L acetoin
Risk classification is based on the European Directive 2000/54/EC

by-product. The isolation of the products is achieved by rectification [159, 166–168].

In recent years, increased interest in microbial production using renewable resources has been seen. Several yeasts and bacteria from various genera such as *Klebsiella*, *Pseudomonas*, *Serratia*, and *Bacillus* have been reported for microbial 2,3-BDO production [165]. Monosaccharides, hexoses, pentoses, and glycerol can be metabolized to 2,3-BDO. The mixed acid-2,3-BDO fermentation pathway is followed so, in addition to 2,3-BDO, a mixture of acetate, lactate, formate, succinate, acetoin, and ethanol may be formed.

For optimizing microbial 2,3-BDO production, genetically modified microorganisms were constructed. Different strategies, for example enhancement of the carbon flux to 2,3-BDO by eliminating the by-product ethanol [169], protoplast transformation and gene deletion of *B. licheniformis* to produce optically pure D-(−)-2,3-BDO [170, 171], and deletion by suicide vector conjugation [172], have been pursued and genetically modified *S. cerevisiae* [173, 174] or recombinant *E. coli* strains [175] have been constructed. Although these strategies look promising, so far they have not shown beneficial production properties compared to wild-type strains.

Until 2010 the best yields and concentrations were obtained with risk class 2 (pathogenic) microorganisms (*Klebsiella* sp. and *Serratia marcescens* with 150 or 152 g/L 2,3-BDO, respectively). However, because of safety requirements, organisms of risk class 2 are unfavorable for industrial scale processes [162, 165]. Therefore, research is presently focusing on the use of risk class 1 microorganism. In recent years, several organisms generally recognized as safe (GRAS) (*Paenibacillus polymyxa*, *Bacillus amyloliquefaciens*, and *Bacillus licheniformis*) have been reported, which show a comparable 2,3-BDO production in comparison to risk class 2 microorganisms (Table 5).

The highest 2,3-BDO concentrations are achieved using pure glucose or sucrose as carbon source. Nowadays, substrates such as glycerol [177, 183], starch [184], corn stover hydrolysate [183], and hydrolysates [185] of cellulose are under research as raw substrates to produce 2,3-butanediol. There are also attempts to produce 2,3-BDO by non-pathogenic acetogenic *Clostridium* species using carbon monoxide or CO_2 from industrial waste gases or syngas as carbon source [186, 187].

The microbial production of 2,3-BDO, although being quite efficient and well-studied, has still not yet been implemented on an industrial scale. However, LanzaTech uses microbial 2,3-BDO as feedstock for the production of bio-butadiene already in pilot/demo scale [13, 188].

Alternatively, 2,3-BDO is formed in a complex mixture together with mono-ethylene glycol, propylene glycols, and other butylene glycols by catalytic hydrogenolysis of sorbitol. The Chinese company Global BioChem operates a 200,000-tons/year bio-polyol plant in which about 10,000 tons/year 2,3-BDO are produced [189].

12 1,4-Butanediol

1,4-Butanediol is used in the production of solvents, fine chemicals, and high performance polymers, such as spandex fibers and polybutylene terephthalate, and has an annual production of approximately 1.3 million tons [158]. Currently it is manufactured industrially through the hydroformylation of propylene, the hydrogenation of maleic anhydride, the Reppe process from acetylene, the dichlorobutene process from 1,3-butadiene, or the 1,3-butadiene-acetic acid process [190].

In 2004, succinic acid was identified by the US Department of Energy as one of the 12 chemical building blocks that can potentially be produced on a commercial scale through biological conversion [191]. It can be transformed to various interesting products, such as 1,4-BDO [192]. Particularly the hydrogenation of bio-succinic acid to 1,4-BDO has attracted attention in recent years with the biotechnology company BioAmber Inc. developing an industrial process. In 2012 they scaled up their hydrogenation catalyst technology under license from DuPont and converted multi-ton quantities of bio-succinic acid to 1,4-BDO [193].

For the hydrogenation of succinic acid to 1,4-BDO, only a few highly active and selective catalyst systems have been reported. They are based mainly on TiO_2-supported noble metals achieving 1,4-butanediol yields up to 89% with THF and γ-butyrolactone as by-products [192, 194]. For example, an 86% yield is described in a patent from DuPont which uses a 1% Pd-4% Re/TiO_2 catalyst at 200 °C [195]. In a patent from BP the highest yield was 89% with a 5% Re/TiO_2 at 177 °C [196].

A biotechnological route to 1,4-BDO has been patented by Genomatica utilizing engineered *E. coli* [197, 198]. The simplified scheme of the potential metabolic routes as extracted from the patents is shown in Fig. 7. In 2012 the Geno BDO® process yielded 2.300 tons during a 5-week campaign at the PDO Tate & Lyle plant in Loudon, Tennessee. The 1,4-BDO produced was provided to several chemical producers, such as BASF, Novamont, Toray, and Lanxess for further testing. Following the campaign, the Geno BDO® process was licensed to Novamont and BASF in late 2013 [199]. Commercial production was expected to start by the end of 2013 at a former (Ajinomoto) amino acid plant in Adria, Italy, with a capacity of nearly 20,000 tons/year. The plant is still under construction and goes on stream in 2016.

13 Miscellaneous

13.1 Higher Alcohols (>C5)

Alcohols with a chain length of >C5 are labeled as higher alcohols or fatty alcohols. They are mainly obtained industrially via five different processes [200]:

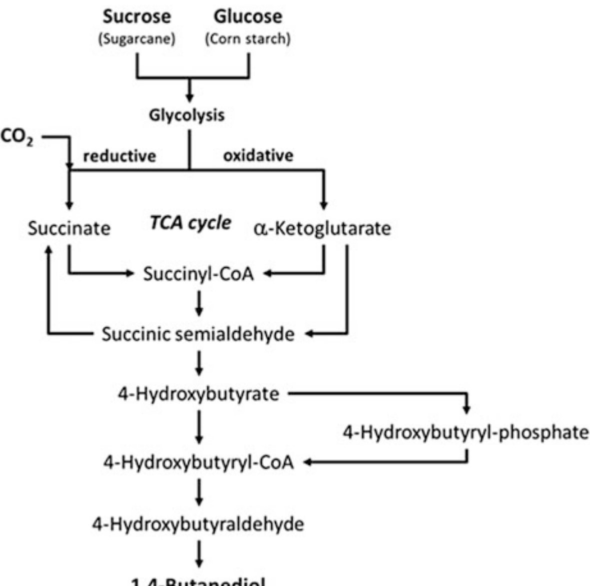

Fig. 7 Simplified scheme of the engineered metabolic pathways for 1,4-BDO production (GENO BDO™ process) in *E. coli*. Modified from [198]

- Hydrogenation of fatty acids and fatty acid esters derived from vegetable and animal fats and oils
- Conversion of ethylene with $Al(CH_2CH_3)_3$ to a mixture of linear, primary alcohols (Alfol or EPAL process)
- Hydroformylation of olefins with CO and H_2 (oxo-synthesis) to a mixture of branched and unbranched aldehydes and subsequent hydrogenation to the corresponding alcohols
- Oxidation of paraffins with boric acid to linear, secondary alcohols
- Condensation of primary alcohols with basic catalysts to α-branched and linear, dimeric alcohols (Guerbet reaction)

Thus higher alcohols are already partially produced from renewable resources. In addition, the substrate ethylene can be obtained from bio-ethanol (dehydration) or bio-methanol (methanol to olefins process, MTO) [2, 44]. A following oligomerization of the ethylene would result in higher olefins which can be further processed in the oxo-synthesis and a succeeding hydrogenation step to higher alcohols [201]. Biomass-derived alcohols, such as methanol and ethanol, can also be used as a substrate for the production of α-branched or linear, dimeric alcohols by the Guerbet reaction [120, 202].

The fermentative production of higher alcohols with natural organisms is not very efficient. Therefore synthetic biology approaches to produce higher alcohols need to increase titers and yields for the realization of industrial processes. Recent work concerning the production of higher alcohols has been reviewed by Lamsen [203]. The authors give a comprehensive overview of several metabolic pathways engineered with common industrially used microorganisms such as *E. coli* or

Table 6 Selected high volume sugar alcohols [204, 221]

Sugar alcohol	Feedstock	Process	Production volume (tons/year)	Reference
Sorbitol	Glucose, sucrose	Hydrogenation	1,700,000	[207]
Xylitol	Xylose	Hydrogenation	161,500	[208]
Palatinit	Sucrose	Two steps: 1. fermentation; 2. hydrogenation	>100,000	Own estimates
Mannitol	Fructose	Hydrogenation	50,000	[209]
Erythritol	Glucose, sucrose	Fermentation	23,000	[204]

S. cerevisiae. In addition, there are numerous research papers whose discussion would go beyond the scope of this chapter.

13.2 Sugar Alcohols

Sugar alcohols, also known as polyols or polyhydric alcohols, with the general formula $H_2(CH_2O)_{n+1}$, are widespread in plants, fungi, and yeasts, sometimes in high concentration. They are produced through hydrogenation or fermentation of mono- or disaccharides [204–206]. Their main application is the replacement of conventional sugars in combination with artificial sweeteners to counter their lower sweetness compared to glucose in the food industry [82]. Furthermore, sorbitol and xylitol were identified by the US Department of Energy as potential biomass-based platform chemicals [74]. Table 6 shows some of the most important sugar alcohols.

Sorbitol is produced with an annual production of 1.7 million tons by the catalytic hydrogenation of glucose or sucrose [207]. It can also be obtained in fermentation processes with *Zymomonas mobilis* or *Candida boidinii*. In particular, the fermentation of high sugar content media with *Zymomonas mobilis* results in coproduction of high yields of sorbitol and gluconic acid [210].

Xylitol is a rare five-carbon sugar alcohol that has potential as a building block for biodegradable polymers, such as poly(xylitol-*co*-citrate) and poly(xylitol-*co*-sebacate) [211, 212]. At industrial level, xylitol is manufactured by chemical hydrogenation of D-xylose [213]. Moreover, it can also be produced in a fermentation process from D-xylose with bacteria, fungi, or yeasts. The feedstock D-xylose is obtained by chemical or enzymatic hydrolysis of hemicellulose [212–217].

Palatinit or isomalt is a mixture of two disaccharide alcohols: gluco-mannitol (α-D-gluco-pyranosyl-1-6-mannitol) and gluco-sorbitol (α-D-gluco-pyranosyl-1-6-sorbitol). It is produced from sucrose in a two-step process starting with a sugar enzymatic transglucosidation into isomaltulose followed by a hydrogenation into isomalt. Aside from being a sweetener, it is also used as a bulking agent, anti-caking agent, and glazing agent [218].

Mannitol is a six-carbon sugar polyol which is an isomer of sorbitol. It is produced by catalytic hydrogenation of fructose which is obtained from inverted sugar or glucose isomerization. A major disadvantage of this process is the low efficiency, yielding only 25% mannitol and requiring a costly and complex purification step. Fermentative processes have been researched extensively. In particular, lactic acid bacteria showed promising results and a complete conversion of D-fructose to D-mannitol in mild conditions has been reported. Besides being a sweetener, mannitol is used as a bulking agent for sugar-free coatings and dusting powder for chewing gum [218].

Erythritol (*meso*-1,2,3,4-butanetetrol) can be found naturally in fruits, vegetables, mushrooms, and fermented foods (e.g., beer and wine). Besides being a sweetener, it is also used in self-tanning agents. Formerly, erythritol was produced by chemical-catalyzed hydrogenation of tartaric acid. The disadvantages of the production process were the complexity and the high costs. A new technology was therefore developed utilizing yeast or fungi to obtain erythritol from the fermentation of sugars [218]. The biotechnological route has been recently reviewed by Moon [204]. Commercial production succeeds by *Moniliella* [219] or by mutants of *Aureobasidium* sp. and *Pseudozyma tsukubaensis*, with high yields and productivities [220].

Moreover, maltitol and lactitol, both disaccharide polyols, exhibit industrial importance. They are manufactured by catalytic hydrogenation of maltose and lactose, respectively. Besides sweetening, maltitol can be used as a fat substitute and lactitol as a thickener and emulsifier [218].

Sugar alcohols can also be used as feedstock to produce short-chain polyvalent alcohols (polyols) through hydrogenolysis. Hydrogenolysis and hydrodeoxygenation can yield ethylene glycol, glycerol, propylene glycols, and butanediols. Glycerol was previously regarded as the most desirable product but is nowadays produced abundantly as a by-product in biodiesel production. As a result, the research focus has shifted to the conversion of sugar alcohols to 1,2- and 1,3-PDO and ethylene glycol [82, 221, 222].

The Changchun Dacheng Group developed a catalytic technology to convert corn-derived sorbitol to a mixture of 1,2-PDO (50–60%), mono-ethylene glycol (25%), and 1,2- and 2,3-BDO (about 25%). After purification, a 5% yield of 2,3-BDO is obtained. A bio-polyol plant based on this new technology has been put into production by the company with an output of 200,000 tons/year [157, 189].

14 Summary

Driven by numerous policies established in the last decade to support the production and use of transport biofuels, both bio-ethanol and biodiesel production increased to 82 and 23 million tons/year, respectively. As a consequence, glycerol, a by-product from biodiesel production, and bio-ethanol were abundantly available as inexpensive feedstock for conversion to chemicals (e.g., mono-ethylene glycol,

Table 7 Current state of the biobased production of the alcohols reviewed in this chapter (combined from the literature and own estimates)

	Carbon number	Alcohol	Status	Production volume (million tons/year)	Process (main feedstock)
Short chain alcohols	1	Methanol	Commercial	0.20	Wood gasification + chemo-catalytic (glycerol)
	2	Ethanol	Commercial	91	Fermentation (sugar-cane, corn, wheat)
		Mono-ethylene glycol	Commercial	0.25	Chemo-catalytic (bio-ethanol)
	3	1-Propanol	Research	–	Fermentation
		Isopropanol	Research	–	Fermentation
		1,2-Propanediol	Commercial	0.115	Chemo-catalytic (glycerol)
		1,3-Propanediol	Research, commercial	Unknown	Fermentation
		Glycerol	Commercial	2.3	Chemo-catalytic (fats/oil + methanol)
	4	1-Butanol	Commercial	Unknown	Fermentation (multiple)
		Isobutanol	Pilot-plant	Unknown	Fermentation (multiple)
		2-Butanol	Research	–	Fermentation
		1,4-Butanediol	Pilot-plant	Unknown	Fermentation (sucrose, xylose)
		2,3-Butanediol	Research/demo-plant	Unknown	Fermentation, hydrogenolysis
Sugar alcohols		Erythritol	Commercial	0.023	Fermentation (glucose, sucrose)
	5	Xylitol	Commercial	0.161	Chemo-catalytic (xylose)
	6	Sorbitol	Commercial	1.7	Chemo-catalytic (glucose, fructose)
		Mannitol	Commercial	0.05	Chemo-catalytic (fructose)
	12	Palatinit	Commercial	> 0.10	Chemo-catalytic + fermentation (sucrose)

1,2-PDO). Furthermore, the interest in butanol and isobutanol as next generation biofuels has led to renewed interest in ABE-fermentation and the development of a proprietary process using GMO yeast to produce isobutanol. However, no commercial process has been realized yet.

In contrast to short chain alcohols, the higher alcohols (>C6) were always partially produced from biomass resources, such as glucose, fructose, and fatty

acids. The predominantly utilized technology for the manufacture of higher alcohols is chemo-catalytic using mostly heterogeneous catalysts. Table 7 gives an overview of the current state of the biobased production of the alcohols reviewed in this chapter.

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Biotechnological Production of Organic Acids from Renewable Resources

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Abstract Biotechnological processes are promising alternatives to petrochemical routes for overcoming the challenges of resource depletion in the future in a sustainable way. The strategies of white biotechnology allow the utilization of inexpensive and renewable resources for the production of a broad range of bio-based compounds. Renewable resources, such as agricultural residues or residues from food production, are produced in large amounts have been shown to be promising carbon and/or nitrogen sources. This chapter focuses on the biotechnological production of lactic acid, acrylic acid, succinic acid, muconic acid, and lactobionic acid from renewable residues, these products being used as monomers for bio-based material and/or as food supplements. These five acids have high economic values and the potential to overcome the "valley of death" between laboratory/pilot scale and commercial/industrial scale. This chapter also provides an overview of the production strategies, including microbial strain development, used to convert renewable resources into value-added products.

Keywords Acrylic acid, Cascade use of renewable resources, Lactic acid, Lactobionic acid, Muconic acid, Succinic acid

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1 Introduction

Biotechnological processes for the production of bio-based chemicals are promising alternatives to petrochemical routes for overcoming the challenges of resource depletion in the future in a sustainable way [1]. The change from a fossil oil-based to a bio-based economy requires the development of innovative utilization processes to exploit fully the potential of biomass. In particular, the cascade use of biomass is essential in the context of efficient utilization strategies, which considers (1) production of food and feed, (2) material use, and (3) energetic use of biomass [2].

It was estimated by Bentsen et al. [3] that around 3.7×10^9 ton (dry matter) of agricultural residues occurred globally per year from barley, maize, rice, soybean, sugar cane, and wheat between 2006 and 2008. Agricultural residues consist of carbohydrates, such as starch, cellulose, and hemicellulose, but also proteins and minerals, which are potential sources of carbon, nitrogen, or phosphorus compounds to be used as nutrients for microorganisms in biotechnological processes [4, 5]. The composition of agricultural residues can be highly variable. Wheat straw, for instance, can contain (w/w) around 40% cellulose, 30% hemicellulose, 20% lignin, 5% proteins, and 5% ash whereas oilseeds consist of around 40% proteins and little cellulose and hemicellulose [5–7].

According to the study by Bentsen et al. [3], the agricultural residues produced yearly between 2006 and 2008 contain 1.376×10^9 ton cellulose and 0.848×10^9 ton hemicellulose. To make the amounts of cellulose and hemicellulose available as feedstocks in biotechnological processes, the recalcitrant structure needs to be broken up and the constituents converted into utilizable nutrients such as glucose, fructose, and xylose. Hydrolysis of the constituents of agricultural residues can be carried out using thermal, chemical, biological, and/or enzymatic methods. The complete hydrolysis of the 1.376×10^9 ton cellulose occurring per year [3] would theoretically give 1.529×10^9 ton glucose. To maintain soil fertility, half of the agricultural residues are left on the field as fertilizer. Nevertheless, 50% of agricultural residues, possessing more than 0.7×10^9 ton glucose in form of cellulose, would theoretically be available for biotechnological processes each year.

Another renewable resource is whey. Whey is a residue from yogurt, cheese, butter, milk, and ice cream production processes and is rich in lactose (4–5%, w/v) and casein (0.6–0.8%, w/v) [8, 9]. For instance, during the production of 1 kg cheese, 9 kg of whey is generated, which is used as animal feed [10]. Castillo [11] estimated that more than 0.145×10^9 ton of liquid whey is produced per year, containing 6,000,000 ton lactose. The great amount of whey generated as a by-product is the reason why it has been used as carbon and nitrogen source in various biotechnological processes for the production of platform chemicals such as ethanol and lactic acid [8, 9].

The strategy of white biotechnology allows the utilization of carbon, nitrogen, and phosphorus compounds even from low value agricultural residues by assimilation in microbial biomass and metabolites. The versatility of microorganisms thereby enables the production of various products, such as food, feed, chemicals, materials, and energy, and allows the utilization of agricultural residues in accordance with the principle of cascade use. The broad range of products obtainable from biotechnological processes, such as alcohols, short- and long-chain organic acids, and polymers, are particularly of interest for the chemical industry as sustainable and green reactants in technical processes [1, 12]. Furthermore, white biotechnology allows not only the conversion of renewable resources into industrially relevant compounds but also the selective production of isomers, such as D(−)- and L(+)-lactic acid [13, 14]. Thus, white biotechnology can produce reactants for highly specialized chemical processes from complex substrates.

Organic acids can serve as feedstocks for many bulk chemicals and polymers [12], and thus are interesting microbial products from a commercial point of view. Therefore, this chapter focuses on the biotechnological production, microbial strain development, and case studies of industrially relevant organic acids from renewable resources. Special emphasis is placed on lactic acid (LA), acrylic acid (AA), succinic acid (SA), muconic acid (MA), and lactobionic acid (LBA), which have great potentials to overcome the "valley of death" between pilot scale and commercial scale, and to be used in the formation of bio-based materials, such as bioplastic and bionylon, and/or food additives. LA and SA are currently produced biotechnologically at commercial scale [15], more research activities being

Table 1 Properties of lactic acid

Molecular structure	
IUPAC name	D(−)-Lactic acid L(+)-Lactic acid 2-Hydroxypropanoic acid
Molecular formula	$C_3H_6O_3$
Molecular weight (g/mol)	90.08
Physical status	D/L-Lactic acid (racemate): liquid
Appearance	Colorless, oily
Melting point (°C)	53
Solubility	Freely soluble in water and ethanol
p*K*a	≈3.8

required to scale up the production of AA, MA, and LBA from laboratory and pilot scales toward demonstration and commercial scale processes.

2 Lactic Acid

LA (2-hydroxypropanoic acid) is one of the most promising platform chemicals [16] with a single chiral center (Table 1), which exists as two isomers, D-LA and L-LA, whereas both of them can be produced biotechnologically. The food, cosmetic, pharmaceutical, and chemical industries have been using lactic acid in many applications, such as pH regulation, antimicrobial agent, flavors, moisturizers, green solvent, and cleaning agent [17]. Furthermore, LA has gained significant attention as a monomer to be used in the production of the biodegradable plastic poly(lactic acid) (PLA), which has the potential to substitute considerable amounts of petroleum-based plastics in the future [18–20] to save fossil carbon resources on the one hand and to contribute to the climate protection goals on the other. LA exists in two isomers, L(+)-LA and D(−)-LA. The pK value of LA is around 3.8 [21]. Although in the synthetic preparation of LA only a racemic mixture of DL-isomers is formed, optically pure isomers can be produced by fermentative processes, depending on the choice of the microorganisms. In PLA synthesis, optically pure lactic acid is required, because small changes in the mixing ratio affect the property of the subsequently produced PLA.

LA is not only an industrially relevant platform chemical and a monomer in PLA production, but also an important product for the bio-based and circular economy. In 2013, the demand of LA was estimated at 714,000 ton. It is expected that the

demand should further increase at an annual rate of 15.5% between 2014 and 2020, based mainly on the demand for bioplastic [22–24].

2.1 Microorganisms for the Production of Lactic Acid

LA can be produced by several microorganisms, such as bacteria, fungi, and yeast [22, 25]. Besides the wide group of *Lactobacilli* [26], other bacteria such as *Bacillus* [27], *Enterococcus* [28], *Lactococcus* [29], *Pediococcus* [30], *Streptococcus* [31], *Candida* [32], and filamentous fungi [33], especially *Rhizopus oryzae* [34, 35], were used as production strains, which are able to convert both hexoses and pentoses into LA.

Different LA producing strains could have significant improvements over the others, such as a broader substrate range, improved yield and productivity, reduction of nutritional requirements, or improved optical purity of LA [22]. In view of complex substrates such as residues and waste materials, the use of mixed cultures in fermentation may also provide useful combinations of metabolic pathways for the utilization of feedstocks containing a mixture of carbohydrates [36–39]. Several genetic-engineering approaches have been exploited to improve fermentation performance such as LA yield and optical purity using various microbial producers [40–42]. An extensive review by Okano et al. [43] provides a broad collection of genetically engineered-microorganisms for LA production, including their characteristics and applicability for fermentation processes.

2.2 Lactic Acid Fermentation Based on Renewable Resources

Worldwide research is focused on the use of renewable raw materials as carbon substrates as well as nutrient sources. An overview of the utilization of different renewable resources for LA fermentation, microorganisms, and yields depending on several process parameters was given by Hofvendahl and Hahn-Hägerdal [44] and Castillo Martinez et al. [18]. In this context, there is strong interest in reducing costs for raw materials and using cheap feedstocks [45].

Lactic acid was produced worldwide at first from glucose or pure starch in fermentative ways. The first efforts in developing bioconversion processes for the production of LA directly from agricultural starchy feedstock were published by Shamala and Screekantiah [46]. During the last few years, starch [47, 48], cassava [49, 50], wheat [51], rice [52, 53], and potatoes [54, 55] have also been tested for their suitability as substrates for LA fermentation.

With respect to the above-mentioned cost aspect of substrates, the utilization of residues and waste materials [56–59] and agricultural by-products [5, 60–62]

became the focus of public attention. Lignocellulosic biomass represents the most abundant global source of biomass, and for this reason it has been utilized in many applications [63–65]. Lignocellulosic materials can be used to obtain sugar solutions that may be usefully exploited for the production of lactic acid through the following steps: (1) pretreatment to break down the lignocellulosic structure, (2) enzymatic hydrolysis to depolymerize lignocellulose to fermentative sugars, (3) sugar fermentation to lactic acid, and (4) separation and purification of the product [66–68]. Besides the carbon source, lactic acid bacteria also need a source of nitrogen and other so-called micro- and macro-nutrients. The standard protein extracts such as yeast and/or meat extract, peptones, etc., are very expensive and their substitution by low-priced nutrient extracts is necessary when large scale production is planned. Although most attention has been paid to the use of plant-based alternatives such as pressed juice out of green biomass [69–71], animal-based materials such as residues from the slaughterhouse could also be used for lactic acid fermentation [72].

2.3 Developments in Lactic Acid Production

Because of the economic pressure and the competition with fossil-based chemicals, there is a pressing need to optimize the entire value chain starting from the feedstock via its pre-treatment and fermentation into LA, up to the downstream processing.

Besides the raw materials and process conditions, modern approaches to introduce new strains with new and/or modified properties can lead to more efficient production of LA. LA can be produced from a wide spectrum of carbon sources as already mentioned (Sect. 2.2) and, together with the cost factor, the competition with food and feed plays an additional role for raw material selection. In this sense, several lignocellulosic materials including wood [73, 74] and agricultural residues have received great attention as possible feedstocks to substitute edible starch material [75]. On the other hand, according to the difficulties mentioned in the mobilization of fermentable sugars, a range of other, easily accessible substrates, such as residues from fruit and vegetable processing, by-products from starch and sugar factories, or from the dairy sector and baking industry, are available.

Together with the need for a low-cost carbon source, there is an additional demand for suitable supplements, which should not cause additional costs and problems connected with impurities. From that perspective, optimization is necessary to find a balance between the substitution of expensive nutrients and the limitation of undesirable components. Therefore, the kind of nutrients as well as the optimization of their concentration is essential. It is likely that one of the future trends in LA production ends up with mixtures of different low-cost raw materials in order to avoid the use of expensive complex supplements [1, 76, 77]. Alternative fermentation strategies (e.g., co-cultivation with microbial strains supplying

essential nutrients) and metabolic engineering are additional tools to resolve or reduce such nutrient requirements [78].

Besides strain optimization and alternative raw materials, the transition from traditional batch fermentation, including repeated batch and fed-batch fermentation, to continuous mode fermentation [79, 80] with cell recycling [81–83] and the use of immobilized cells in different reactor types (fixed or fluidized beds) could lead to further performance improvement of the entire process.

The use of complex alternative raw materials as substrates in fermentation results in the introduction of impurities. To obtain pure LA formulations from fermentation broths, extensive downstream processing is required. Downstream processing can include micro- and nanofiltration, electrodialysis with monopolar and bipolar membranes, and concentration by water evaporation [84]. Another process, which is currently considered as state-of-the-art, is the reaction of LA with $Ca(OH)_2$ and subsequent titration with H_2SO_4. The product is pure LA and $CaSO_4$ as chemical effluent [85].

2.4 Summary

LA has been produced in fermentative processes based on renewable resources. The great opportunity to produce a bio-based plastic from LA makes this monomer an interesting chemical feedstock. However, further research on downstream processing is needed to separate optically pure L(+)- and D(−)-LA from impurities. The number of downstream processing steps including costly water evaporation at the end or the production of chemical effluents strongly influence the quality and price of the product, and the overall sustainability. Thus, further efforts in R&D to achieve the provision of lactic acid biotechnologically and commercially within the lowest cost are needed.

3 Acrylic Acid

AA (prop-2-enoic acid, Table 2) is the simplest unsaturated carboxylic acid and one of the most important industrial chemicals [86]. It is used for the formulation of acrylate esters, superabsorbent polymers, detergents, and dispersants [87]. Currently, 4,200,000 ton of AA are produced worldwide, totally based on fossil oil [88]. The petrochemical route includes the partial oxidation of propene at relatively low yield of 50–60% or via acrolein at a yield of 90% [86]. AA consumption is expected to increase to 6,974,000 ton with a global market value of USD 16,456 million by 2019 [89]. Therefore, new and innovative processes are required to establish a sustainable production of AA and to overcome fossil oil dependence in the future.

Table 2 Properties of acrylic acid

Molecular structure	$H_2C=CH-C(=O)OH$
IUPAC name	Prop-2-enoic acid
Molecular formula	$C_3H_4O_2$
Molecular weight (g/mol)	72.06
Physical status	Liquid
Appearance	Colorless
Melting point (°C)	14
Solubility	Miscible with water
p*K*a	4.25

3.1 Chemical Production of Acrylic Acid from Lactic Acid

To move the production toward sustainability, microbial metabolites produced from renewable resources can be converted into AA. For instance, the dehydration of LA has been shown to result in AA, but also in side products such as aldehydes, acetic acid, and propionic acid [90–92]. Generally, the selectivity of catalytic dehydration reactions to AA is rather low (Table 3). Selectivity in this context is defined as moles of AA obtained per mole of LA applied. Matsuura et al. [93] were able to obtain a selectivity of 63% with an Sr–P hydroxyapatite catalyst at 350 °C, an LA flow rate of 0.45 mL/h and an Ar flow rate of 40 mL/min, which means that almost two-thirds of the reacted LA was converted into AA. It should be admitted here that, in the examples listed in Table 3, conversion was never 100%. Using a catalyst (93% $Ba_3(PO_4)_2$ + 7% α-$Ba_2P_2O_7$) at 380 °C, 93% of the supplied LA reacted, which is considerably higher than the 23% when the reaction was carried out at 450 °C and 100 MPa without a catalyst. In most of the studies listed in Table 3, commercially available LA was used and much higher selectivities (>40%), were achieved in comparison to studies carried out with fermentatively produced LA (<20%) [91–94]. The quantity of side products seems to increase and consequently the selectivity to AA decreases when fermentatively produced LA is used. This is probably because of the presence of remaining salts and sugars in LA formulations, which may affect the performance of applied catalysts [92]. Nevertheless, this hybrid approach is promising as AA produced from fermentatively obtained LA can be considered as a sustainable product.

Table 3 Biotechnological production of acrylic acid from lactic acid by hybrid approaches

Strain	Substrate	Lactic acid (mol)	Acrylic acid selectivity (%)	Catalyst/reaction conditions	Reference
–	–	4.2[a]	63[b]	Sr–P at ≈350 °C	[93]
–	–	–	49[c]	93% $Ba_3(PO_4)_2$ + 7% α-$Ba_2P_2O_7$ at 380 °C	[94]
–	–	0.05[a]	44[d]	450 °C, 100 MPa	[91]
Lactobacillus plantarum	Sugarcane molasses	50.6[e]/ 152[f]	16.7	KI/NaY at 300 °C under CO_2	[92]

[a]Experiments carried out using commercially available lactic acid
[b]72% of lactic acid converted
[c]93% of lactic acid converted
[d]23% of lactic acid converted
[e]Fermentatively produced lactic acid
[f]Concentrated fermentatively produced lactic acid

3.2 Microbial Formation of Acrylic Acid

Because the chemical conversion of LA into AA is energy intensive and has a relatively low selectivity, microbial formation of AA has been investigated as an alternative production strategy. Even when AA is part of the metabolism of many microorganisms, it is immediately utilized and does not appear as a metabolic end product. *Desulfovibrio acrylicus* uses acrylate as electron acceptor and forms propanoate, *Alcaligines faecalis* M3A hydrates AA to 3-hydroxypropanoate, which is then utilized [95, 96]. *Propionibacterium shermanii* forms acrylyl-coenzyme A from AA and converts it into succinyl-coenzyme A via propionyl-coenzyme A [97]. Thus, the biotechnological production of AA at high titer is challenging. Furthermore, AA is toxic and microorganisms used should be able to tolerate high AA concentrations.

Advantageously, the biotechnological production of AA can be based on the conversion of many metabolites derived from sugars because of the versatility of metabolic pathways (Fig. 1). Danner and Braun [90] suggested possible routes to produce AA via biotechnological pathways. One route includes the production of LA and its chemical or biological conversion into AA. The chemical conversion has already been explained. For the microbial conversion, a strain or enzyme that is able to dehydrate LA is needed. For instance, it was found that AA was formed by *C. propionicum* from LA in the presence of 3-butynoic acid, an inhibitor of lactate dehydrogenase [98]. Another route is the formation of propionic acid and its conversion into AA. This route includes the oxidation of propionate, which can be carried out by *P. shermanii*.

Even though several routes are known to produce AA solely by biotechnological processes from renewable resources, the AA concentrations obtained were rather low and reached only 1% of the initial substrate concentration [90, 98]. No significant improvement of direct AA fermentation using viable cells has been reported

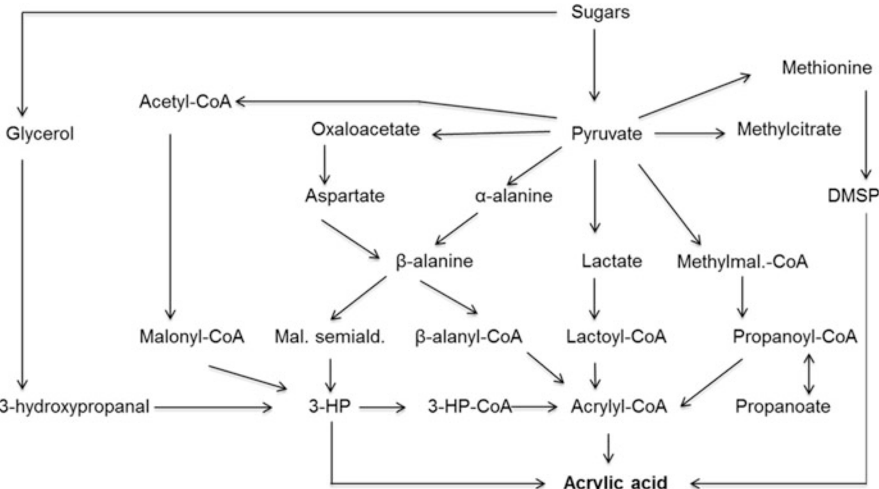


Fig. 1 Possible pathways for the chemical synthesis of acrylic acid from renewable resources derived sugars (*CoA* coenzyme A, *mal. semiald* malonic semialdehyde, *DMSP* dimethylsulfoniumpropionate, *methylmal.* methylmalonyl) [86]

in the last decade. Nevertheless, genetic engineering may provide the tools to create strains that can effectively convert renewable carbon sources into AA and to increase the tolerance toward high AA concentrations. It should, however, be admitted here that Nagasawa et al. [99] reached a concentration of 390 g/L AA in a fed-batch culture using resting cells of *Rhodococcus rhodochrous* strain J1. The resting cells were periodically fed with 200 mM acrylonitrile as substrate for AA formation. The molar conversion yield was 98.5% [99]. This interesting approach has its drawback as it is based on acrylonitrile, which is derived from propylene and a potential carcinogen.

3.3 Summary

Because of the little success to produce AA at considerable concentrations directly from sugars with microbes as biocatalysts, the indirect production via LA is the most realistic approach. LA can be produced from sugars derived from renewable resources. The simplicity of the LA pathway provides an effective opportunity to convert carbon sources from renewable resources in AA. Theoretically, 2 mol LA can be formed from 1 mol glucose, which serves as feedstock for the chemical conversion into AA.

The opportunity to produce LA from renewable resources derived sugars (see Sect. 2) supports the concept of AA production from sustainable resources. Processes to release fermentable sugars from agricultural residues have been investigated, which include acid hydrolysis and/or enzymatic hydrolysis. Another

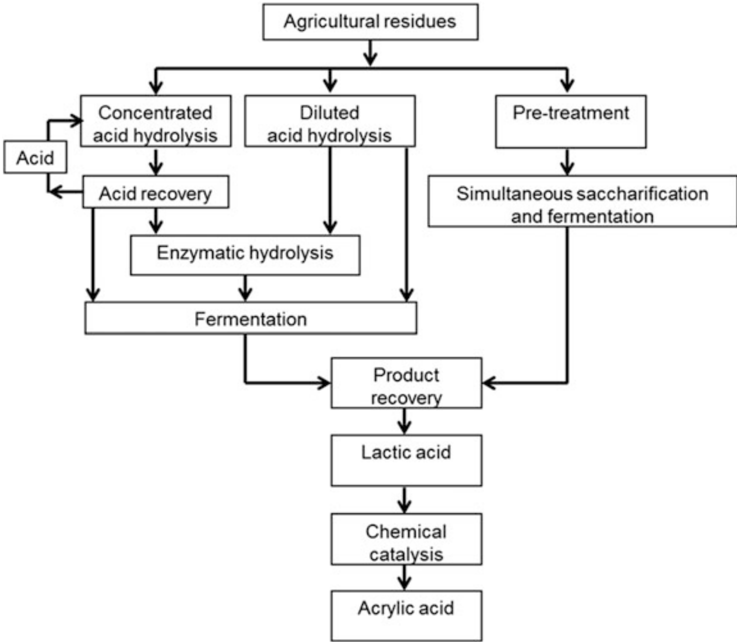


Fig. 2 Conversion of sugars derived from renewable resources into acrylic acid via fermentative lactic acid approach

approach is the pretreatment by mechanical or thermal methods of the agricultural residues, followed by simultaneous saccharification and fermentation (Fig. 2). After recovery and purification, LA can be chemically converted into AA by catalytic processes. However, more research is needed to increase the selectivity of catalytic conversion of LA into AA and to avoid or minimize the presence of unwanted side products which negatively affect the carbon balance.

4 Succinic Acid

SA, a four-carbon dicarboxylic acid (Table 4), was ranked as one of the top 12 promising platform chemicals from biomass by the US Department of Energy in 2004 because of its versatile applications, such as an additive in polyesters, paints, fuel, herbicides, pharmaceuticals, and detergents [16, 100]. Current global production ranges from 30,000 to 50,000 ton/year, and the expected market growth is 654,000 ton valued at USD 3.5 billion by 2020 [101]. The traditional chemical synthesis via hydrogenation of maleic anhydride mainly requires heavy metal catalysts, organic solvents, and high temperature and pressure, which makes the petrochemical-based SA production process costly and ecologically questionable [102]. Additionally, the increasing cost of crude oil and the imminent need for

Table 4 Properties of succinic acid

Molecular structure	
IUPAC name	Butanedioic acid
Molecular formula	$C_4H_6O_4$
Molecular weight (g/mol)	118.09
Physical status	Solid
Appearance	Colorless
Melting point (°C)	184
Solubility	Soluble in water
pKa$_1$ and pKa$_2$	4.2 and 5.6

green and renewable chemicals are the drivers for the demand of bio-based SA. Efforts to expand production capacity of bio-based SA should help foster market growth in the coming years. Bio-based SA production has many benefits such as lower carbon footprint, higher cost efficiency, reduced price volatility, and the ability to reduce dependency on crude oil. Currently, demonstration plants have been built in North America, Europe, and Asia Pacific by several leading chemical companies such as BioAmber, Reverdia, Myriant, and BASF for upscaling the fermentative SA production from renewable feedstock. However, only 3% of succinic acid market volume was bio-based in 2011 [103]. This calls for more research and development on bio-based SA production and particularly on SA production from renewable resources.

4.1 Succinic Acid Fermentation

Fermentative SA can be obtained using either natural producers or metabolic engineered strains [104]. Some of the SA production hosts, such as recombinant *Escherichia coli*, *Actinobacillus succinogenes*, *Mannheimia succiniproducens*, and *Corynebacterium glutamicum,* demonstrated high productivity and yield and have been extensively studied in recent years [105]. However, these strains have certain disadvantages such as being potentially pathogenic, having poor growth, low tolerance toward acidity, osmotic stress, and high glucose levels needed, limiting their industrial applications [106]. Additionally, SA purification from these fermentations requires complicated downstream processing. More recently, research and industrial production have appeared to switch to the use of yeast as production hosts, such as *Saccharomyces cerevisiae*, *Yarrowia lipolytica*, and *Candida krusei*. Yeast is commonly used in organic acid production and its application in bio-based SA production has several advantages [106, 107]: (1) generally regarded as safe

status, which eases waste disposal, product and process approval, (2) genetically and physiologically well-characterized and tools for genetic optimization are established, (3) good tolerance to low pH, which ensures low risk of bacterial contamination and eliminates the need of neutralization of acidic products, and (4) good tolerance to fermentation inhibitors, which might be present in biomass hydrolysates. Its high tolerance toward acidity is a major advantage over bacterial succinic acid production hosts as it allows succinic acid production in free acid form, which enormously facilitates the downstream process and avoids the generation of chemical waste such as gypsum by traditional processes [108]. In this part of the chapter, our focus is on fermentative succinic acid production by yeasts such as *S. cerevisiae*, *Y. lipolytica*, *Zymomonas mobilis*, and *Issatchenkia orientalis* through metabolic engineering (Table 5).

S. cerevisiae is a common industrial host for production of enzymes, pharmaceuticals, nutritional additives, biofuels, and commodity chemicals [106]. Succinic acid can be accumulated by disturbing the tricarboxylic acid (TCA) cycle via metabolic engineering. Raab et al. [109] disrupted the TCA cycle in *S. cerevisiae* to produce SA by deletion of the genes *SDH1*, *SDH2*, *IDH1*, and *IDP1*, which encode succinate dehydrogenase subunits and isocitrate dehydrogenase subunits. In shake flask cultures, this TCA cycle disrupted mutant did not exhibit serious growth constraints on glucose, and resulted in up to 3.62 g/L SA at a yield of 0.11 mol/mol glucose [109]. Construction of a modified pathway in yeast is another strategy to improve SA production [110]. Yan et al. [110] constructed a modified pathway to enhance SA production through the overexpressing of *PYC2*, *MDH3R*, *E. coli FumC*, and *FRDS1* in a *pdc*- and *fum1*-deficient strain of *S. cerevisiae*. This engineered strain produced up to 6.17 g/L SA. Furthermore, succinate production was improved to 8.09 g/L by the deletion of *GPD1*. Under optimal supplemental CO_2 conditions in a bioreactor, the engineered strain produced 12.97 g/L SA at pH 3.8 [110].

Modern genetic engineering approaches, such as metabolic profiling analysis [111], genome scale metabolic network reconstruction [112], and microarray gene transcription analysis [113], were integrated with metabolic engineering to increase SA production. SA was produced intracellularly by elimination of the ethanol biosynthesis pathway of *S. cerevisiae*. To export intracellular SA outside of cells and hence to increase SA production in *S. cerevisiae*, the *SDH1* and *SDH2* genes were deleted and an *mae1* gene encoding the *Schizo saccharomyces pombe* malic acid transporter was introduced [111]. The final engineered strain S149 sdh12/pNV11-mae1 produced 2.36 C-mole SA/C-mole glucose. Otero et al. [112] used a model-guided strategy to predict in silico gene deletion targets and an evolutionary programming method to couple biomass and succinate production. After deletion of *SDH3*, two genes, *SER3* and *SER33*, were deleted to force the yeast to produce glycine from glyoxylate derived from glyoxylate cycle. The mutant coupled SA production with growth by subsequent directed evolution. Moreover, succinic acid production increased by over 60% when *ICL1* gene was overexpressed [112]. Agren et al. [113] demonstrated another model-guided metabolic engineering to obtain low-level SA production at anaerobic conditions by deletion of the

Table 5 Overview of fermentative succinic acid (SA) production by metabolic engineered yeast strains

Strain	Modification	Substrate	Titer (g/L)	Yield (mol SA/mol carbon source)	Productivity (g/L/h)	Reference
S. cerevisiae AH22ura3.	Deletion of the genes *SDH1*, *SDH2*, *IDH1*, and *IDP1*	Glucose	3.62	0.11	0.02	[109]
S. cerevisiae	Overexpression of *PYC2*, *MDH3R*, *E. coli FumC*, and *FRDS1* in a *pdc*- and *fum1*-deficient strain, and subsequent deletion of *GPD1*	Glucose	12.97	0.21	0.11	[110]
S. cerevisiae S149 sdh12/pNV11-mae1	Deletion of the genes *SDH1* and *SDH2*, and overexpression of a *mae1* gene from *Schizo saccharomyces pombe*	Glucose	N/A	2.36[a]	N/A	[111]
S. cerevisiae CEN. PK113-5D	Deletion of *SDH3*, *SER3*, and *SER33*, subsequent directed evolution and overexpression of *ICL1* gene	Glucose	0.9	0.08	N/A	[112]
S. cerevisiae BY4741	Deletion of the inner dicarboxylate mitochondrial transporter (*DIC1*).	Glucose	N/A	0.02[a]	N/A	[113]
S. cerevisiae	Overexpression of phosphoenoylpyruvate carboxylase from *A. succinogenes* or from *M. succiniciproducens*, native malate dehydrogenase glyoxylate shunt enzymes, and malic acid transporter from *S. pombe*,	Glucose	19.5	N/A	N/A	[114, 115]
I. orientalis IoΔura3 + SA	Expression of genes *pyc*, *mdh*, *fumr*, and *frd*,	Glucose	11.63	0.18	0.11	[126]
Z. mobilis ZM4	Deletion of *pdc* and *ldhA*	Glucose	N/A	1.46	N/A	[127]
Y. lipolytica	Deletion of *SDH2* gene in the impaired *SDH1* mutant	Glycerol	45.5	0.28	0.27	[116]
Y. lipolytica H222-AZ2	Exchange of the native promoter of the *SHD2* by inducible promoters	Glycerol	25	0.23	0.15	[117]
Y. lipolytica PGC01003	Distribution of *SDH* gene	Crude glycerol	160.20	0.40	0.40	[125]

[a]The SA yield was calculated by dividing C-mole SA with C-mole glucose

inner dicarboxylate mitochondrial transporter (*DIC1*), which coupled to mitochondrial redox balancing.

Several patents have also been filed specifically on enhancing SA production from *S. cerevisiae* by metabolic engineering [114, 115]. Verwaal et al. enhanced SA production by introduction of a series of genes to modify the metabolic pathways of *S. cerevisiae* [17]. The resulting strain, with overexpression of phosphoenoylpyruvate carboxylase from *Actinobacillus succinogenes* or from *Mannheimia succiniciproducens*, native malate dehydrogenase glyoxylate shunt enzymes, and malic acid transporter from *S. pombe*, produced 19.5 g/L succinate in anaerobic fed-batch fermentation with supplementation of carbon dioxide [114, 115].

Y. lipolytica can utilize a broad range of substrates, such as glucose, glycerol, sucrose, ethanol, and fatty acids, to grow and produce organic acids [107]. Therefore, *Y. lipolytica* has the potential for the overproduction of SA by redirecting metabolic pathways upon gene knockout. The first approaches for metabolic engineering of *Y. lipolytica* to enhance SA production were presented by Yuzbashev et al. [116]. First, a temperature-sensitive mutant with an impaired *SDH1* gene was constructed and then the *SDH2* gene was deleted. Subsequent chemical mutagenesis was applied to increase cell viability and a maximum SA concentration of 45.5 g/L with pH control and 17.4 g/L without pH control were obtained [116]. These amounts of SA were higher than those produced by an engineered *S. cerevisiae* strain with four gene deletions [109]. Jost et al. [117] found that the exchange of the native promoter of the *SHD2* gene by inducible promoters allowed the recombinant strains to accumulate more SA from glycerol. In a 1-L fermentor, the production of SA increased to 25 g/L under oxygen limitation conditions by using a less active promoter in *Y. lipolytica* H222-AZ2.

In contrast with these studies, Kamzolova et al. [118] successfully developed a two-stage process for succinic acid production. First, α-ketoglutaric acid was produced via yeast fermentation using *Y. lipolytica* VKM Y-2412 and subsequent decarboxylation of α-ketoglutaric acid by addition of hydrogen peroxide to SA [118–120]. Under strictly controlled conditions, up to 71.7 g/L succinate was produced from ethanol and 69.0 g/L succinate was produced from rapeseed oil.

4.2 Fermentative Succinic Acid Production from Renewable Resources

To reduce the production costs and promote the bio-based economy of succinic acid production, utilization of cheap raw materials, such as agricultural residues and food waste, was investigated [100, 121]. The annual global production of food wastes is 1.3×10^9 ton [122], and these food wastes are rich in carbon and nitrogen sources. Our previous studies have clearly demonstrated the possibility of SA production from food wastes by *A. succinogenes* [123] or recombinant *E. coli*

[124]. For the advantages in SA production, *Y. lipolytica* was reconstructed as SA producer by our group through the disruption of *SDH* gene. The mutant *Y. lipolytica* PGC01003 prefers glycerol rather than glucose as the major carbon source for SA production [125]. In shaking flask experiments, 5.4 g/L SA was obtained using YPG media containing 2% glycerol, 2% tryptone, and 1% yeast extract, whereas 1.1 g/L SA was produced using YPD-rich media consisting of 2% glucose, 2% tryptone, and 1% yeast extract. Furthermore, fed-batch fermentation was carried out using biodiesel industrial residue crude glycerol as substrate in a 2-L fermentor to increase SA production [125]. The oxygen level was limited by fixing the aeration rate at 2 L/min and the stirring speed at 600 rpm. After 400 h of cultivation with six times feeding with crude glycerol, the final SA production was up to 160.2 g/L with the yield of 0.40 g/g glycerol (62.4% of theoretical yield). The SA average volumetric productivity was up to 0.40 g/L/h with a maximum of 1.36 g/L/h [125].

Several other yeast strains have been shown to produce SA via metabolic engineering. Xiao et al. [126] isolated a low pH tolerance yeast *I. orientalis* SD108, drafted the genome sequence, and used the sequencing information for guide pathway design. An engineered four-gene expression cassette related to the reductive TCA cycle was assembled and integrated into the genome of a uracil auxotroph of SD108, which resulted in 11.6 g/L succinic acid with a productivity of 0.11 g/L/h in batch cultures using shake flasks. *Z. mobilis*, a bioethanol-producing yeast, was also shown to be able to produce succinic acid [127]. Gene deletion targets for increased SA production were predicted by reconstructing a genome scale metabolic model of *Z. mobilis* ZM4. Double gene deletion of *pdc* and *ldhA* was performed to improve SA yield to 1.46 mol/mol glucose, which agreed well with the model simulation [127].

4.3 Summary

To have an economically viable bio-based SA production process, the selection of appropriate production hosts and feedstocks are the two key criteria. Yeasts could be one of the promising hosts for fermentative SA production in combination with metabolic engineering. Genetic modification of yeast for enhancing SA production should be conducted at the genomic level by combining metabolic engineering and evolutionary engineering. Coupled biological and chemical processes would be an alternative strategy to improve SA yield. Thanks to the development of genome, transcriptome, and synthetic biology, rational designs can be used to regulate the global metabolic pathways to improve succinic acid production.

Table 6 Properties of *cis*,*cis*-muconic acid

Molecular structure	
IUPAC name	(2Z,4Z)-Hexa-2,4-dienedioic acid
Molecular formula	$C_6H_6O_4$
Molecular weight (g/mol)	142.11
Physical status	Solid
Appearance	White powder
Melting point (°C)	194–195
Solubility	1 g/L is soluble in water, freely soluble in anhydrous ethanol
p*K*a	2.7 and 4.66

5 *cis*,*cis*-Muconic Acid

Worldwide sales of *cis*,*cis*-MA (Table 6) were worth approximately USD 54 million in 2013 with an anticipated annual growth of 7% [128]. The compound has a high commercial potential when produced from biomass. Integrated biochemical production routes are being developed with glucose and lignin as the first and second generation biomass, respectively as carbon source [129, 130]. To convert glucose into *cis*,*cis*-MA, the substrate is first converted via the shikimic acid pathway to dehydroshikimate, which can be further converted into *cis*,*cis*-MA via, subsequently, 3-dehydroshikimic acid dehydratase (AroZ), protocatechuic acid decarboxylase (AroY), and catechol 1,2-dioxygenase (CatA) [131–136]. The latter three enzymes are heterologously expressed. On the other hand, *Arthrobacter* sp. and *Pseudomonas* sp. can convert benzoate into *cis*,*cis*-MA via the *ortho*-cleavage pathway, which is a part of the ß-ketoadipate pathway [137–144]. To accumulate *cis*,*cis*-MA, further metabolic conversion needs to be prevented by the knockout of muconate cycloisomerase encoded by *catB*. Benzoate is a petrochemically-produced small aromatic compound. Its chemical structure is used as a model substance to study the metabolic conversion of small aromatics which exist in nature derived from depolymerized lignin. Momentarily, the further industrial usage of *cis*,*cis*-MA is hampered by its high price of 27.800 €/kg (Sigma-Aldrich, United States). This is reflected by the fact that the compound is sold as a specialty chemical by Sigma Aldrich (United States) and Alibaba (China). Compared with the top sugar-derived building blocks and new top chemical potentials from biorefinery carbohydrates, *cis*,*cis*-MA is relatively unknown [16]. Of all the *cis*,*cis*-MA produced, 85% is processed into adipic acid, an intermediate for nylon-6,6 [128]. Caprolactam and terephthalic acid are the second and third bulk chemicals which, it is expected, can significantly increase the market for *cis*,*cis*-MA.

5.1 *Microbial Production of* cis,cis-*Muconic Acid*

Various microorganisms have been used to develop an integrated biochemical process to accumulate *cis*,*cis*-MA [130]. The highest concentration has been obtained with *E. coli* [132, 145]. Through heterologous gene expression, the strain was enabled to utilize glucose (USD 300/ton [146], 180 g/mol) and catechol (USD 5,000/ton, 110.1 g/mol) as substrate for the production of *cis*,*cis*-MA. From both compounds a concentration of 59 g/L was reached that corresponded to yields of 30 and 100%, respectively. Because glucose was also used for growth and maintenance, the yield was lower as for catechol. Similar to benzoate, catechol is petrochemically produced. It can, however, be defined as an interesting compound for the microbial production of *cis*,*cis*-MA, as it is an intermediate metabolite for both described conversion routes. From glucose it was even shown to be possible to accumulate catechol in the medium via the shikimic acid pathway [147]. Furthermore, catechol and proto-catechuate form central metabolites of the ß-ketoadipate pathway, which are converted via the *ortho*- and *meta*-cleavage pathways, respectively [140]. Via the *ortho*-cleavage pathway *cis*,*cis*-MA can be formed from catechol in one enzymatic step [148]. Strains containing the ß-ketoadipate pathway, such as *Pseudomonas* sp. and *Arthrobacter* sp., are known for their ability to channel aromatic molecules, which enables the biological funneling of impure aromatics [140, 148, 149]. These kind of conversions can be separated fully from the central metabolism, when glucose or another carbon source is fed separately for growth and maintenance [143, 150]. With the two described strains using toluene (USD 1,100/ton, 92.14 g/mol) and benzoate (USD 1,000/ton, 122.12 g/mol) as carbon source, respectively, a concentration of 45 g/L *cis*,*cis*-MA was reached [138, 139]. For *Arthrobacter* sp., a yield of 96% was described. Hexoses such as glucose can be seen as representatives of the first generation biomass. Aromatic compounds are rather expensive when produced petrochemically. Biorefinery processes are now being developed to depolymerize lignin (USD 145/ton) in small aromatic compounds [149, 151]. As a result, it is expected that such compounds can function as model substrates obtained from lignin, which is a second generation biomass [129]. Large quantities should be accumulated as "waste" from lignocellulose when second generation biofuels produced from C5 and C6 sugars become processed on a commodity scale. The valorization of lignin, as a consequence, plays a crucial role in the economic success of the biofuels industry [152, 153]. It is therefore expected that for *cis*,*cis*-MA a large scale employment in future processes can be sought.

5.2 *Market Potential of* cis,cis-*Muconic Acid*

The "drop in" bulk chemicals adipic acid, caprolactam, and terephthalic acid represent applications for which *cis*,*cis*-MA can be used (Fig. 3). To express the

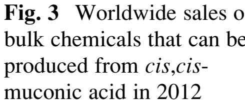
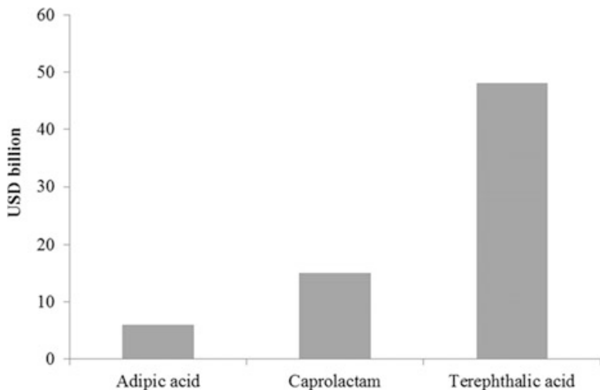


Fig. 3 Worldwide sales of bulk chemicals that can be produced from *cis*,*cis*-muconic acid in 2012



market potential of integrated biochemical routes relative to the petrochemical routes, processes have to be compared economically and ecologically in more detail. Prices of organic acids given in this section are based predominantly on information provided by Alibaba.com in May 2015.

5.2.1 Adipic Acid

Worldwide sales of adipic acid were nearly USD 5.5 billion with a production of 3,200,000 ton in 2012. The predicted annual growth rate (CAGR) and volume are expected to grow gradually until 2018 at 5.8 and 4.5%, respectively [128]. Around 85% of the worldwide volume of adipic acid is converted together with hexamethylenediamine (HMDA) to nylon-6,6 [154]. This fiber was discovered by Wallace Carothers in 1935 who was working for Du Pont (USA) [155]. To lower the environmental burden for production of adipic acid, new non-fossil-based raw materials are being investigated. In this context it was shown that a purified fermentation broth, containing 150 mM *cis*,*cis*-MA, could easily be hydrogenated into adipic acid. A yield of 97% was reached in the presence of 10% Pt on activated carbon (5% mol/mol) as catalyst, with H_2 (34 bar) for 2.5 h at 25 °C [131, 133, 144, 156–158]. Additionally, it was shown that HMDA (USD 3,000/ton) can be obtained by catalytic conversion of *cis*,*cis*-MA at a yield of 58% via hydrogenation of adipic acid to 1,6-hexanediol followed by an amination with ammonia [159, 160]. If the *cis*,*cis*-MA production path is followed, the demand for *cis*,*cis*-MA could be increased significantly in relation to the production of nylon-6,6. This can be underlined by the market value of USD 5 billion for HMDA [161]. In addition to nylon-6,6-based resins and fibers, adipic acid can be used in polyurethanes, non-phthalate plasticizers, and new polyesters such as biodegradable ones. In general, nylon-6,6 (USD 3,000/ton) is made from adipic acid of high purity, and adipic acid with a lower purity is mainly used for the production of polyurethanes (USD 2,000/ton) [154, 162]. Companies that produce adipic acid are, among others, Invista, Ascend, and Honeywell in the United States, BASF in Germany, Radici in

Italy, and China Shenma and PetroChina in China. The average price was about USD 1,600/ton in 2012. Of the global adipic acid production capacity, 93% is produced by oxidation of cyclohexane to KA oil (cyclohexanone and cyclohexanol), followed by oxidation with nitric acid [163, 164]. In recent years, Rennovia, Verdezyne, BioAmber, Celexion, and Genomatica in the United States developed new processes for the production of bio-based adipic acid. Given the relatively low price of glucose at USD 300/ton [146] (180.16 g/mol) compared to cyclohexane at USD 1,250/ton (85.16 g/mol), bio-based processes are highly interesting. Furthermore, N_2O is formed when KA oil is oxidized to adipic acid by nitric acid. The greenhouse gas N_2O has a life time of 120 years and a 310 times stronger IR absorption compared to CO_2 [165]. The compound also causes environmental problems as it is converted to NO, which destroys the ozone layer and causes acid rain. To avoid N_2O emission, abatement techniques can be introduced in petrochemical processes [166]. In cases where bio-based resources are used as substrate, a completely new process can be generated. Based on a limited life cycle assessment (LCA), it was calculated that the CO_2 equivalent (CO_{2eq}) emission can be reduced by 65–80%, when adipic acid is produced from sugars or lignin, respectively [129, 165]. The first commercially implemented process concerns the established chemical catalytic process of Rennovia, which reached a yield of 65% from glucose [161]. A two-step chemical catalytic process was developed, which involves an oxygenation and a hydrogenation [167–169]. Under high pressure and temperature, glucose is converted via glucaric acid in adipic acid. Based on this process, it has been determined that the operational (OPEX) and capital expenditure costs (CAPEX) can be reduced significantly. Specifically, the capital costs can be reduced by 20%, and the utilities and manufacture costs can be reduced by 15 and 30%, respectively. The yield in combination with the high molar weight of glucose are crucial parameters, which define the profitability of the process. Given the described low yield in the case where whole cell biocatalysis is applied, the application of metabolic engineering is a central issue in developing a platform process with sugars. Another commercial applied process was developed by Verdezyne. By whole-cell biocatalytic conversion with *Candia tropicalis*, adipic acid is directly produced from alkanes and fatty acids via the ω-oxidation pathway [170, 171]. Depending on the carbon chain length of the substrate, the number of carbons can be reduced by the ß-oxidation pathway to obtain adipic acid.

5.2.2 Caprolactam

The market value of caprolactam was estimated at USD 14.5 billion for 4,600,000 ton in 2012 [172, 173]. The CAGR is 3.9% for the period 2014–2019. The average price was about USD 2,500/ton in 2012 and 2013 [174]. Caprolactam is used for nylon-6 fibers and resins used in packaging, electrical goods and electronics, consumer goods and appliances, and automotive products [175]. To get functionalities similar to those of nylon-6,6 and to avoid the patent for its production, the production of polyamide nylon-6 based on caprolactam was

developed in 1938 by Paul Schlack from IG Farben (Germany) [176]. Examples of companies that produce caprolactam include BASF in Germany, DSM in the Netherlands, UBE Industries in Japan, Honeywell International in the United States, and Sinopec, China Petrochemical Development Corporation, China Petroleum & Chemical Corporation, and Shandong Haili Chemical Industry in China [172]. Most of the caprolactam is synthesized from cyclohexanone (USD 1,800/ton, 98.15 g/mol), which is catalytically produced from phenol [177]. After addition of $NH_2OH \cdot H_2SO_4$, cyclohexanone is converted to its oxime, which is further converted into caprolactam via the Beckmann rearrangement by addition of H_2SO_4. In the last step, ammonia is added for neutralization, leading to ammonium sulfate production. To optimize the process, reduction of the production of ammonium salts was tried [178, 179]. When looking at an LCA assessment concerning the production of caprolactam from sugars, it was predicted that greenhouse gas savings can be as much as 95% [180]. To produce caprolactam from biomass, a yield of 55% was obtained from 70 mM *cis*,*cis*-MA in the presence of 5% Pd-Al_2O_3 (5% mol/mol of Pd) as catalyst, dissolved in dioxane (0.07 M) together with ammonia (3.4 bar) and hydrogen (34 bar) at 90 bar, 250 °C for 2 h [181]. Caprolactam can also be produced from adipic acid, whereby a yield of 64% was generated in the presence of 5% Ru-Al_2O_3 (5% mol/mol) as catalyst in tetrahydrofuran with ammonia (3.4 bar) and hydrogen (69 bar) at 250 °C for 2 h [182].

5.2.3 Terephthalic Acid

In total, 43,600,000 ton of terephthalic acid were produced worldwide in 2012 [183]. With an average price of USD 1,100/ton in 2012 and 2013, this represents a market value of USD 48 billion [174]. A CAGR of 5.3% is foreseen until 2020 [183]. Half of this chemical (50%) is converted to polyester fibers and 33% to polyethylene terephthalate (PET) [184]. Dickson and Whinfield, who worked for the textile company Calico Printers Association (UK), discovered the production and usage of this fiber in 1941 [185]. Terephthalic acid is produced via the Amoco process [186]. *p*-Xylene (USD 1,000/ton [187], 106.16 g/mol) is dissolved in acetic acid and oxidized by air in the presence of a catalyst (mostly a combination of cobalt, manganese, and/or bromine). Many companies are involved in the production of terephthalic acid. As an alternative bio-based process, *trans,trans*-MA and *cis*,*trans*-MA can be used. Via a thermal inverse electron demand Diels-Alder reaction, *trans*,*trans*-MA (1 equiv.) and acetylene (USD 2,090/ton [188], 26.04 g/mol) (10 equiv.) can be converted to cyclohexa-2,5-diene-1,4-dicarboxylate (PI) in 1:1 ratio [189]. After flushing with nitrogen, *trans*,*trans*-MA and acetylene are charged and heated at 200 °C for 12 h at 34 bar. The PI formed can easily be oxygenated to terephthalic acid by exposure to air. The preliminary LCA case study for PET shows that the CO_{2eq} emissions are not reduced by this conversion in relation to its petrochemical production [190]. However, the feedstock had a great impact on the overall assessment. Therefore, it is expected that the outcome for the environmental impact can be reduced when agricultural waste products are used. As

indicated, *cis*,*cis*-MA is formed by degradative enzymes from catechol. To obtain the other isomers, it was shown that 35.2 mmol *cis*,*cis*-MA was converted in 4 h to *trans*,*trans*-MA with a yield of 90% in a reflux process, dissolved in tetrahydrofuran (44 mL/0.8M) with 5% H_2O in the presence of I_2 (45 mg; 0.5 mol present) as catalyst [191]. In another approach, *cis*,*cis*-MA was isomerized in 1.25 h into *cis*, *trans*-MA at pH 5 at 60 °C, 2N sulfuric acid being added over half an hour [132]. Furthermore, *cis*,*cis*-MA and *cis*,*trans*-MA were converted into *trans*, *trans*-MA with a yield of 80%. This was done in 50 mL methanol and 50 g MA in the catalytic presence of 5% Pd/C. After 1 h reflux the Pd was removed at room temperature by filtration. Next the volume was reduced by 50% by evaporation, the volume being adjusted by the addition of acetonitrile. To precipitate *trans*,*trans*-MA, the residual methanol was removed by evaporation under reduced pressure. Then the product was washed with acetonitrile and extracted by filtration.

5.3 Summary

The opportunity to develop processes for the production of MA from lignin paves the way for the biotechnological utilization of the lignocellulosic materials-derived components which are predominantly burned under current practice. By converting lignin into MA, products with a 10–20 times higher value than lignin can be created. Furthermore, expensive aromatic substrates can be substituted by lignin-derived phenolic compounds. With further microbial strain and process optimizations, the concentration of MA obtained from lignin-based biotechnological processes might reach an industrially relevant value and allow the utilization of lignin at an industrial scale.

6 Lactobionic Acid

LBA is a carboxylic acid (Table 7) produced by lactose oxidation (Fig. 4). Because of its versatile properties as antioxidant, chelating, humectant, and emulsifying agent, LBA has attracted growing attention on the market with a high potential for application in the pharmaceutical, food, cosmetic, and chemical industries. The advantages that lactose is available in surplus and its low cost promote the research on novel and innovative processes for producing value-added lactose derivatives, such as lactitol, lactulose, lactosucrose, LBA, and galacto-oligosaccharides [192].

It was first reported by Stodola and Lockwood [193] that some *Pseudomonas* strains are able to form LBA by oxidation of lactose (Fig. 4). Nowadays, however, LBA is produced by chemical synthesis, which requires expensive metal catalysts and is energy intensive. However, as the commercial relevance of LBA grows, its biotechnological production also receives growing attention. The possibility of

Table 7 Properties of lactobionic acid

Molecular structure	
IUPAC name	(2*R*,3*R*,4*R*)-2,3,5,6-Tetrahydroxy-4-[[(2*S*,3*R*,4*S*,5*R*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)-2-tetrahydropyranyl]oxy]hexanoic acid
Molecular formula	$C_{12}H_{22}O_{12}$
Molecular weight (g/mol)	358.30
Physical status	Solid
Appearance	White powder
Melting point (°C)	128–130
Solubility	Freely soluble in water, slightly soluble in anhydrous ethanol and methanol
p*K*a	3.6

Fig. 4 Schematic biocatalytic oxidation from lactose to lactobionic acid [196]

utilizing renewable and/or waste material for LBA production further increases the interest of the biotechnological route, both environmentally and economically.

6.1 Application of Lactobionic Acid

Because of its non-toxic and biodegradable characteristics, LBA is widely used in the pharmaceutical and chemical industries. LBA is used as the major ingredient of cold-storage solutions to preserve organs prior to transplantation, known as "University of Wisconsin solution" because of its metal-chelating property. In the field of drug-delivery systems, LBA is used as nanoparticle vehicle in clinical

chemotherapy targeting liver cancer and as carrier for calcium supplementation. In addition, LBA has been used as a novel magnetic resonance imaging contrast agent. The functionalized LBA can efficiently detect liver cancer cells in complex matrices such as blood. LBA proves to be the key active compound of novel anti-aging and regenerative skin-care products, resulting in its commercial application as a cosmetics ingredient. Moreover, LBA exhibits strong moisturizing and antioxidant properties, preventing photoageing. Compared to classical hydroxyacids such as glycolic acid, LBA displays the absence of both skin irritation and skin barrier impairment [194].

LBA is used as a starting chemical for detergent production. Especially LBA amide compositions, formed by reacting LBA with mixtures of primary fatty acids, show good foam stabilizing, cleaning, emulsifying, and softening properties [195]. More applications of LBA in the chemical industry include its use as a sugar-based surfactant, as an additive in anti-corrosive coatings, and as a reactant for synthetic amides [196].

A novel antibacterial agent containing LBA is also reported, which is suitable for use in foods. These compositions have excellent antibacterial properties at low concentration and very little flavor, aroma, or color. The use of calcium lactobionate as food preservative in the USA has been approved by the Food and Drug Administration, but the approval by the European Food Safety Agency is still pending because of the lack of assessment of its long-term effect on human health [196]. More potential uses for LBA as food additives include filler in cheese production, calcium carrier in food and beverages, and gelling agent in dessert products.

6.2 Biotechnological Production of Lactobionic Acid

At present, LBA can be produced chemically via the oxidation of lactose with bromine and by electrocatalytic oxidation of lactose in alkaline medium at platinum and gold electrodes [197, 198]. The biotransformation of lactose to LBA is conducted by means of specific enzymes or by using microorganisms. The enzymatic approach of LBA production involves two different steps: (1) the cultivation of the enzyme source and (2) cell purification and subsequent cell-free biotransformation. The enzymatic transformation, however, can only be performed with refined lactose. On the other hand, the microbial whole cell conversion enables the use of cheaper feedstock, such as cheese whey. However, the longer cultivation time and lower yield are still challenging. This review focuses on "green" production of LBA, aiming at yield and productivity improvements.

The microbial production of LBA was first discovered by Stodola and Lockwood [193]. From the 15 *Pseudomonas* species investigated, only *Pseudomonas graveolens* showed the ability to oxidize lactose to LBA. Within 165 h, 75% of the initial lactose was converted in rotating drums. LBA production by filamentous fungi *Penicillium chrysogenum* was observed by Cort et al. [199] and by marine red

alga *Iridophycus flaccidum* by Bean and Hassid [200]. However, LBA appeared to be an intermediate in lactose utilization, as it was also subsequently used. This consumption phenomenon was also determined by Kluyver et al. [201] by *Pseudomonas* strains, where LBA slowly disappeared under prolonged nutrient starvation conditions in the latter stages of cultivation (>16 days). Nevertheless, this issue is negligible, as the formation rate of LBA is much higher than its degradation rate, especially in short-term cultivation processes [202].

In *Pseudomonas* sp., a membrane-bound dehydrogenase enzyme first catalyzes the oxidation of lactose to a lactone intermediate (lactobiono-δ-lactone) using flavin adenine dinucleotide (FAD) as electron transfer system. The lactone intermediate is subsequently hydrolyzed by a lactonase into LBA (Fig. 4). The process is generally carried out at 25–50 °C and at a pH of around 6. The product formed can be lactobionic acid or its salts. When lactobionate salts are produced, the solution is passed through cation exchange resins to obtain LBA solutions which are then further concentrated and crystallized to pure LBA [196].

Currently, several microorganisms are under investigation for their suitability as LBA producer. These are *P. aeruginosa* [193], *P. taetrolens* [203], *Pseudomonas* sp. [204], *Burkholderia cepacia* [205], *Acetobacter orientalis* [206], and *Halobacterium saccharovorum* [207]. Among these microorganisms, *P. taetrolens* has recently been studied to convert whey to LBA. However, the pathogenicity of *P. aeruginosa* and *B. cepacia* makes them unsuitable for food production and discourages any further industrial implementation.

Miyamoto et al. [204] isolated a bacterial strain able to convert LBA from whey by screening 300 soil samples. This strain was identified as *Pseudomonas* sp. LS13-1. The addition of 5 or 10 g/L peptone enhanced LBA production and shortened the cultivation time. The effect of various whey concentrations was also studied, showing that the production of LBA increased with increasing whey concentration. In an experiment, 197 g/L of LBA was produced from 200 g/L lactose with calcium carbonate to control the pH above 5.0. Fed-batch cultivation showed a high LBA concentration of 175 g/L when *Pseudomonas* sp. LS13-1 was grown in the presence of 207 g/L whey and three intermittent additions of 69 g/L whey after 180 h cultivation.

LBA was described as non-growth associated product and the production followed concomitantly after the growth phase [203]. The production of LBA using *P. taetrolens* and whey was signaled by an increase in pH and a drop in dissolved oxygen concentration during the exponential growth phase. The pH decreased again as LBA was produced (two-stage pH shift). The higher the initial biomass concentration, the higher the productivity. A high inoculation level of 30% (v/v) increased the productivity to 1.12 g/L/h. Coupling a two-stage pH shift with a 30% (v/v) seed culture resulted in an LBA production of 42.4 g/L within 32 h. With this two-stage pH shift, the pH was uncontrolled above 6.5 during the growth phase and maintained at 6.5 during cumulative production. At pH values lower than 6.0, *P. taetrolens* showed reduced cellular proliferation and a subsequent delay in the onset of the LBA production. The behavior and bioconversion from *P. taetrolens* were also negatively influenced by pH values over 7.0 [208].

Alonso et al. [209] studied the effect of dissolved oxygen and agitation rate on the production of LBA. Although the cell growth increased with increasing aeration rate, the use of high aeration rate was detrimental to LBA production. The optimal aeration ratio for a complete lactose bioconversion was 0.5–1.5 L/min. Likewise, high agitation schemes stimulated the cell growth of *P. taetrolens*, increased pH shift values and the oxygen uptake rate by cells, but LBA production was negatively affected. The best culture performance and the best LBA volumetric productivity were obtained at 350 rpm.

LBA microbial production was further studied by optimizing the physical heterogeneity of *P. taetrolens* seed culture using a multi-parameter flow cytometry approach [210]. "Old" seed cultures (older than 12 h) used in bioreactor cultivation displayed poor productivities because of the presence of damaged and dead cells in these cultures. To achieve a high yield of LBA concentration, fed-batch cultivations with different feeding strategies were studied: co-feeding, continuous and multi-pulse feeding, coupled with multi-parameter flow cytometry to monitor the cell physiology [211]. Unlike the other previous studies that used diluted whey, the fed-batches were conducted with concentrated whey as substrate. The best result was obtained using a fed-batch culture under co-feeding conditions: 164 g/L LBA with 2.05 g/L/h volumetric productivity. The co-feeding consisted of a combination of multi-pulse feeding with lactose and continuous feeding with concentrated whey, yeast extract, and peptone.

6.3 Summary

The versatile application of LBA makes the production of this lactose derivative very profitable. Presently, this product is mainly produced via chemical synthesis. However, the recent advances in LBA bio-production, especially with whey as feedstock, enable this process to compete with conventional methods.

7 Conclusion and Future Tendency

The strategies of white biotechnology allows the utilization of recovered nutrients from renewable resources for the production of a wide range of bio-based, value-added, and industrially relevant compounds. The fact that renewable resources are available in large amounts and at low costs makes them particularly interesting as nutrient sources in biotechnological processes. The use of renewable resources may thereby contribute to creating economically feasible processes and overcoming the "valley of death" between laboratory and pilot scales to commercial and industrial scales. It has been shown in this chapter that renewable resources have been used for the production of LA, AA, SA, MA, and LBA, which find use as monomers for bio-based material and/or as food supplements. Particularly for LA and LBA,

production strategies based on renewable resources have already been developed, which allow a final product titer higher than 100 g/L. On the other hand, the biotechnological production of AA, SA, and MA still needs further research and strain development to obtain high titers and effectively utilize renewable resources. It is expected that renewable resources can play an important role as nutrient sources in biotechnological processes in the future because of increasing environmental concerns regarding the petrochemical industry and products, as well as the finite supply of fossil oil.

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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

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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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Bioplastics

Hans-Josef Endres

Abstract The number of newly developed bioplastics has increased sharply in recent years and innovative polymer materials are increasingly present on the plastics market. Bioplastics are not, however, a completely new kind of material, but rather a rediscovered class of materials within the familiar group of materials known as plastics. Therefore, existing knowledge from the plastics sector can and should be transferred to bioplastics in order to further increase their performance, material diversity and market penetration.

Keywords Biobased, Biocomposites, Biodegradability, Bioplastic types, Bioplastics, Processing, Properties, Utilization

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1 Introduction

The number of newly developed bioplastics has increased sharply in recent years and innovative polymer materials are increasingly present on the plastics market. Bioplastics are not, however, a completely new kind of material, but rather a rediscovered class of materials within the familiar group of materials known as plastics.

The first polymer materials synthesized by man were all based on renewable materials or on transformed natural materials (e.g., caseins, gelatine, shellac, celluloid, cellophane, linoleum, rubber, etc.) because at that time there were simply no petrochemical materials available. Apart from a few exceptions (cellulose and rubber-based materials), these first bio-based plastics were almost completely displaced from the middle of the last century onward by petrochemical polymer materials.

Bioplastics are now experiencing a renaissance: this is particularly because of ecological aspects as well as limited petrochemical resources and also, in part, innovative property profiles. This is combined with an increasing awareness amongst the public, politicians, industry and, in particular, research and development.

At the start of the 1980s, the then newly developed biopolymers were at first received with euphoria as possible future polymer materials which were non-reliant on oil. However, because of the as yet generally very underdeveloped material properties and unsatisfactory cost/benefit relationships, disillusionment soon set in. This was, however, followed by continuous successful development and improvement of innovative biopolymer materials. In recent years, bioplastics have shown a dynamic market growth – in double figures each year – although still at a relatively low level in comparison to the volume of the plastics market as a whole.

In Europe and America in particular, the early development and use of biopolymer plastics were concentrated almost exclusively in the area of compostable packaging and other products with a short lifespan. Initially posed in Asia, the question of the availability of the raw materials is now also being pushed increasingly into the foreground in Europe.

Biopolymers are, however, still very much at the start of their development. As with conventional plastics, future material developments should concentrate not only on new monomers or new kinds of polymers but also on the further development of existing polymers through the creation of copolymers and terpolymers, blending and additivation. The relationships between microstructural composition and macroscopic processing, applications, and disposal properties apply in exactly the same way to biopolymers as they have always applied to conventional plastics. This means that the extensive wealth of experience which has already been gained in the field of conventional plastics can – and indeed should – be utilized.

In this chapter, bioplastics are not only described and discussed exclusively in the narrow sense of *bio-based* materials but in a very general context. This is done for comprehensiveness but also because, in most cases, the materials used in practice are combinations, that is, copolymers or blends of bio-based and petrochemically-based monomers or polymers and additives such as plasticizers.

2 State of the Art

Unfortunately, a range of different terms are also in use for bioplastics, such as biopolymers, green plastics, ecoplastics, etc., and at the same time there is varying and confusing discussion regarding the correct usage of these terms.

This discussion concerning the nomenclature of bioplastics is strongly reminiscent of the beginnings of conventional plastics, when a range of terms in various languages were discussed over many years. The fast technical development of plastics, however, rapidly overtook these early discussions on terminology. In the end, the term “Kunststoff” became accepted in the German language and in English the name “plastics.” Whilst for the German name “Kunststoff” it was the synthetic, artificial creation of these materials which was definitive, it was the plastic behavior of these materials which formed the foundation of the Anglo Saxon term [1]. In German usage this has analogously led to the term “Biokunststoff,” whilst English speakers mainly refer to “bioplastics.”

Whilst the word “plastics” offers no opportunity for misunderstanding, the syllable “bio” unfortunately leads to the term “bioplastics” still frequently being associated with a number of partially related, intersecting, or confusing terms (Fig. 1). For example, the prefix “bio” means that bioplastics are automatically linked with properties such as biodegradability, compostability, and biocompatibility, or subjects such as biotechnology or bioeconomy, sustainability, renewable resources, etc.

2.1 *Biopolymers and Bioplastics*

To achieve a clearly-defined terminology and unified nomenclature in the field of bioplastics, the differentiation between bioplastics as useful materials and

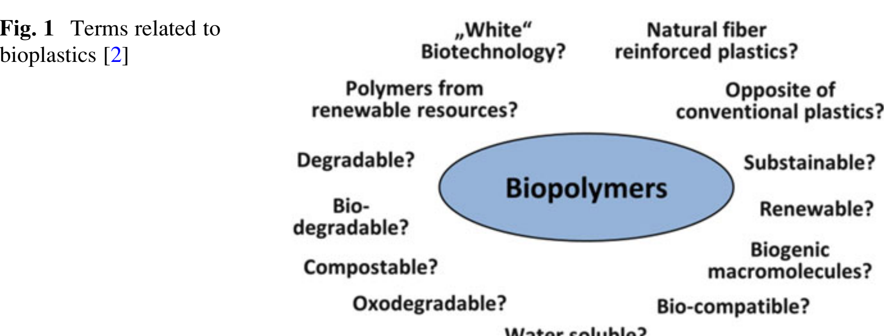


Fig. 1 Terms related to bioplastics [2]

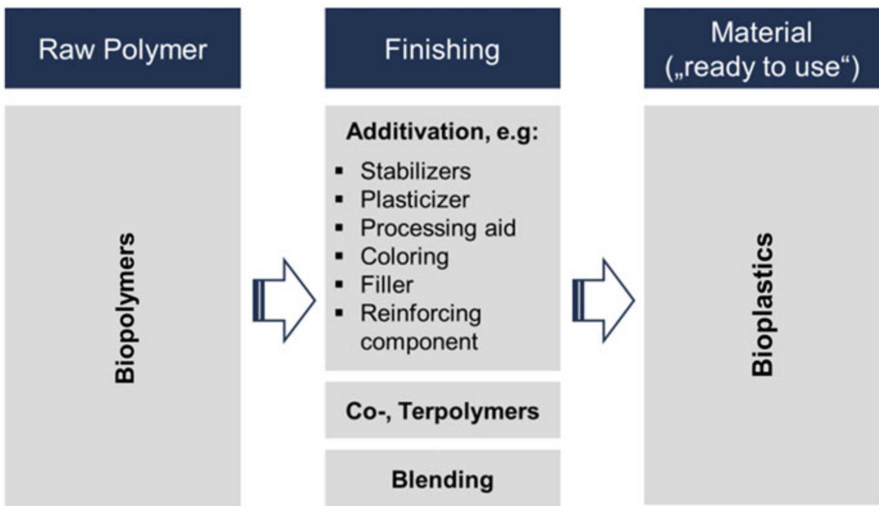


Fig. 2 From raw polymer to bioplastic

biopolymers as macromolecular substances is very important. Whilst biopolymers are indeed the basis for the creation of bioplastics, the term “biopolymer” does not describe the resulting material itself. As with conventional polymers, in the vast majority of cases biopolymers have to be “upgraded” to varying degrees, that is, modified or subjected to additives (stabilizers, plasticizers, colors, processing agents, fillers. . .) and blended to make “ready-to-use materials” with satisfactory processing and performance properties (see Fig. 2). In this chapter, the term “biopolymer” therefore describes the macromolecule, whilst the term “bioplastic” represents the material used for the generation of semi-finished and final products. As with conventional plastics, in bioplastics there are now a variety of materials based on the same basic polymer types. For the sake of a unified nomenclature, it is suggested that this differentiation between the initial polymer and the “finished” material also be applied with appropriate consistency when speaking about bioplastics.

2.2 *White Biotechnology*

The term “white biotechnology” is even younger than green (agriculture) and red (pharmaceutical) biotechnology, although the related processes have been used by humankind for millennia, for example, in the fermentation of alcohol or lactic acid. White biotechnology refers to the industrial production or modification of organic basic or fine chemicals and substances as well as biogenous fuels using optimized species of microorganisms, enzymes, or cells. However, this covers only a portion of biopolymers and bioplastics, that is, exclusively the biotechnological creation of

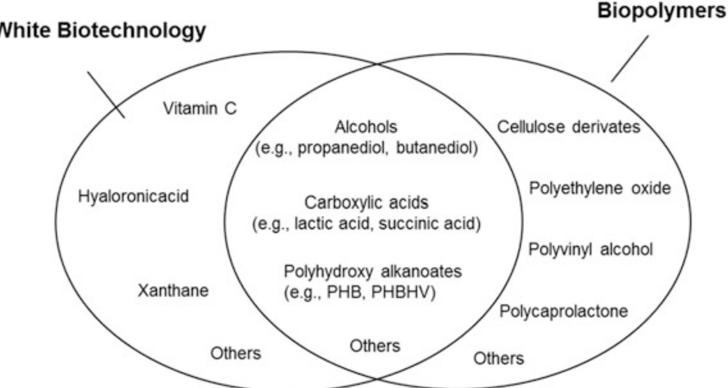


Fig. 3 Intersection between white biotechnology and biopolymers [2]

monomer or polymer building blocks for bioplastics. The purely biotechnological creation of molecular substances which do not (cannot) serve as plastics raw materials, for example exopolysaccharides, xanthan, pullulan, gellan, cordulan, alginate, oligosaccharides, and various acids (hyaluronic acid, citric acid. . .) and vitamins, also come under the umbrella of white biotechnology. Because these are not materials or base products for materials production but functional polymers or macromolecular chemical substances, these biotechnological products are not further considered in this chapter within the context of bioplastics.

The large group of biopolymers based on biotechnologically-created monomer or polymer raw materials, such as lactic acid, alcohols, or polyhydroxyalkanoates, forms the intersection between the two terms "biopolymers" and "white biotechnology" and simultaneously represents the relationship between the bioeconomy and bioplastics (Fig. 3).

Similarly, the biogenous macromolecules or biomolecules, such as the large group of polyamino acids, are organic substances occurring in nature or in living beings and thus are not raw ingredients for technical materials. Therefore, in the view of the author, these biogenous macromolecules do not form part of the bioplastics field either, even if in biology or medicine they are frequently referred to as biopolymers for the sake of simplicity.

2.3 Biocompatible Plastics

In the case of biocompatible plastics, there similarly exist for certain materials crossovers between these materials used in medicine and bioplastics but, once again, these two terms do not describe the same materials. The term "biocompatible" generally refers to materials which can come into direct contact with living beings without leading to interactions with negative consequences for the living

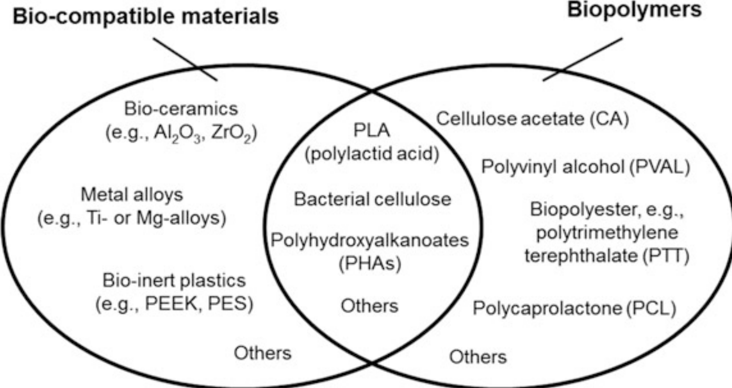


Fig. 4 Intersection between biocompatible materials and bioplastics [2]

being. This does not, however, mean that it is always necessarily a bioplastic. Similarly, bioinert materials such as ceramic and titanium-based implants or siloxane, as well as special petrochemical plastics (e.g., certain types of PEEK, PET, or PE-UHMW), also belong to the group of biocompatible materials because of their minimal interactions with human tissue [3–5]. With bioresorbable or bioactive polymers as biocompatible plastics (e.g., suturing materials or medical implants based on polylactide) there is also an intersection between bioplastics and biocompatible materials here; however, the terms are a long way from meaning the same thing as there is also a large number of materials which respectively belong to only one of these two material groups (Fig. 4).

2.4 *Biocomposite Materials*

Another aspiring group of materials are biocomposites. These terms usually describe fiber-reinforced plastics in which at least one material component (matrix or reinforcing component) is biologically-based or is made of a bioplastic. Bioplastics can therefore serve as the matrix but can also be reinforcing fibers. This means that, in the case of a petrochemical-based non-biodegradable thermoplastic or thermoset polymer matrix, at least the reinforcing component must be biologically-based. Well-known biocomposite materials from this group are natural fiber reinforced plastics (NFP) and wood plastics composites (WPC), that is, polyolefins reinforced or filled with natural plant fibers or wood fibers and wood dust. Superior bio-based synthetically-produced reinforcing fibers, that is, bioplastic fibers (e.g., PLA (polylactic acid), bio-PA or bio-PET fibers), could also be used (Fig. 5).

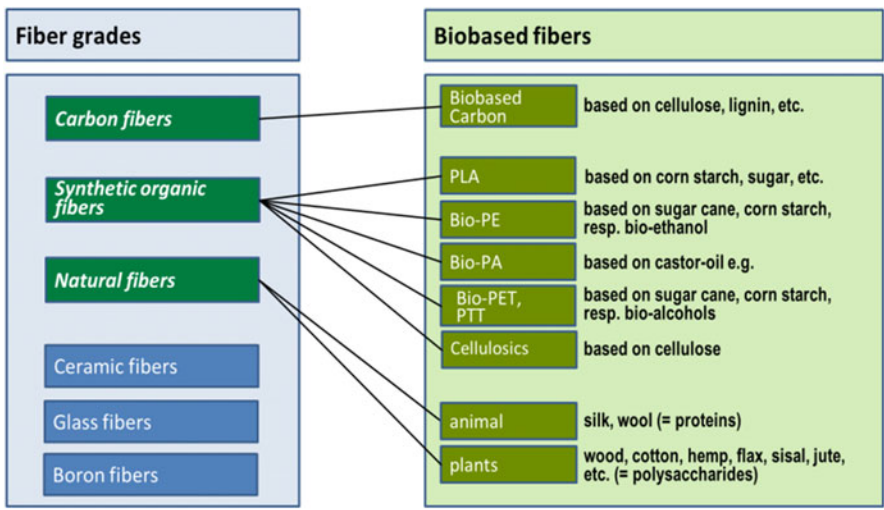


Fig. 5 Bioplastics and bio-based fibers [6, 7]

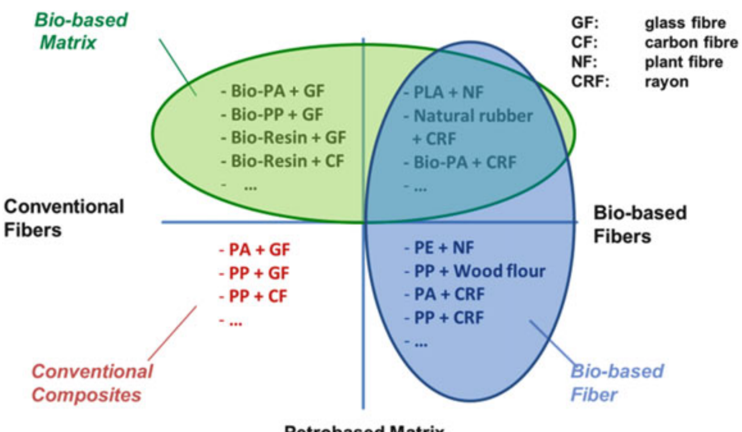


Fig. 6 Biocomposite materials [6, 7]

Conversely, however, conventional non-bio-based fibers can also be used for biocomposite materials. In this case, the matrix must consist of a bioplastic (e.g., glass fiber-reinforced bio-PA or carbon fiber-reinforced bio-based duromers).

Figure 6 provides another overview of the classification of biocomposite materials in comparison with conventional composite materials.

Alternatively, both components can, of course, have a bio-based origin, such as wood fiber-reinforced polylactide, viscose fiber-reinforced bio-PA, or components made from natural-fiber weaves and bio-based resins.

2.5 Bioplastics

In the author's opinion, currently the best general definition for the term "bioplastic" is a polymer material which possesses *at least one* of the two following properties:

1. Is made from bio-based (renewable) raw materials
2. Is biologically degradable

If this definition is adhered to, the following three fundamental bioplastic/biopolymer groups exist:

1. Degradable petrochemical-based bioplastics
2. Degradable (primarily) bio-based bioplastics
3. Non-degradable bio-based bioplastics

Biologically-degradable plastics can be based on petrochemical raw materials as well as on renewable raw materials. The degradability of the biopolymer materials is influenced exclusively by the chemical and physical microstructure and not by the origin of the raw materials used or the manufacturing process used in producing the polymers. This means that biopolymers need not necessarily be made exclusively from renewable materials. Biologically-degradable biopolymers can also be produced from petrochemical ingredients such as polyvinyl alcohols, polycaprolactone, various polyesters, polyesteramides, etc. (Fig. 7, bottom right). Conversely, not all biopolymers based on renewable ingredients are necessarily biologically-degradable; for example, highly-substituted cellulose acetates, vulcanized rubber, casein plastics, linoleum (Fig. 7, top left). Typical examples of the last group of bio-based and biologically-degradable bioplastics are starch-based plastic blends, polyhydroxyalkanoates (PHA), and PLA (polylactic acid).

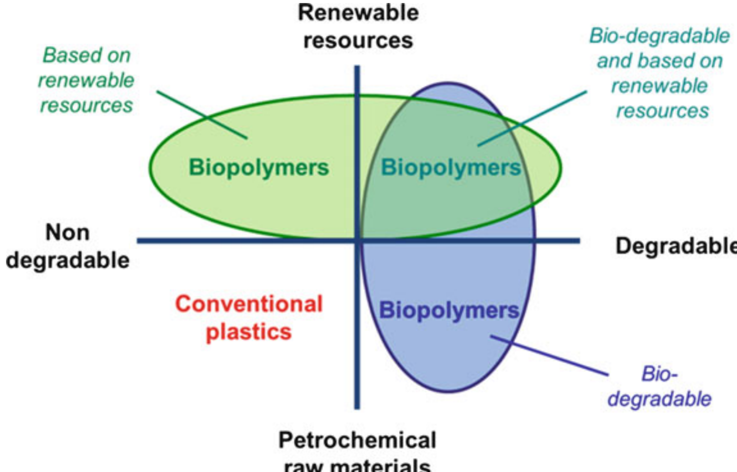


Fig. 7 Bioplastics and the three fundamentally-different biopolymer groups [2]

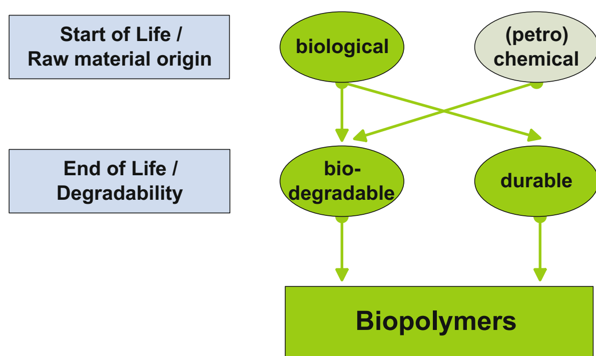


Fig. 8 Raw material basis and degradability of bioplastics

To avoid misunderstandings when speaking of biopolymers or bioplastics, it is imperative that the most precise nomenclature possible is used, that is, it is advisable to speak specifically of degradable or bio-based bioplastics. Degradability here means a functional property or disposal option at the end of the material"s life cycle, irrespective of the origin of the raw materials, whilst, conversely, bio-based describes exclusively the origin of the raw ingredients of the polymer and provides no statement whatsoever regarding its degradability. These two different approaches are still being pursued and form the technical basis for a variety of bioplastics (Fig. 8).

2.5.1 Degradable Petrochemical-Based Bioplastics

Biopolymers based on petrochemical raw materials are, similar to conventional plastics, based on the various hydrocarbon monomers and oligomers produced from crude oil, natural gas, or coal through fractionated distillation and targeted cracking processes as well as their derivatives (e.g., polyols, carboxylic acids). Just as in the past, the property profile of conventional polymers could be varied and adjusted to suit an enormous range of applications through the use of a wide variety of starting monomers, polymerization reactions, process parameters, resulting polymer microstructures, and additives. The property profile of polymer materials can be further expanded, in particular by the inclusion of various heteroatoms in the molecule (primarily oxygen and nitrogen). A significant property that can be influenced in this way is degradability. Whilst with conventional plastics the focus in the past has mostly been on durability, that is, a high level of resistance to chemical, microbiological, or other environmentally-defined influences, with degradable, petrochemical-based biopolymers an appropriate molecule and material design is pursued with the objective of creating a polymer material that is not very resistant to environmental influence. The purpose of this is to achieve a material that, as a result of environmental influences or as part of a targeted (industrial) composting process, can be broken down and achieves the simplest possible depolymerization with further ultimate degradation of the molecule fragments.

2.5.2 Degradable Bio-Based Bioplastics

The renaissance of bio-based bioplastics has been initiated over the last few decades by the second subgroup of these polymer materials, that is, by polymer materials based on renewable raw ingredients that are at the same time compostable (see Fig. 7, top right). These activities led to the recognition and naming of bioplastics as innovative materials around 30 years ago.

Renewable raw materials that can be used to create degradable polymers and bioplastics include, in particular, oligo- and polysaccharides such as cellulose, starch, sugar, and vegetable oils as well as some lignins and proteins and chemical and biotechnological derivatives based on them (e.g., acids and alcohols).

2.5.3 Non-degradable Bio-Based Bioplastics

These biopolymer materials are, in part, materials produced from renewable raw materials. The final polymer structures are not biodegradable, even though they are based on a bio-based degradable feedstock. In particular, in this context there is currently (as yet) no minimum share of bio-based material components for polymer blends and copolymers or terpolymers to be declared as bio-based bioplastics, although in recent years suitable methods have been developed for determining the share of bio-based carbon in bioplastics (see Sect. 2.7).

In addition, there also exist – similarly to conventional plastics – many copolymers and terpolymers as well as polymer blends in which a combination of various monomers or a mixture of the various aforementioned biopolymer groups are created in order to optimize the resultant properties.

Alongside the biopolymers as primary components, in the vast majority of cases bioplastics also contain appropriate auxiliary products or additives in order to achieve an appropriately satisfactory property profile and to produce useful materials which can be processed. In principle, the same system is used for classifying these additives as for the classification of the biopolymers, as mentioned above. If, for example, the amount of bio-based but non-degradable oligomer or polymer as additive or material component is increased, the result is an increase in the share of bio-based materials with a simultaneous decrease in the degradability of the bioplastic or bioplastic blend. Conversely, the increasing use of non-bio-based blend components or petrochemical monomer materials leads necessarily to a reduction of the share of bio-based materials in the polymer material.

2.5.4 Old and New Economy Bioplastics

The first technical, industrially-used polymer materials, the development of which began over 100 years ago, were all bio-based as there were no petrochemical raw materials available at that time. These so-called old economy bioplastics were

based on the renewable plant-based raw materials cellulose and natural latex or on animal proteins. With increasing industrialization at the end of the nineteenth century, the availability of the raw materials was of great concern for the production of these materials. The natural raw materials available at that time were modified as part of the material manufacturing process in such a way that they resulted in the first really durable polymer materials with a completely new property profile for that time, without being explicitly described as bioplastics. These old economy bioplastics therefore belong to the group of bio-based, non-degradable bioplastics. Of the old economy bioplastics, the only ones still of economic significance on the plastics market are natural rubber, regenerated cellulose, and cellulose derivatives (cellophane, viscose, celluloid, cellulose acetate) as well as linoleum in smaller volumes (Fig. 9, 10).

The renewed development of novel bioplastics (new economy bioplastics), which began around 30 years ago, was at first faced with the waste problem as it existed at the time and the unsatisfactory disposal situation with regard to conventional plastics. The objective of these developments was degradable bioplastics as a solution to the waste problem. There are currently increasing applications in medicine, landscaping and gardening, wastewater treatment, etc., for which degradability permits an additional function under the respective environmental conditions, such as films for use in agriculture which can be plowed in after use, bioresorbable implants or suture materials for use in surgery, or the targeted release of active substances (fertilizers, medical substances).

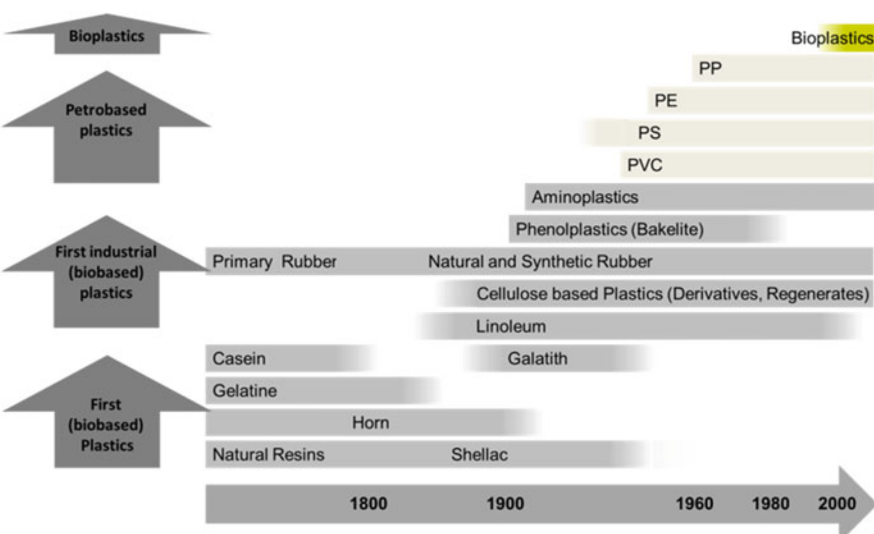


Fig. 9 Timeline of bioplastics with eras

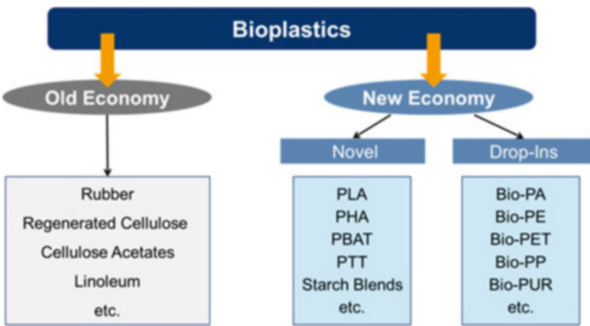


Fig. 10 Traditional (old economy) and novel bioplastics (new economy)

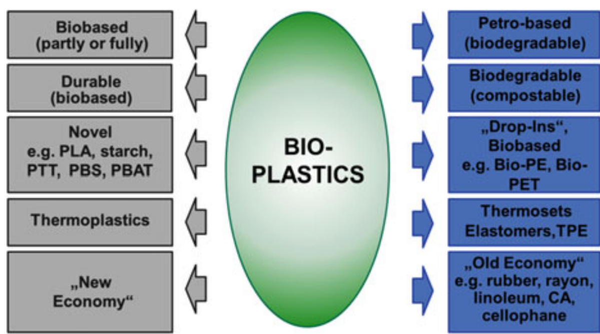
2.5.5 "Drop-in" Bioplastics

In the current developments in bioplastics, the availability of raw materials is once again becoming of concern. Within this group of non-degradable, bio-based bioplastics, one development which has made strong and technically very successful advances in the last 5–10 years is what is known as drop-in solutions. These are, simply put, an effort to retain the established methods of synthesis based on petrochemical raw materials whilst substituting as completely as possible petrochemical feedstock by biogenous raw materials. The objective here is the creation of bio-based plastics which are similar to familiar conventional plastics. Because of the identical chemical structure, with the same additivation the drop-ins have completely the same property profiles as their petrochemical equivalents. This means that when conventional plastics are substituted by the respective drop-ins, no changes are to be anticipated in the areas of processing, usage and, in particular, recovery and recycling. Here, however, it is less about an acute or current availability of raw materials and more about a strategically-assured, long-term availability, that is, using bio-based and renewable raw materials rather than non-renewable petrochemical materials for the creation of plastics. Examples of these are bio-based polyethylene (bio-PE), polyethylene terephthalate (bio-PET), polyamides (bio-PA), and polyurethanes (bio-PUR) based on a variety of renewable raw materials or their respective bio-based derivatives (Fig. 10).

This means that the new economy bioplastics are made up of two basic groups: (1) the chemically-novel biopolymers, that is, unknown in the field of plastics from a chemical point of view until a few years ago (e.g., novel bio-based polyesters such as PLA (polylactic acid) or PBAT (polybutylene adipate terephthalate)) and (2) so-called "drop-ins" which are identical in chemical structure but partially or completely bio-based plastics. Currently, with regard to the commercial market share, the most prominent examples of these are bio-PET and bio-PE. Alongside these, work is currently being carried out on further drop-ins, including that in the field of thermoset (e.g., bio-based EP resins) or elastomer polymer materials (e.g., bio-based EPDM or bio-based polyurethanes).

Depending on the perspective, this means that there are a number of different types of bioplastics (see Fig. 11). To avoid misunderstandings, bioplastics should therefore generally not be mentioned without further specifying, through additional information, which group is meant.

Fig. 11 Various types of bioplastics



2.6 Biological Degradability

Biologically-degradable plastics consist of natural (renewable) or petrochemical raw materials and, as polymer materials, are amenable to biological degradation reactions – that is, they break down under the influence of microorganisms and/or enzymes. In general, decomposition processes in plastics in the starting phase initially lead to a change in specific properties, such as the reduction of the mechanical values, the optical appearance (surface structure, coloration, etc.), the development of an odor or an increase in permeability, before further material degradation then occurs.

2.6.1 Primary and Ultimate Degradation

As regards material dissociation, it is necessary to differentiate more precisely between primary degradation (splitting of the macromolecules) and ultimate degradation of the fission products into water, carbon dioxide, methane, and biomass [8–11] (Fig. 12).

The metabolic potential of the (macro-) molecular fission products formed as part of the primary degradation defines here whether the process is simply a macroscopic disintegration process of a component or material or whether it is in fact a complete ultimate degradation. When the ultimate degradation of fission products is not assured, in the case of an exclusively primary degradation the decomposition products can accumulate, for example in compost or in groundwater. Therefore, in this case the term “biological degradability” should not be used. One prominent example of a product exclusively coming from primary degradation is microplastic, which is increasingly accumulating in groundwater and consequently also in living beings. The respective test standards for certifying degradability therefore also usually include as a significant core element the quantification of the decomposition products formed in the ultimate degradation and/or a record of the oxygen required for this. The oxygen requirement or the amount of CO_2

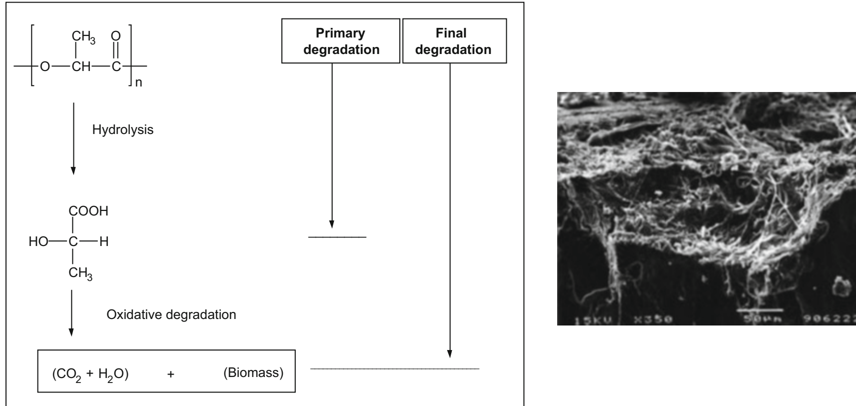


Fig. 12 *Left*: Primary and ultimate degradation. *Right*: PLA surface populated by microorganisms [2]

produced is then compared to the amount theoretically expected in a complete chemical conversion of the material/product to be degraded.

2.6.2 Environmental Conditions During Degradation

As well as differentiating between a macroscopic decomposition of the material (primary degradation) and a microscopic ultimate degradation, information about the respective environmental conditions is also essential for a complete description of the degradation process. Biological decomposition can vary enormously under a variety of environmental conditions (soil, water, salt water, compost, human body, etc.) as, apart from the material itself, the degradation process depends on a variety of other (environmental) factors such as microorganisms present, humidity, temperature, available oxygen, pH value, time, etc. (Fig. 13).

As an example, the biological decomposition of the material in an industrial composting plant with continuous irrigation and turning of the heap takes place more quickly than under "normal" ground or anaerobic conditions in a biogas plant, or even on the ocean floor at temperatures of approx. 4°C with no light and a completely different microflora in the salt water (Fig. 14). This is also very evident for a product such as wood. Under dry conditions, wooden furniture in a house, for example, has an almost unlimited lifespan, whilst in the forest, biological degradation progresses relatively rapidly. Another example is fossilization. Here, too, environmental conditions have prevented a complete degradation of the organic mass.

There is an increasing number of appropriate test standards for testing the stability of plastics or the degradability of bioplastics under various environmental conditions [4], although not all areas have yet been reflected satisfactorily, for example, the degradability of biologically-degradable plastics in the ocean, in biogas plants, or in the ground.

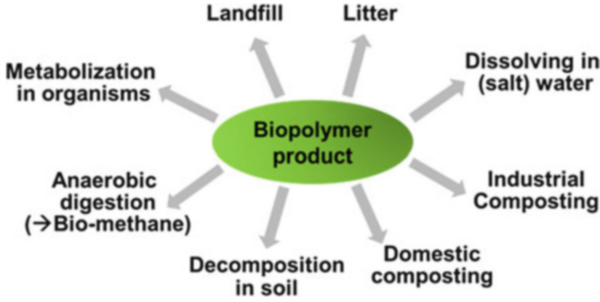


Fig. 13 Various degradation scenarios for bioplastics [2]

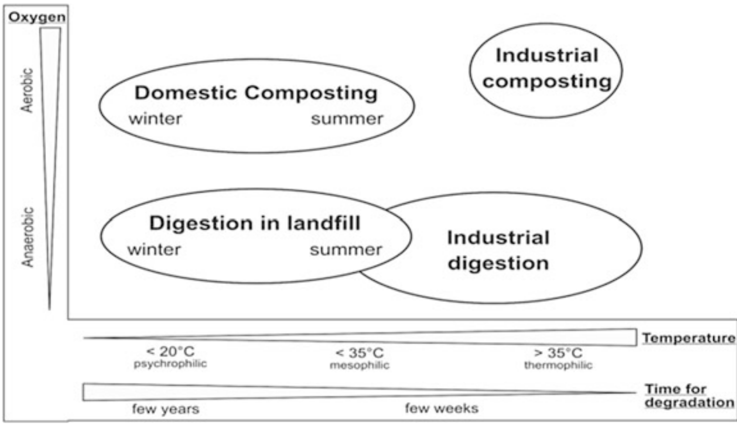


Fig. 14 Conditions in industrial and domestic composting and biogas plants [2]

2.6.3 Influence of the Material Microstructure on Biodegradability

As part of a complete biological degradation process, microorganisms can essentially initially only further process biodegradable plastics and/or their molecular primary fission products with extracellular enzymes. The extracellular enzymes substantially break down the material through processes of oxidation and hydrolysis into even smaller components, which can then be taken up by the cell for further intracellular degradation [10, 12] (Fig. 15). The reason is that the enzymes are too voluminous to be able to penetrate the rotting material effectively, which means that this process can only take place as surface erosion or as a diffusion-controlled process with liquid carrier media, in particular water.

Biological degradability is mainly based on the presence of heteroatoms (no carbon) in the main chains of the macromolecules (see Fig. 16). These heteroatoms allow the microorganisms and their enzymes present under the respective conditions access to the splitting of the chains at this point and thus induce the

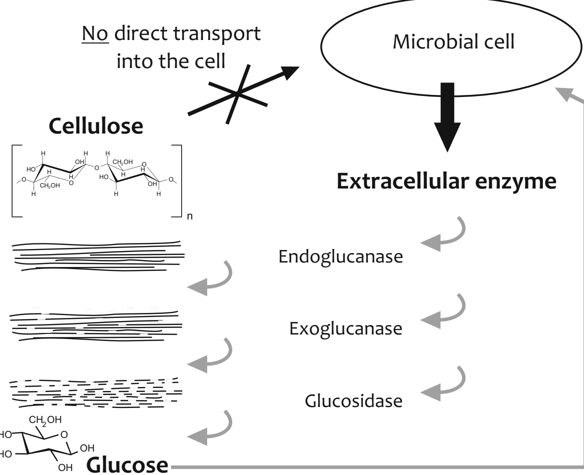


Fig. 15 Cellulose degradation through cellulase-enzyme complexes [2]

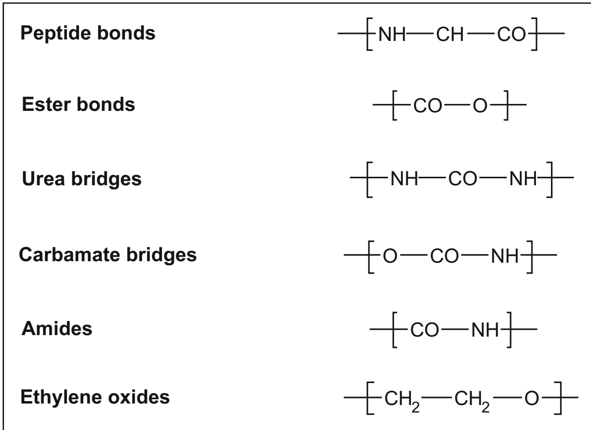


Fig. 16 Heteroatoms in the macromolecules of biologically-degradable polymers [2]

degradation process through primary degradation. The ultimate degradation of the biopolymer decomposition products generally continues through intracellular metabolic reactions of the relevant microorganisms [4, 9].

It can be very generally said that, with an increasing ratio of heteroatoms to carbon, particularly in the main chain, the degradability fundamentally increases (Fig. 17).

In addition to the heteroatoms, various other material-technical microstructure parameters play an important role in the biodegradability. In principle, it also applies here that the biological degradability and compostability by microorganisms increases with easier accessibility and fissionability of the molecules. For

example, an increasing crystallinity with an otherwise identical molecular biopolymer structure and the same environmental conditions therefore lead to a deterioration of biological degradability. If, as an example, the crystallinity of PLA is increased through a tempering which is above the glass transition temperature (approximately 55°C), the biological degradability decreases significantly.

Figure 18 again illustrates the influence of various material-technical microstructure parameters on the biodegradability of bioplastics.

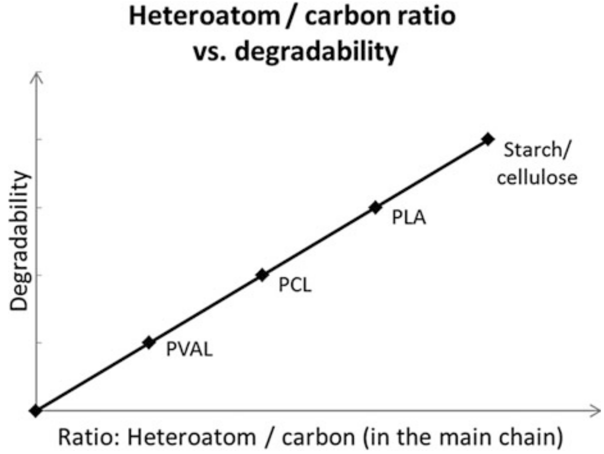


Fig. 17 Carbon:heteroatom ratio in the main chain vs degradability [2]

Microstructural parameter	**Degradability**
Flexible, branched molecular structures ↑	↑
Intermolecular interactions, crystallinity ↑	↓
Heteroatoms ↑	↑
Quantity of unsaturated compounds ↑	↑
Aromatics proportion ↑	↓
Swelling capability/polarity ↑	↑
Molecular weight ↑	↓
Specific surface ↑	↑

↑ = increase, ↓ = decrease

Fig. 18 Degradability dependent on various microstructure parameters [2]

2.6.4 Compostability of Bioplastics

Even the complete biological degradability of a material does not automatically mean that the material and, in particular, the components produced therefrom are also compostable. A material is designated as being biologically degradable if all the organic components generally succumb, within reasonable periods of time, to primary and ultimate biodegradation caused through biological activity. With the term composting, however, a statement is made as to whether a material or a mixture of substances and components derived therefrom can, under defined conditions in a composting system and within a more precisely-specified period of time, usually a composting cycle of a few weeks or months, be completely converted into CO_2, H_2O, CH_4, or biomass [8]. The verification and certification of the compostability of a material and component is carried out according to relevant standards. In particular, the environmental conditions, the period of time, and, for product certification, the component geometry (surface, wall thickness, size) are thereby precisely defined.

2.6.5 Oxodegradability

During degradation of a component, primary degradation can be initiated not only by a biologically-induced decomposition reaction but also through other degradation mechanisms. These include fission of the macromolecules through radiation. The most important natural radiation in this context is the UV proportion in sunlight. In particular for polymers with chromophoric groups in the molecular structure such as aromatic polyesters or polyamides, the effect of the sunlight can lead to a direct fission of the polymer chains (photodegradable polymers) [13].

Catalyst residues, impurities, peroxides, and other oxygen-containing components can also absorb sunlight and initiate a primary degradation. Similarly, indirect fission processes have been identified during which “host molecules,” such as aldehydes or conjugated double-bond systems, are initially excited through the radiation; in the next step, these excited host molecules then transfer the energy necessary for bond fission to the actual polymer molecule.

In addition to this pure photodegradation, sunlight, in combination with oxygen, also causes photooxidative degradation. Through heat or the effect of light, the process of oxodegradation can be started by formation of radicals. Alkyl radicals can subsequently thereby be formed as well as, through their reaction with oxygen, photosensitive hydroperoxides as an intermediate stage of the photooxidative degradation. Through the ongoing effects of light and temperature, a renewed formation of radicals (alkoxy, peroxide, and alkyl radicals) subsequently occurs based on the hydroperoxides formed previously and, finally, degradation of the polymer chain [4]. If the reaction products are carboxylic acids or alcohols, they are subject to a further ultimate degradation.

Currently, work is once again being intensified on the oxodegradability of polyolefins, in particular PE, through, for example, the incorporation of specific metal ions for the initiation of a radical oxidation mechanism. The method of oxodegradation is, however, very controversial. Within the academic field, it is assumed that a complete microbiological ultimate degradation generally only takes place for oligomeric fission products with less than approximately 20–25 carbon atoms [13]. The fission products of polymer oxodegradation are, however, normally significantly larger. To obtain smaller, completely-degradable oligomers as the basis for a complete degradation, a correspondingly high doping of the initiator components is necessary. This, however, leads to very considerable – and usually unacceptable – losses in quality in the material properties.

A further possibility for the initiation of the primary degradation is a chemical dissolving process, for example in water with subsequent or parallel hydrolysis for water-soluble polymers (Fig. 19).

These different reaction mechanisms, however, have one thing in common: they can initially only lead to a macroscopic primary degradation without ensuring a definite ultimate degradation of the fission products.

It is therefore particularly important in the case of macroscopic disintegration or macrobiological damage with a reduction or loss in the mechanical material characteristics, alteration of the surface, or odor development, that a complete biological degradability or compostability of the materials is not automatically assumed. The respective additive-enhanced plastics, whose macroscopic decay or primary degradation is initiated solely through oxodegradation, may therefore not be designated as being degradable or even compostable bioplastics, as ultimate degradation is the crucial process as regards ensuring degradability. From a scientific perspective, there is no further need for discussion as regards oxo-induced or solution-induced primary degradation because here, in accordance with the testing standards, the quantification of the resultant final degradation products and the oxygen or possibly hydrogen demand necessary for the metabolization form the basis for an accurate statement concerning complete degradability.

The additives for the initiation of degradation do not ensure ultimate degradation. They can, however, instead lead to a reduction in the stability of the primary materials and also to a contamination of recycled products and thereby, for example, to a reduction in the stability of secondary polyolefins. Additives which solely

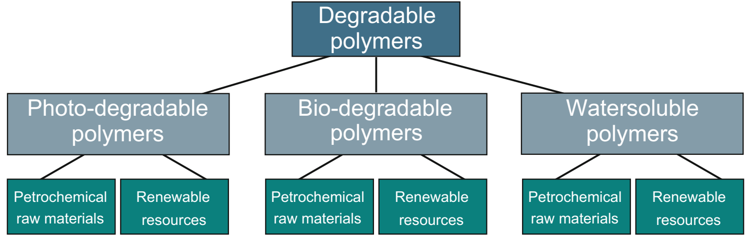


Fig. 19 Degradation mechanisms in degradable polymers [14]

initiate an oxodegradation do not therefore necessarily lead to a complete degradation of the material, but present a potential problem for the established recycling of polyolefins.

2.7 Bio-Based Material Share

As, on the one hand, both petrochemical and renewable raw materials can be used for biopolymers, copolymers and blends can be produced from both raw materials groups and, in particular, the structurally-similar bio-based drop-ins are entering the market, and, on the other hand, statutory special regulations for bio-based biopolymers are coming increasingly into effect, the question as to how high the proportion of renewable or biogenic raw material in a biopolymer is of increasing importance for the future.

This question can currently be best answered by means of radiocarbon dating (also known as the C^{14} method or radiocarbon method) in accordance with ASTM D6866. ^{14}C–dating is based on the radioactive decay of the carbon isotope ^{14}C and actually served as the historical age-determination of carbonaceous organic materials with an age of up to approximately 50,000 years. In nature, carbon occurs in three isotopes: ^{12}C, ^{13}C, and ^{14}C. In contrast to ^{12}C and ^{13}C, which occurs at higher concentration in inorganic compounds, ^{14}C is not stable and is, for this reason, also known as radiocarbon. Its half-life according to Libby is around 5,568 $\pm$ 30 years [15]. It is reformed in the upper atmosphere at the same speed as the decay rate and integrated into the biomass during the metabolic processes of photosynthesis. In dead and mineralized biomass, however, the quantity of ^{14}C decreases over time through radioactive decay as no new ^{14}C is absorbed. This means that petrochemical raw materials or petrol-based plastics, because of the relatively short half-life of the carbon isotope ^{14}C, no longer contain "young" ^{14}C. The ^{14}C proportion or the ^{14}C:^{12}C ratio is therefore a measure for the bio-based carbon and thereby an indicator for the proportion of renewable raw commodities in the material.

However, this method has the disadvantage that only the biogenic carbon and not the hydrogen or other elements are recorded. For example, a biopolymer filled with glass fibers (such as glass fiber reinforced bio-PA) would, according to this method, be comprised of 100% renewable raw materials, or a polypropylene-starch blend would, because of the (in comparison with PP) lower carbon content in the starch phase, only contain approximately 18 wt% bio-based relative to total carbon.

3 Process Technologies

For the production of biopolymers there are a number of varying manufacturing routes. As already demonstrated, biopolymers can, in principle, be based on both biogenic and petrochemical feedstocks. A biodegradable polymeric material does

not, however, automatically result from a biogenic raw material, and a non-degradable material does not necessarily result from a petrochemical raw material. The actual polymerization reaction, however, regardless of raw material origin, can occur both chemically, that is, brought about by human hand, and in biological or natural, primarily fermentative, ways. In the end, the degradability is in turn only dependent on the resulting molecular structure and not on the raw material origin or the formation reaction of the polymers.

As an example, a polylactide as biopolymer is based on the biologically-produced raw material lactic acid, which is subsequently polymerized using chemical methods. Furthermore, this manufacturing route of man-made polymerization of biotechnologically produced monomers is also representative of drop-in solutions such as, for example, a PE or PET based on biogenic ethanol.

In contrast, polyhydroxy fatty acids, for example, are produced naturally as biopolymers based on biogenic raw materials by microorganisms for energy storage. It is, however, also conceivable to "feed" microorganisms with specific petrochemical-based raw materials for polymer synthesis, such as when using petrochemical-based alcohols as a nutrient for the fermentative production of polyhydroxy fatty acids. Natural/biological production of non-degradable polymers does not exist as this would result, contrary to natural evolution, in biological production of polymer substances which, because of their subsequent biological durability, would accumulate in the Earth's ecosystem.

In contrast, the modification of natural substances can lead to materials where the originally degradable, native molecular structure is altered to such an extent that the resulting polymers are no longer degradable, as they can no longer be metabolized in the modified form. Examples of this include the modification of cellulose to cellulose acetate or natural latex to vulcanized rubber.

For the production of biopolymers, the following fundamental production methods therefore apply (Fig. 20):

1. Chemical synthesis of petrochemical raw materials
2. Chemical synthesis of biotechnologically manufactured polymer feedstock
3. Direct biosynthesis of polymers
4. Modification of molecular, renewable feedstock
5. Production of blends and co−/terpolymers from these groups

In the following overview, the most important associated bioplastics are assigned to the various process routes (Fig. 21).

3.1 Chemical Synthesis and Polymerization of Petrochemical Raw Materials

If exclusively petrochemical raw materials are utilized as polymer feedstock during production, these must be degradable materials for the products to be designated as

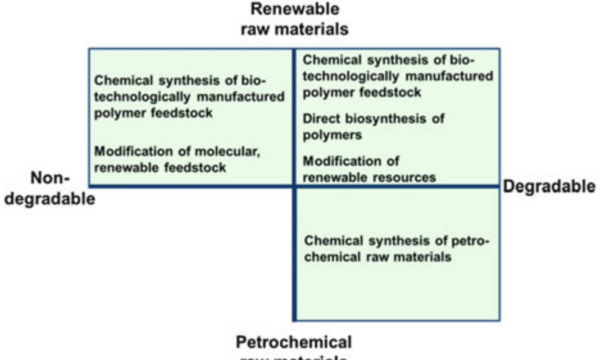


Fig. 20 Synthesis routes in biopolymers [2]

Synthesis process	Examples for biopolymers/bioplastics
Chemical synthesis and polymerization of petrochemical raw materials	– Polyesters – Polyester amides – Polyester urethanes – Polyvinyl alcohols (PVOH) – Polycaprolactone (PCL)
Chemical synthesis and polymerization of bio-technologically manufactured polymer feedstock	– Polyethylene (Bio-PE) – Polyester (e.g. PLA) – Polyamide (e.g. bio-based PA 11)
Direct biosynthesis of polymers	– Polyhydroxyalkanoates (e.g. PHB)
Modification of molecular, renewable feedstock	– Cellulose regenerates – Starch derivates – Cellulose derivates (e.g. CA)
Co-/Terpolymers	– Polybutylene Terephthalate (Bio-PBT) – Polybutylene Succinate (PBS) – Polybutylene Adipate Terephthalate (PBAT) – Polybutylene Succinate Terephthalate (PBST) – Polyethylene Terephthalate (Bio-PET) – Polytrimethylene Terephthalate (PTT) – Polyamide (e.g. Bio-PA 4.10, 6.10, 10.10) – Polyurethane (Bio-PUR) – Ethylene Propylene Diene Rubber (EPDM)
Blends	– Starch or cellulose blends – Polyester blends

Fig. 21 Production routes for various bioplastics

bioplastics. This group of degradable polymers synthesized from petrochemical raw materials includes, for example, polycaprolactone (PCL) and some polyvinyl alcohols (PVOH).

PCL is produced through ring-opening polymerization of ε-caprolactone (6-hydroxy-hexanoic acid lactone, 6-hexanolide, or oxepan-2-one), whereby a diol, that is, a bivalent alcohol, and tin(II) or tin(IV) salts are used as an initiator. The monomer ε-caprolactone is obtained industrially through the conversion of cyclohexanone with peroxyacetic acid. Analogous to polycaprolactam, which is better known under the name polyamide (PA 6) and which is produced from caprolactam through ring-opening polymerization, polycaprolactone contains five methylene groups between the points of attachment. In the case of PCL these attachment points are formed from ester and in the case of PA from amide groups. PCL fundamentally has similar mechanical properties to other conventional, non-degradable, synthetic polymers. It is non-toxic and, with a molecular weight of less than 15,000 g/mol, the material is brittle. At higher molecular weights of around 40,000 g/mol, it is partially crystalline in structure and soft, that is, it has a particularly high elasticity. One important limitation in the applicability results from the sharply-defined but very low melting point of around 60°C. Polycaprolactone is compatible with almost all plastics and, in particular, with starch or lignin [2].

The preparation of PVOH cannot be effected via direct polymerization but is instead carried out, because of the volatility of the vinyl alcohol monomer (keto-enol tautomerism), via the hydrolysis of an ester; particularly preferred is that of the acetic acid vinyl ester, that is, polyvinyl acetate (PVAc) [16]. Polyvinyl alcohol is produced as a granulate or (ground) as a powder, is white-to-pale ivory in color, and odorless. As a dry cast film (from water), polyvinyl alcohol is brittle in the anhydrous state. The brittleness is reduced by any remaining acetyl groups (partially-saponified polyvinyl alcohols), polymerized monomers, or water absorption. Further special features of PVOH include good film-forming ability, excellent gas barriers (against oxygen, carbon dioxide, nitrogen, aromas), high stability of the films, high adhesion and cohesion, high pigment-binding capacity, viscosity increase of suspensions, foamability, curability, applicability as a protective colloid, partial approval under food legislation, water solubility, insolubility in many organic solvents, and a highly variable property profile (co- and terpolymers, variable degree of saponification, variable molecular weight, etc.). When examining the biological degradability of PVOH, a distinction must be made between an aqueous solution and an undissolved product. Whilst for a dissolved PVOH in an adapted treatment plant the metabolization occurs rapidly, composting is not possible. Because of its hydroxyl groups, the PVOH is highly adhesively-bonded to the components of the Earth and is not sufficiently convertible in the aqueous phase. Bonded PVOH could therefore not be degraded under standard conditions (e.g., ISO Standard 17088 or EN 13432). In the analysis of the compostability of PVOH, a rapid primary degradation and dissolution in aqueous systems could usually be observed whilst, in contrast, the secondary or ultimate degradation takes place very slowly, usually because of the lack of heteroatoms in the molecular chain. The complete biological degradability/compostability of PVOH is therefore controversial [2].

3.2 Chemical Synthesis and Polymerization of Bio-Technologically Manufactured Polymer Feedstock

3.2.1 PLA (Polylactic Acid)

In terms of quantity, the lactic acid-based polylactide is currently the most important completely bio-based plastic and belongs to the polyhydroxy acids. For the production of PLA, varying starch- or sugar-containing raw plant materials are used. These include maize, wheat, sugar beet, and sugar cane. Other plants, such as tapioca, are currently also being tested as starch suppliers for PLA production, as is the use of waste materials. The largest production capacities can currently be found in the USA at Nature Works, which uses maize for the fermentative production of lactic acid. Further PLA capacities are currently being built, particularly in Asia.

There are fundamentally a multitude of microorganisms which qualify for the bio-technological production of lactic acid. Within the framework of the industrial production of lactic acid, Gram-positive, non-spore-forming, facultative anaerobic homo- and heterofermentative lactic acid bacteria in particular are used. During fermentative lactic acid production, specific optically-active forms of lactic acid are produced. Whilst for the generally less-productive homofermentative lactic acid bacteria the sole fermentation product is L(+)-lactic acid, heterofermentative lactobacteria produce a racemic mixture of L- and D-lactic acid with a dominating D-proportion. The ratio of L- to D-lactic acid therefore depends essentially on the bacterial culture itself and its age as well as the pH [17–20]. Widely varying carbohydrates, for example, short-chain saccharides such as sucrose, maltose, lactose, or starch (which is enzymatically saccharified to glucose) are thereby offered to the bacteria as a nutrient source, and are then metabolized during fermentation to lactic acid. The subsequent isolation of the lactic acid currently usually takes place by means of a neutralization reaction. A different, more favorable method, for which work is still ongoing regarding the continuous isolation of lactic acid, uses micro-filtration and electrodialysis with specific bipolar membranes from the aqueous phase. Because of the comparatively high cost of membrane, a CO_2-supported trialkylamine extraction is predominantly used for the separation of the lactic acid from the culture broth. This process is currently favored by Cargill, the largest PLA manufacturer.

As the fermentative processes have become more cost effective and the demand for naturally-produced lactic acid has risen, only small amounts of lactic acid are still being synthetically produced today, particularly in Asia. In Fig. 22, an overview of the fundamental process steps for the fermentative production of lactic acid and PLA is provided.

From the lactic acid, low molecular weight prepolymers (DP = 30–70, d.h. $M_n < 5{,}000$ g/mol) are created through a so-called oligocondensation which are then depolymerized at high temperatures and reduced pressures to form dilactides. Because of the enantiomeric configuration isomerism of the lactic acid, if no special precautions are taken a stereoisomeric mixture of meso-(di-)lactides with a high

L-proportion occurs. In the next step, a temperature- and pressure-assisted, catalyst-controlled (organometallic compounds such as tin octoate) so-called ring-opening polymerization is applied, under vacuum-technical removal (vacuum distillation) of the non-polymerized monomers (demonomerization), to produce the high-molecular weight polylactide (DP = 700–15,000, d.h.M_n >> 50,000 g/mol) [2] (Fig. 23).

Another way of polymerizing the lactic acid is the direct production of a high molecular weight PLA from the lactic acid using a polycondensation reaction in an (organic) solvent. The solvent thereby also serves in the absorption and removal of the water resulting from the condensation process.

Through subsequent compounding of the PLA and the addition of further additives and/or blend components, the polymer material polylactide (PLA) then results in the commercial granular form.

The resulting final microstructure (conformation) of the PLA and thereby the resulting product quality (crystallinity, mechanical properties, T_g) can, in addition to the costly production of pure monomers or dimers (L, L-lactide, D, D-lactide) or the purification of the racemic mixtures as initial monomers, also be partially influenced by the controlled ring-opening polymerization. As with conventional polymers, increasing racemic purity also leads in the PLA to molecular structures with increasing crystallinity. As a result, there is an increase in the stability, the elastic deformation resistance, the swell resistance, the glass transition temperature, and the melting temperature, as well as an increasing resistance against environmental influences such as humidity or biological degradation.

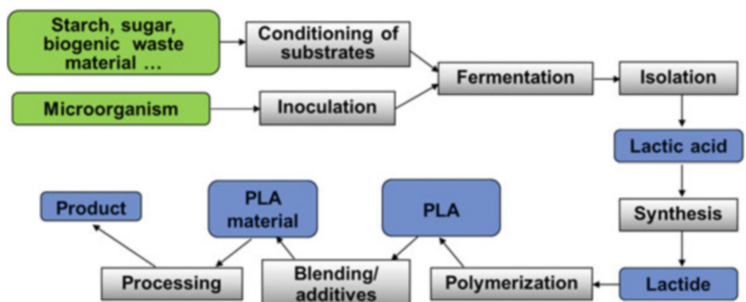


Fig. 22 Production of PLA [2]

Catalyst + heat

L-lactic acid Dilactide Polylactides

Fig. 23 Polymerization reaction of PLA [2]

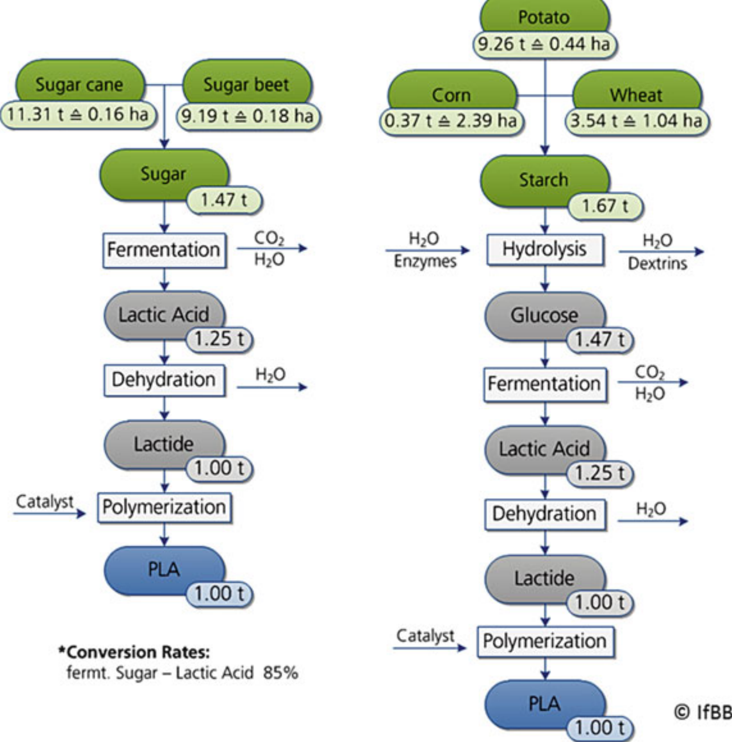


Fig. 24 Process routes and material flows in PLA production [21]

Figure 24 illustrates the process routes for PLA with the significant material flows and the associated conversion rates. These process routes are a very good and transparent basis for the calculation of resource requirements. To produce 1 tonne of PLA, 1.47 tonnes of sugar or 1.67 tonnes of starch are necessary. This means that, depending on the utilized starch or sugar plant, a land requirement of between 0.16 and 1.04 ha/tonne of PLA is required. From this it also becomes clear that, in the case of sugar, the sugar cane and for starch, maize have the lowest land requirement or the highest PLA yield per field area. These are also currently the most important PLA raw materials.

3.2.2 Bio-Based Polyethylene Terephthalate (Bio-PET)

Beside aliphatic polyesters such as PLA, there also exist aromatic polyesters within the group of biopolymers that are synthesized in part or completely from materials produced by means of biotechnology. For this group of biopolymers, and for all future New Economy bioplastics as well, partially bio-based polyethylene terephthalate (Bio-PET) is the most important bioplastic material economically. Its

bio-based basis is supplied by bioethanol, which is so far mainly produced from sugar cane or corn starch. In a series of chemical reactions, it is converted to bio-based monoethylene glycol (MEG) as alcohol component. The next step is to start the esterification (also known from conventional PET) with the petrobased terephthalic acid (PTA) to produce Bio-PET (see Fig. 25).

In this case, the percentage of bio-based feedstock is 30 wt% (therefore named Bio-PET 30). In the final product Bio-PET 30, however, only about 23% of the carbon is bio-based because of the differing portions of carbon in the two polymer components as feedstock.

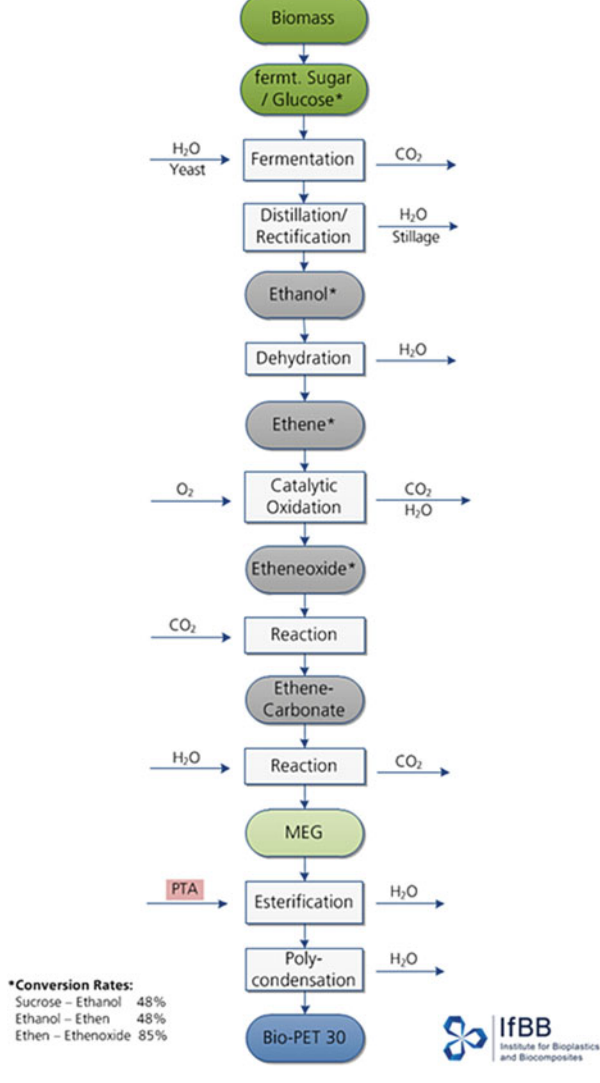


Fig. 25 Process routes and material flows in Bio-PET 30 (i.e., 30% bio-based) production (source: [21])

Research is currently underway to develop entirely, that is, 100%, bio-based PET (Bio-PET 100). This means that the alcohol component as well as the aromatic acid component are fully bio-based (see Fig. 26).

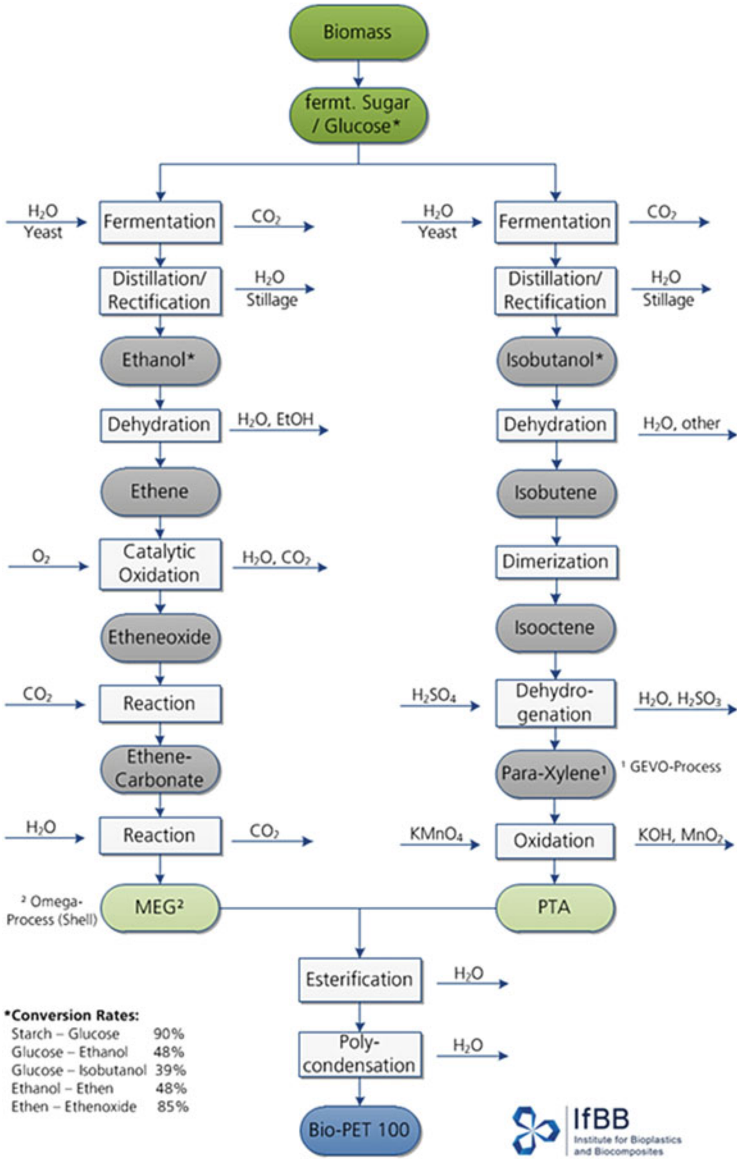


Fig. 26 Process routes and material flows in Bio-PET 100 (i.e., 100% bio-based) production (source: [21])

3.2.3 Bio-Based Polyethylene Furanoate (Bio-PEF)

A different approach for developing a fully bio-based aromatic polyester involves the production of polyethylene furanoate (PEF). This is a promising new type of polyester developed specifically by Avantium Co. in collaboration with Mitsui and put on the market using the buzzword “yxy technology.” Here also, one of the polymer components is bio-based MEG based on bio-ethanol. The other component is bio-based furandicarboxylic acid (FDCA) based on methoxymethyl furfural (MMF) or hydroxymethyl furfural (HMF) (Fig. 27).

The result is a new type of polymer, seemingly with a somewhat different property profile compared to bio-PET. First comments suggest that PEF has much better barrier properties for CO_2, O_2, and H_2O compared to PET and also has improved mechanical properties as well as better heat resistance.

A similar path is being followed by DuPont Industrial Biosciences in cooperation with Archer Daniels Midland (ADM). They have developed a method for producing furan dicarboxylic methyl ester (FDME) from fructose. FDME is a high-purity derivative of furandicarboxylic acid (FDCA). Utilizing FDME, one of the first polymers under development is polytrimethylene furandicarboxylate (PTF) based on FDME and also DuPont’s Bio-PDO™ (1,3-propanediol).

3.2.4 Bio-Based Polyethylene (Bio-PE)

Bio-based PE is a drop-in; as with conventional PE, for bio-PE the synthesis conditions of the polymer formation reactions (temperature, pressure, monomer concentration, catalysts, inhibitors, etc.) also ultimately determine the resulting microstructure and thereby the macroscopic properties. As expected, the properties of the bio-PE can also, through further measures such as use of co-monomers, additives, blending, and cross-linking, be configured in exactly the same way as is known from conventional PE. The only significant difference between conventional and bio-based polyethylene lies in the feedstock or the process route. More specifically, the process routes differ from one another only as far as the source of the bio-based ethylene differs compared to the petrochemical variant.

Depending on the selected raw material, for 1 tonne of bio-PE, between 0.48 and 3.1 ha of land are required (Fig. 28). Because of the higher starch and sugar yields for sugar cane in the sugar plants and corn starch in the starches, these also demonstrate here the highest land-use efficiency. In principle, the values for the land requirements for bio-PE are slightly higher than for PLA, because with bio-PE the oxygen as integral part of the polysaccharide starting product is not present in the molecular structure (Fig. 24, 28).

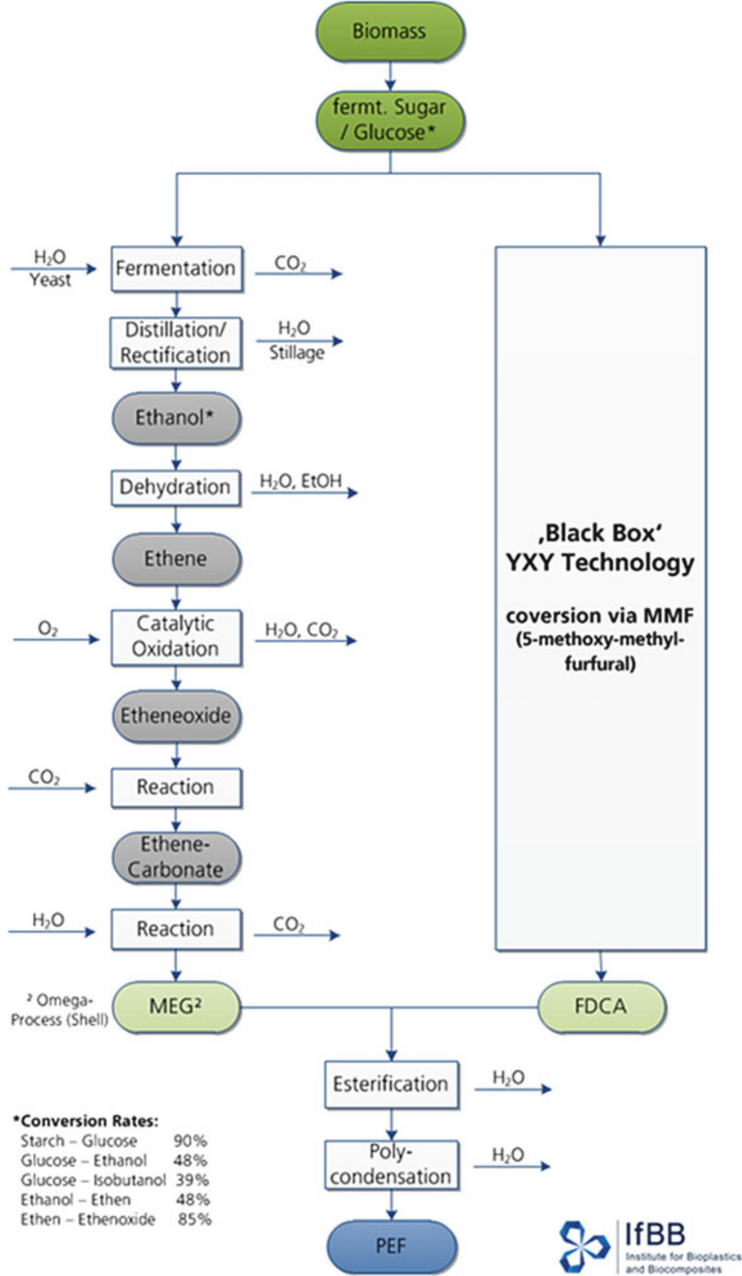


Fig. 27 Process routes and material flows in Bio-PEF production (source: [21])

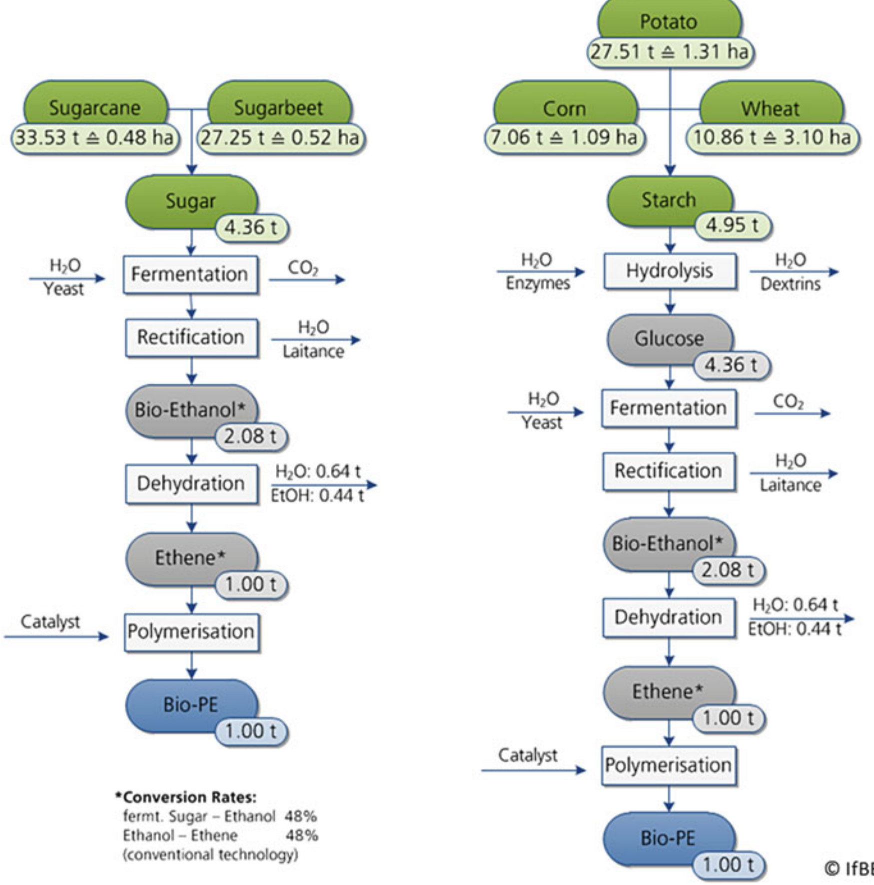


Fig. 28 Process routes of bio-based polyethylene (bio-PE) [21]

3.2.5 Fully Bio-Based Polyamides

The fully bio-based (homo-) polyamides include PA 11, which is based on castor oil or undecanoic acid, and PA 6, which is based on fermentatively-produced ε-caprolactam (6-amino hexanoic acid lactam, 6-hexan lactam, azepan-2-one) as an initial raw material. In addition, there are also a number of other partially bio-based (co-)polyamides such as PA 4/4, 4/10, 6/4, 6/6, 6/9, 6/10, and 10/10. Strictly speaking, these belong to the group of mixed co- and terpolymers which is comprised of differing bio-based and petrochemical raw materials (see Fig. 29).

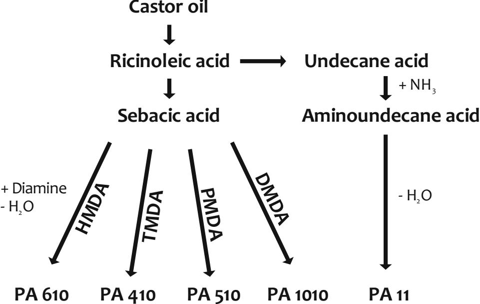


Fig. 29 Bio-based polyamides based on castor oil [2]

3.3 Direct Biosynthesis of the Polymers

In the direct fermentative production of biopolymers, a polymerization of the biopolymers occurs during the fermentation process. In contrast to the aforementioned fermentative production of the monomers, where the subsequent polymerization is induced by synthetic methods, the additional synthesis step of the polymerization is not necessary here because of the natural biosynthesis. Within these biopolymer groups, which are produced through direct biosynthesis, the best-known and most important representatives are the so-called polyhydroxy fatty acids and polyhydroxyalkanoates. Polyhydroxyalkanoates are polyesters which are intracellularly accumulated by bacteria as storage or reserve material. These are polymers which are composed primarily of saturated and unsaturated hydroxyalkanoic acids; hence the name polyhydroxyalkanoates. In addition to unbranched 3-hydroxyalkanoic acids, monomeric components also occur which are branched or have a substituted side chain, as well as 4- or 5-hydroxyalkanoic acids. Based on these varying monomers, PHAs are created as homopolymers, copolymers, and terpolymers. Because of the variety of the monomers, the constitution isomerism, variable molecular weights, and the additional possibilities for the production of blends or a chemical/physical modification of the microstructure, a major potential of widely-varying biopolymers with differing property profiles results within this family of polymers.

From a chemical perspective, PHAs are optically-active, aliphatic polyesters possessing the structure shown in Fig. 30. In the case of $R = CH_3$, the result is so-called polyhydroxybutyrate or so-designated polyhydroxybutyric acid (PHB). At $R = C_2H_5$, polyhydroxyvalerate (PHV) occurs, at $R = C_3H_7$, polyhydroxyhexonate (PHH), and at $R = C_4H_9$, appropriately, polyhydroxyoctanoate (PHO), etc. In view of the large number of theoretically possible PHAs, it can be assumed that a maximum of ten different industrially-interesting PHAs should find implementation in the future [12, 20, 23].

Generally, PHAs are easy to use in injection molding, are insoluble in water but nevertheless biodegradable, and are biocompatible. Furthermore, they have a very

Fig. 30 General structure of polyhydroxyalkanoates (PHAs) [22]

$$\left[-O-\underset{\displaystyle |}{\overset{\displaystyle R}{CH}}-CH_2-\overset{\displaystyle O}{\overset{\|}{C}}- \right]_n$$

good barrier effect against oxygen and, compared to other biopolymers, a somewhat better barrier effect against water vapor. This, in the opinion of the author, together with their variable molecular structure with different resulting property profiles and a broad range of raw materials for PHA production, means that these polymers form a promising material group for future material developments. In addition, PHAs also provide an interesting source for the production of small molecules or chemicals such as hydroxy acids or hydroxyalkanols [2].

3.4 Modification of Molecular, Renewable Feedstock

The various biopolymers in this group are based in particular on the polysaccharides starch and cellulose. Around 100 years ago, when petrochemical raw materials were not available, cellulose-based biopolymers constituted the first polymers and thereby, from today's perspective, biopolymers. However, starch-based biopolymers, because of low commodity prices, good availability, and very good degradability, occupy the leading role in modern biopolymers, which have now been researched for around 30 years.

3.4.1 Starch-Based Bioplastics

For the production of bioplastics from starch, there are a number of fundamentally different methods. These are shown in Fig. 31.

In the use of starch as a raw material for *fermentatively-produced polymers*, the process is a metabolism of starch for the microbiological formation of other polymeric raw materials (see Sect. 3.2).

During extrusion-technological production of *starch-filled, thermoplastic composites*, the particulate or granular starch serves as both a low cost and a functional filling material. The starch granules enable an improvement in the mechanical properties, such as the elastic modulus, and accelerate the degradation behavior [14, 24, 25].

In contrast, in the case of so-called *thermoplastic starch*, the *starch granules* are *destructured extrusion-technologically* and a thermoplastic is created based on the starch macromolecules amylose and amylopectin. Depending on the ratio of the added quantity of water, shear forces, and temperature, this results in a predominantly thermomechanical destructuring of the granules or, rather, a thermochemical gelatinization of the starch, caused by water [26]. Because of the polarity of the

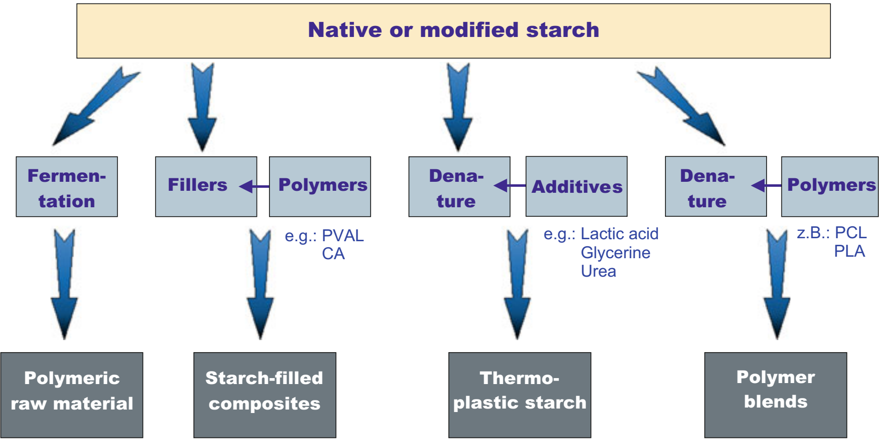


Fig. 31 Bioplastics based on starch [2]

macromolecules amylose and amylopectin, corresponding intensive molecular interactions are formed following the destructuring of the granules. The consequences of this are, as with cellulose, a difficult extrudability and brittle mechanical material properties. The destructuring and plasticizing of the starch in the extruder is therefore often carried out with the addition of water and other processing agents as well as plasticizers such as glycerin. Pure thermoplastic starch has, for example, a glass transition temperature T_g of 80°C at an equilibrium water content of approximately 14%, which makes it very brittle at room temperature. Through the addition of various plasticizers, such as hydroxycarboxylic acids, glycerin, polyols, or water, T_g can be reduced and the material can be made more ductile.

A further problem is presented by the hydrophilic properties of starch and starch polymers. In addition to the so-called external plasticization through additives, such as sorbitol or glycerin, and/or an internal plasticization through the starch modification, the thermoplastic starch is generally blended with other biopolymers, such as PLA or other polyesters. As the polyesters are often petrochemical polyesters with a generally higher material price, the starch blend manufacturers endeavor to optimize the starch proportion to achieve maximum material performance.

3.4.2 Cellulose-Based Bioplastics

There are two main groups of cellulose polymers: the so-called regenerated cellulose, which is present predominantly as fiber or film, and the cellulose derivatives, which are further divided into the two main groups of cellulose esters and cellulose ethers; see Fig. 32 [27, 28].

Regenerated cellulose is essentially cellulose which has been physicochemically dissolved and re-assembled in the form of fibers or films. There is a wide range of designations for regenerated cellulose. The best-known names for fibrous products

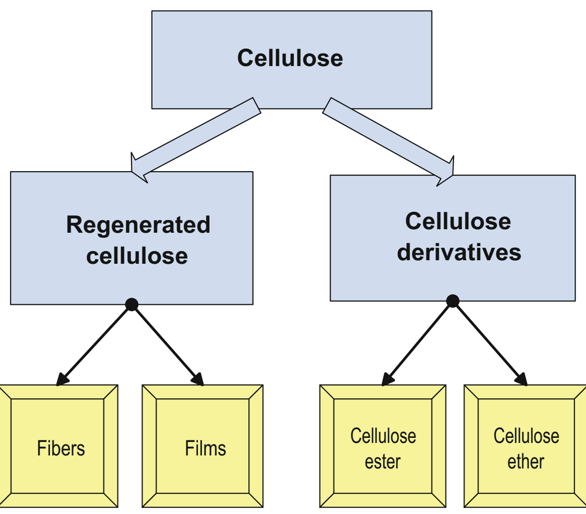


Fig. 32 Cellulose-based polymeric materials [2]

are viscose, viscose silk, rayon, spun rayon, Modal, Lyocell, copper silk, and artificial silk and for films from regenerated cellulose cellophane, cellulose hydrate, hydrated cellulose, and cellulose film. Significant differences include the solvents used in the production processes and the corresponding process management as well as the differing resultant properties. Copper silk is based on Schweizer's reagent, whilst for the Lyocell fiber, NMMO is used as solvent for the cellulose. The quantitatively most significant regenerated fiber is viscose or viscose silk. For this, and for curled spun rayon (shrinkage of stretched viscose fibers in a hot liquid), the manufacturing path leads through an alkalization of the cellulose so that alkali cellulose is produced, which is subsequently transformed with carbon disulfide and then precipitated in an acid bath to viscose fibers. For the higher-strength Modal fiber, additional chemical additives (particularly Zn salts) are used in the viscose process. Rayon and artificial silk are non-standardized collective terms for fibers made from regenerated cellulose or cellulose acetate.

As regards the cellulose derivatives, differentiation is made between the two main groups, cellulose ethers, and cellulose esters. The various cellulose ethers, which are predominantly produced through etherification with halogenated hydrocarbons or epoxides, are mainly used as additives for viscosity stabilization or as water retention agents in construction materials, adhesives, cosmetics, detergents, paints, drilling fluids, or in the paper industry. In contrast, cellulose esters are predominantly thermoplastic molding compounds. As a starting material for cellulose esters, the cellulose can, because of its form with anhydroglucose units with three reactive hydroxy groups respectively, theoretically form unlimited numbers of esters of organic acids. The complex structure of the cellulose molecule, however, drastically restricts the number of technical possibilities. In applied technology, organic esters with a high degree of esterification are therefore only produced by a few aliphatic fatty acids with up to four carbon atoms. Of importance today are,

Fig. 33 Chemical structure of cellulose esters [22]

in particular, the organic cellulose esters of acetic acid, butyric acid, and propionic acid. For the esterification reaction, the corresponding anhydrides of these acids are usually applied.

With :	Cellulose acetate (CA)	$R = —CO—CH_3$
	Cellulose propionate (CP)	$R = —CO—CH_2—CH_3$
	Cellulose butyrate (CB)	$R = —CO—CH_2—CH_2—CH_3$

Mixed polymerizates such as cellulose acetate propionate (CAP) can also be formed. Furthermore, substantial quantities (3–35 wt%) of plasticizers are required to improve the thermoplastic melt processability and the resultant mechanical performance properties of the cellulose esters. Through the degree of esterification, the solubility and compatibility with plasticizers, coating resins, etc. are primarily determined and the mechanical properties thus ultimately also influenced [29, 30]. In addition to the quantity and type of plasticizer, the resultant property profile of the cellulose ester is determined particularly by the acid group, the number of ester groups (degree of substitution), and their distribution (tacticity). As a total of three hydroxy groups per glucose ring are available as functional groups, a maximum degree of substitution of three, that is, a cellulose triacetate, is possible. The term triacetate is, however, often applied to a substitution of the hydroxy groups of >92%, that is, of a degree of substitution of >2.75 [31–33].

Cellulose nitrate (often mistakenly referred to as nitrocellulose) is obtained from nitric acid and cellulose and is also known as, amongst others, celluloid (75% cellulose nitrate +25% camphor). As a cellulose ester it was the oldest thermoplastic material and was discovered in 1870 (Fig. 33).

3.5 Co−/Terpolymers

In representation of the extensive group of generally partially bio-based co- or terpolymers, the quantitatively most important types of these biopolymers are (co-) polyamides (see Sect. 3.2.5) and polyesters. In most cases, these polyesters are produced from a bivalent alcohol and a dicarboxylic acid or an ester produced therefrom.

For the alcohol component, propanediols (PDO) such as 2,2-dimethyl-1,3-propanediol, 1,3-propanediol, or 1,2-propanediol as well as various butanediols (BDO) such as 2,3-butanediol or 1,4-butanediol are applied. Whilst in the

so-designated biopolyesters the aliphatic alcohol component is usually of biogenic, that is, fermentative origin, for the second reaction component, petrochemical dicarboxylic acids such as terephthalic acid or dimethylterephthalate, succinic acid, or adipic acid are still preferred. In the future, more and more of these acids, particularly non-aromatic acids such as succinic acid, should become bio-based, that is, produced with biotechnology.

As these polyesters, depending on composition and raw material, also contain differing proportions of bio-based material components and, simultaneously, their biodegradability varies widely, no clear boundary can be defined for this family of polymers.

The group of best-known biodegradable polyesters includes polybutylene succinate (PBS). The degradable PBS is currently usually produced from petrochemical raw materials. If, however, a bio-based butanediol and bio-based succinic acid are applied as monomer components, the polycondensation results in a fully bio-based PBS. Other well-known degradable, aliphatic-aromatic terpolyesters include polybutylene adipate terephthalate (PBAT), formed from adipic acid, terephthalic acid and butanediol, polybutylene succinate adipate (PBSA) as a linear terpolyester from polybutylene succinate and polybutylene succinate adipate, that is, polymerized with succinic acid and adipic acid, and polybutylene succinate terephthalate (PBST). These degradable polyesters also serve as important blending components for many other biopolymers, in particular for starch and PLA blends.

In addition to these currently still predominantly petroleum-based, degradable polyesters, there are also various partially-durable, bio-based polyesters. The best-known durable biopolyesters are the partially bio-based polyethylene terephthalate (bio-PET) and polytrimethylene terephthalate (PTT). For PTT, the bio-based polyester component is biogenic 1,3-propanediol, whilst bio-PET is based, in addition to petrochemical phthalic acid, on monoethylene glycol (MEG) produced from bio-based ethanol.

3.6 Blends

Similar to the diverse material developments in conventional plastics over the last few decades, blending has led to the emergence of a number of new bioplastics with improved property profiles. However, homogeneous blends (homogeneous polymer alloys, homogeneous polymer mixtures), in which two or more blend components are thermodynamically-miscible as far as the molecular level, only occur when the blend components have very good compatibility. For biopolymers, however, homogeneous blends are rather the exception; morphological multiphase systems (heterogeneous blends) usually result. In contrast to the homogeneous blends (or copolymers), these heterogeneous blends usually demonstrate no uniform properties, that is, they usually have two identifiable glass transition or melting temperatures for the individual components (Fig. 34).

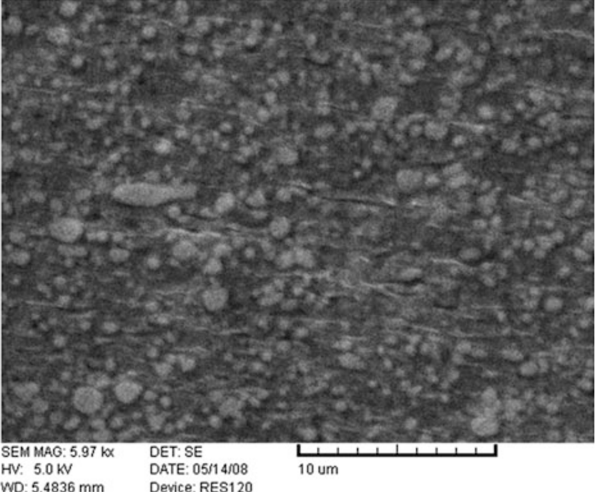


Fig. 34 Section through a starch blend (discontinuous phase = thermoplastic starch) [2]

For the blending of biopolymers, biphasic blends (bi-blends or binary blends) are currently preferentially generated, that is, two different biopolymers are mixed. Work is being focused on the compatibility and the finest-possible dispersion or distribution of the different phases. As an example, for the hydrophobizing of a hydrophilic biopolymer, the continuous phase must be formed through the hydrophobic component. The procedural minimal realizable phase size for these heterogeneous blends is 0.5 μm.

In addition to the optimization of the blend morphology, suitable compatibilizers are usually applied to increase the compatibility of the blend components, which often have differing polarity. The compatibilizers favor accumulation at the interfaces of the participating polymers or even penetrate the components slightly. They increase the interface quality, that is, the adhesion between the different phases. Simultaneously, they reduce the surface tension and thus the particle size as well, as the coagulation of the dispersed phase.

Most biopolymer blends are based on thermoplastic starch, which is rendered hydrophobic through the addition of other biopolymers such as cellulose acetate, polycaprolactone, or other polyesters, and which exhibits a significantly higher ductility. Thermoplastic starch is therefore only processable for the formation of films through the compounding of other blend components. A further important group of biopolymer blends are mixtures based on the copolyester PBAT or PBS.

4 Properties

During the course of these development stages, various biopolymers and bioplastic material types based on these biopolymers have been developed which exhibit widely-differing property profiles. The range of bioplastics thereby ranges – similar

to conventional plastics – from less-expensive bioplastics which are produced in large quantities through to high-quality and higher-priced materials for technical applications.

The pyramids in Figs. 35, 36, and 37 compare the material performance of the most important bio-based and biodegradable bioplastics and show the range of materials now available.

Similar to conventional bulk plastics, economically-priced bioplastics are now available with prices of around €2/kg. Bio-based polyamides, however, are higher-priced materials. The prices for bio-based polyamides are currently still 20–50% higher than the prices for conventional polyamides. These bio-based polyamide materials, however, partially offer innovative or specifically better property profiles. As regards drop-ins, such as bio-based PE or PET, the technical properties of the bioplastics are identical with those of their petrochemical equivalents. In terms of price they are, because of the currently modest production scales, approximately 20% more expensive than their petrochemical equivalents. Drop-ins can currently therefore only be marketed on the strength of their sustainability.

As regards the thermal properties, the biopolyamides are durable bioplastics with a higher thermal resistance; there are, however, currently no bioplastics which achieve the level of high-temperature-resistant conventional plastics such as PEEK (HDT/B = 240°C) or PPS (HDT/B = 215°C). It must, however, be noted that the

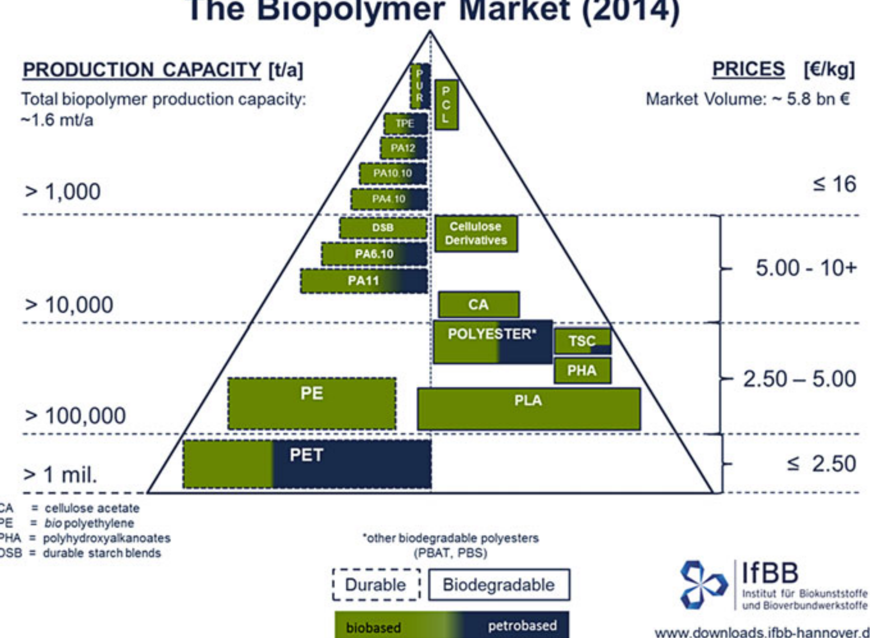


Fig. 35 Production capacities and prices for various bioplastics [7]

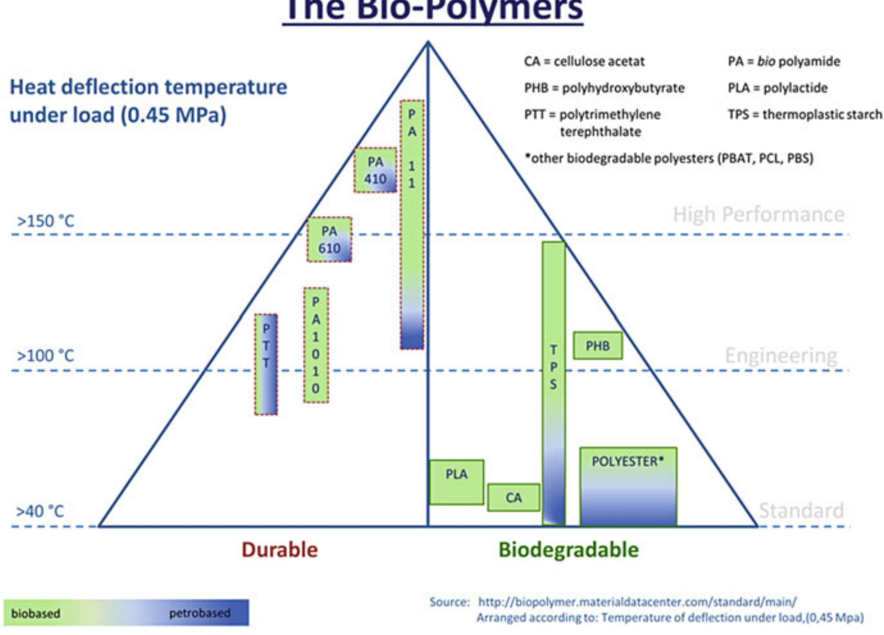


Fig. 36 Heat distortion temperature (HDT/B) for various bioplastics [7]

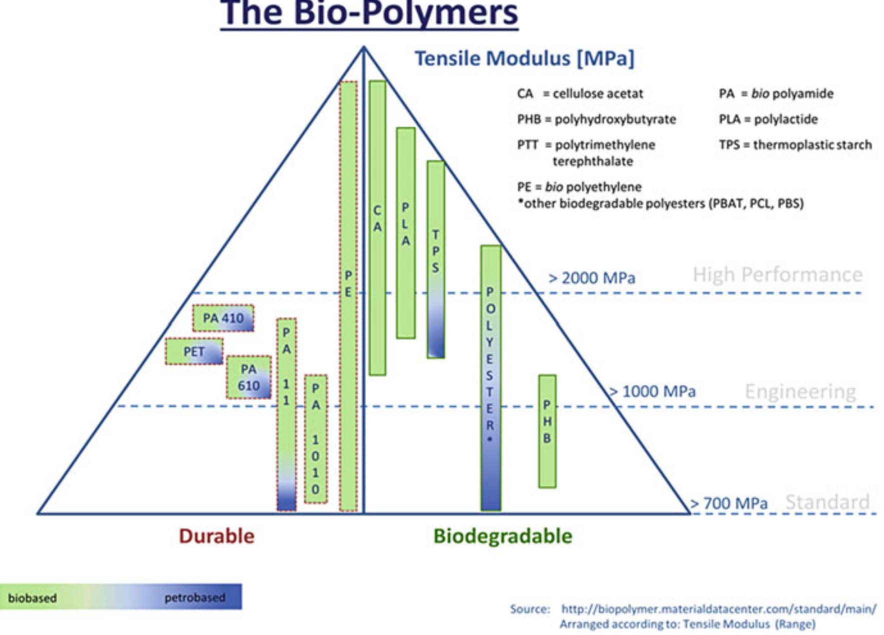


Fig. 37 Tensile modulus for various bioplastics [7]

developments in bioplastics until now have not been targeted at their use at high temperatures.

As regards the mechanical properties, bioplastics now also cover a greater range, but there is still need for further optimization. To optimize further the thermal and mechanical property profiles of bioplastics with regard to technical applications, the known methods used in conventional plastics, such as the production of specific blends, reinforcement with glass fibers or cross-linking, can and should be used.

5 Utilization of Residual Materials

The use of residual materials in the production of bioplastics or raw materials for bioplastics production is the subject of intensive research. Whilst the energetic recovery of residual materials such as lignin or bark as well as plant debris (bagasse, straw, hurds, etc.) or the use of these residues as a filler or reinforcement has been established, the use of lignocellulose as a polymer raw material for fermentation or polymerization has not yet advanced beyond theoretical status. Although there have been numerous scientific studies on this subject, as well as promising research approaches, most of the transformation processes have not yet achieved an economic status. The targeted cultivation of renewable raw materials such as starch or sugar, however, currently represents the most economical and land-efficient method for the provision of bio-based raw materials.

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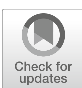
Biotechnological and Biochemical Utilization of Lignin



Dominik Rais and Susanne Zibek

Abstract This chapter provides an overview of the biosynthesis and structure of lignin. Moreover, examples of the commercial use of lignin and its promising future implementation are briefly described. Many applications are still hampered by the properties of technical lignins. Thus, the major challenge is the conversion of lignins into suitable building blocks or aromatics in order to open up new avenues for the usage of this renewable raw material. This chapter focuses on details about natural lignin degradation by fungi and bacteria, which harbor potential tools for lignin degradation and modification, which might help to develop eco-efficient processes for lignin utilization.

Keywords Aromatics, Enzymes, Lignin, Lignin degradation, Lignin utilization

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1 Biosynthesis and Chemical Structure of Lignin

Detailed knowledge about the structure and biosynthesis of lignin is central for developing new strategies to degrade the complex lignin molecule and precisely modify its single components. Additionally, this information can help to develop lignin with specific characteristics by engineering plants.

The development of tracheids, and thereby water-connecting tissues, is considered to be a keystone process in the evolution of terrestrial plants. This process is associated with the development of the biopolymer lignin. Because of its hydrophobic nature, lignin makes the tracheids impermeable to water, which is essential for water transport. Furthermore, because of its complex structure, it stabilizes the aerial organs and enables erect-growth. Lignin was recently also found in red algae, assuming its original role in ancestors of higher plants and algae was the protection against microbial degradation or UV radiation [1–4].

Lignin is mainly composed of the monolignols sinapyl-alcohol, cumaryl-alcohol, and coniferyl-alcohol (see Fig. 1). Generally, lignins from gymnosperms contain mainly coniferyl units (>95%) with a minor amount of cumaryl units (<5%), whereas in angiosperms coniferyl, sinapyl, and marginally cumaryl units occur at 25–50%, 46–75%, and <8%, respectively. Lignins of grasses are also composed of all three components, but differ in higher portions of cumaryl units (up to 33%) [5, 7].

The phenylalanine-derived lignin precursors are synthesized in the cytoplasm and exported to the apoplast. The transport mechanism across the plasma membrane is still unknown. However, reaching the apoplast, the monolignols undergo single-electron oxidation and form reactive radical species. The radicalization process is most likely mediated by laccases and/or peroxidases (see Fig. 1) [8]. In

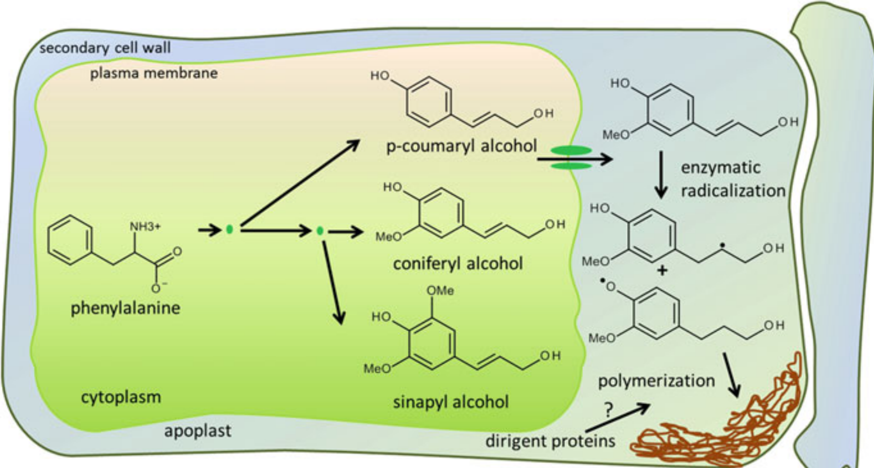

Fig. 1 Lignin synthesis in plants (modified after [5, 6])

this regard, just recently a triple knock-out of laccases in *Arabidopsis thaliana* showed an almost completely abolished lignin deposition in roots in vivo, hinting on the participation of laccases in lignin biosynthesis [9].

The lignin polymer is built by the crosslinking of the radical monolignols. Herein the combinatorial random coupling of the radical monomers is the widely accepted mechanism for the formation of lignin. In contrast, a protein-directed synthesis including dirigent proteins has also been hypothesized [10]. The model of directed polymerization of lignin is supported by some experimental findings. For instance, the specificity of a peroxidase for only one monolignol was shown, suggesting a possible regulation of the lignin composition by plants. Accordingly, experiments with knock-out and down-regulation of certain peroxidases resulted in an alteration of sinapyl or coniferyl unit content in lignin [11]. In contrast, a deletion of sinapyl or coniferyl alcohol-delivering enzymes resulted in lignins with high amounts of coniferyl or sinapyl units, contradicting a strict control of monolignol assembly [6]. Additionally, formation of a defined primary lignin structure mediated by protein templates was suggested [12]. This finding is under discussion as lignin shows no optical activity and genetic data are missing [6, 13]. A precise delivery of laccases to the secondary cell wall within the apoplast was shown recently [8]. This indicates a protein-directed synthesis of lignin, especially for the localization of polymerization. Despite that, the dirigent role of proteins within the selectivity for monolignols and building of determined bonds remains in doubt.

According to the random coupling hypothesis, the monomers are coupled either one by one or different oligomers are linked together at the same time, termed "endwise polymerization" and "bulk polymerization." Herein the availability of the monolignols may influence the prevalence of the two mechanisms. However, the nature of the monolignol probably exerts the most influence. Besides being reactive at the phenolic oxygen (hydroxyl group at the C4-position), the monolignol-derived radicals are reactive at the C1, 2, 3, 5, and at the β–C (see Fig. 2), although steric

Fig. 2 Cumaryl, coniferyl, and sinapyl alcohols with computed electron spin densities (modified with permission from [14], Copyright 2012, American Chemical Society)

hindrance may limit or prevent the reactivity in some of these sites [15]. Conferring to the highest computed electron spin density, the *p*-coumaryl radical in the C1-position has supposedly the highest reactivity, followed by the phenolic oxygen, C3, β–C, and C5. In coniferyl and sinapyl radicals, phenolic oxygen has the highest spin density succeeded by C1, C3, β–C, and C5. Nevertheless, C1-coupling occurs at low frequency in natural lignin and coupling at the methoxylated C-positions happens with an irrelevant frequency, maybe because of steric hindrance. Generally, the phenolic hydroxyl group and the β–C appear as the most likely coupling sites [14].

Coupling at the β-position is preferred for coniferyl and even more for sinapyl alcohol monomers. In dimerization reaction experiments, the coniferyl alcohol led to dimers with β–β, β–O–4 and β–5 linkages. The portion of the β–O–4 bond was less than one-third with coniferyl and only about 9% when sinapyl alcohol was used as substrate [16]. In contrast to dimerization, the oligomers are unable to couple only at the β-position during lignification. The cross-coupling of the monolignols coniferyl or sinapyl alcohol with a guaiacyl unit of the lignin polymer (where the β–C site is already coupled) gives two possible bonds: β–O–4– and β–5. Moreover, in coupling reactions of monolignols with a sinapyl unit (where the β–C site is already coupled) only a β–O–4 unit occurs (see Fig. 3b). This shows why β-ethers are formed more frequently in lignification than in monolignol dimerization experiments. Additionally, these findings explain why lignins with higher amounts of sinapyl monomers contain more β-ether bonds. In an approach of peroxidase-mediated in vitro polymerization of coniferyl monomers, addition of sinapyl monomer to the reaction led to a switch from a bulk to an endwise mechanism [11]. The coupling of two lignin oligomers is uncommon in lignins with high sinapyl unit

Fig. 3 (**a**) Prominent bonds in lignin (modified after [17, 18]). (**b**) Possible coupling site of incorporated guaiacyl (*left*) and sinapyl units (*right*). *Red arrow*, OH-group at the C-4-position; *blue arrow*, C-5-position (modified after [16] with permission from Springer)

Table 1 Major bond types in beech and spruce lignin[a] (%) [17, 20]

Bond type	β–O–4	β–5	β–1	β–β	5–5′–O–4	4–O–5[a]	5–5[a]
Beech	60.3	1.0	3.5	8.0	0.5	2	2
Spruce	44.7–49.4	10.6–13.1	1.1–2.6	1.6–5.8	2.0–3.8	6–7	7–9

[a]Bond only indicated in [17]

content, but is often found in lignins with high coniferyl unit ratios, where, for example, 5–5 coupling occurs more often [5].

Lignin is a very complex molecule and the analysis of its structure and linkages is a considerable challenge, even with modern NMR methods. The most abundant linkage in lignin is the β–O–4 ether bond. However, the composition of the numerous bond types varies significantly between different plants [19]. The structure of the major bonds in lignin is illustrated in Fig. 3a. In Table 1 the occurrence of these bonds in beech and spruce lignin is shown, which represent hard and soft wood. The proportions of β–1 and β-ether linkages in beech lignin are considerably higher than those in spruce lignin, whereas the content of 5–5 and 4–O–5 linkages is lower. This means that spruce lignin is more condensed, resulting in lower solubility and degradability [17].

A better understanding of the lignin biosynthesis mechanism is important for the development of lignocellulose biomass-utilizing technologies [15]. Detailed knowledge about lignin synthesis pathways is essential for the engineering of plants with a modified lignin polymer structure. A less recalcitrant lignin makes lignocellulose biomass more accessible by treatment methods [21]. Moreover, the lignin structure may be designed in a way that makes lignin itself more suitable for fuel and chemical applications by enhancing its homogeneity and optimizing its chemical properties [22].

2 Technical Lignins

Here, a short overview of existing lignocellulose pretreatment processes and the resulting lignin types is given.

Kraft lignin (KL) and lignosulfonates are the most common commercially available technical lignins [23]. Both these lignins contain sulfur residues as a result of the underlying lignocellulose pretreatment processes [24]. Within the kraft process, biomass is cooked in the presence of sodium hydroxide and sodium sulfide and the lignin is degraded and solubilized in the alkali solution. In contrast, lignosulfonates are gained by cooking with sulfite, at which sulfonating, degrading, and solubilizing of the lignin occurs [25]. Additionally, the soda process and the organosolv process, both working without sulfur, are also described as main industrial lignocellulose pretreatment processes, although the latter is currently not operated on a commercial scale [26]. The soda process was the first lignocellulose pulping method and is similar to the kraft process, but uses solely sodium

hydroxide [25, 27]. Within the organosolv process biomass is cooked in a mixture of organic solvents and water and the separation of lignin is performed via solubilization. Common solvents for this method are ethanol, formic acid, and acetic acid.

By means of soda and organosolv pulping processes sulfur-free lignins are obtained [24], which are in many respects superior to sulfur-containing lignins. Sulfur-free lignin has an advantage in environmental applications [23], can be heat-processed without odor release, and is preferred as raw material for several products [28]. For instance, in carbon fiber production, sulfur leads to inferior melt spinning characteristics [28, 29]. Sulfur-free lignin is, moreover, rather suitable as raw material for activated carbon and other aromatic added-value chemicals and does not cause air pollution problems [30].

Besides organosolv and soda pulping, sulfur-free lignin can be obtained by other processes such as steam explosion, ball milling, pyrolysis, and processing with ionic liquids, which have however not yet reached the marketplace [31]. Organosolv processes deliver lignin with the highest purity in the above-named processes [32]. Because of its relatively high homogeneity, purity, and reactivity, organosolv lignin may be the most promising technical lignin for further processing and direct applications [33]. Commercializing of sulfur-free lignins opens new potentials for utilizing lignin in value-added products [34].

Nevertheless, in spite of their lower homogeneity and purity, sulfur-containing lignins can be preferable in certain applications because of sulfur or their higher molecular weight. Thus, for example the addition of lignosulfonates in gypsum paste resulted in a better dispersibility, especially at higher molecular weights and sulfur content [35].

Summing up, the technical lignins vary strongly in their physical and chemical features. Therefore, a specific type of lignin has to be chosen depending on the particular application [36].

3 Lignin Applications

In this section an overview of current industrial lignin usage, details of the challenges in processing technical lignins, and an outlook on potential future applications are given. Thereby, biotechnological and chemical methods for processing lignin components are described.

The utilization of lignin in an economically viable manner is one of the most important tasks of lignocellulose biorefineries. Currently, only about 2% of the 50 million tons of lignin produced by the pulp and paper industry every year are used for industrial purposes, and the residual lignin is burned [18]. In most biorefinery concepts, which focused mainly on hydrolysis and sugar fermentation to ethanol or other fuels, lignin was also underutilized. Remarkably, the refineries produce 60% more lignin than necessary for their own power supply, resulting in a high amount of unused lignin [22]. However, a separation process for KL from pulp and paper mills, called lignoboost, was recently launched on an industrial scale in

Finland and the USA. Lignoboost is able to separate lignin efficiently with higher quality, meaning low ash contents. Traditional pulp mills can be transformed into combined biorefineries [37]. Consequently, new high value applications for lignin are needed [22].

A few industrial applications for lignin do already exist, such as the production of synthetic vanillin and dimethyl sulfoxide. However, lignin is mostly used for its chemical properties as electrolytic material, or advantage is taken of its polymeric structure. Thus, lignin is utilized as sequestrant, binder, dispersant, and emulsifier, or, to a lesser extent, used as a filler and in adhesives. For these types of applications, lignin is not modified or modified only slightly, and therefore used in its naturally-occurring structure [38]. A successful example for value-added commercializing of lignin is its processing by injection molding, extrusion, and compression molding, using polymeric lignin, wood meal, and additives such as flax fibers. The thermoplastic material named “arboform” can be used as shells for mobile phones or computers and in components of cars such as steering wheels [39, 40].

Moreover, the use of lignin as carbon fibers, polymeric modifiers, resins, and the expansion of lignin adhesives bears a high potential for a valorization of this raw material [41].

Numerous lignin-converting processes are still on the road toward commercialization. Perez-Cantu et al. were able to prepare an aerogel with lignin as the only phenolic component. Lignin was crosslinked with oligo (ethylene or propylene glycol)-diglycidyl ethers, which results in gels with promising properties for thermal insulation [42]. Engelmann and Ganster also used glycerol-diglycidyl ether for crosslinking low molecular weight fraction lignin. They produced solvent-free resins with lignin contents up to 50%. The lignin resins exhibited a better thermal stability than conventional resins made with pyrogallol [31].

Because of its properties, lignin may not be suitable for applications requiring thermal stability and melting processes, and therefore many studies were carried out on the incorporation of lignin into polymer blends with other synthetic or other bio-based polymers. For example, a combination of lignin and fish protein or wheat gluten was described [43]. Lignin could successfully be introduced in styrene-butadiene rubber as a lignin-layered double hydroxide (L-LDH). Mechanical analyses indicated that L-LDH/styrene-butadiene rubber was superior to LDH/styrene-butadiene rubber concerning elongation at break, modulus, tensile strength and hardness [44]. Recently, Spiridon et al. produced a polylactic acid material with an organosolv lignin content of 7%, which showed improved thermal stability and mechanical properties compared to neat polylactic acid [45]. Chung et al. were able to produce a lignin-g-poly(lactic acid) copolymer, which can additionally be blended with polylactic acid, leading to a material with UV-blocking properties and improved mechanical features. The polymer length could be influenced by an acetylation pretreatment of the lignin [46]. Although much research on transformation of lignin into chemicals, materials, and fuels was carried out, realization into a commercial process is still rare [22].

The most striking difficulties, which restrict the conversion of lignin into high-value products, are the non-uniform structure, chemical reactivity, and impurities of technical lignins. The lignin polymers vary in their size, polymer composition, and

degree of crosslinking, as well as in the abundance of functional groups, which results in non-uniform structures. This issue could be overcome by controlled depolymerization of the lignin, which can be performed chemically or by enzymatic pathways. Additionally, technical lignins often have different reactive groups, causing several diverse reactions. When technical lignins are used for polymer applications such as for producing resins, only one of these reactions might be desired and the other side reactions might hamper the polymerization process. Moreover, lignins are limited in chemical reactivity because of their small number of *ortho* and *para* reactive sites. Degradation of lignin might help to gain access to the reactive groups but this does not improve the general reactivity of lignin. The introduction of reactive sites into the lignin molecule might be another possibility to increase the reactivity of this inert molecule [25].

One feasible method for increasing the reactive sites of lignin, for instance, is the grafting of functional molecules onto lignin. An example for functionalization of lignin via hydroxyl groups is the esterification with oleic acid. Thereafter, it is possible to epoxidize the double bounds of the fatty acids followed by a ring-opening reaction to produce polyols. These building blocks, together with isocyanate prepolymers, were used to produce polyurethanes, showing advanced properties, and may be utilized as durable materials in the building and automotive industry [47].

Lignin can be functionalized chemically, but eco-efficient biotechnological approaches are also feasible. Some potential examples for a functionalization of lignin by ligninolytic enzymes are given here. Laccases are well-known to generate radicals and can be used to link phenolic compounds, such as vanillic acid diisocyanate or acrylamide, to the lignin polymer. Horseradish peroxidase can also be used in this way and lead to copolymers when incubated with straw pulp lignin and cresol. This process might replace the use of phenolic resins [48–50]. Other examples of enzymatic approaches with industrial lignins are the manufacturing of paints or polymer–template complexes and the optimization of chelating properties of lignin. For producing paints, including protective coatings, lignin is mixed with a dye or a pigment. There it reacts with a peroxidase or laccase and the process is stopped when the desired viscosity is reached. Polymer–template complexes are obtained by polymerization of a template (e.g., lignosulfonate) and a monomer through an enzyme (e.g., peroxidases). These complexes can be used for various applications as lightweight energy storage devices (e.g., rechargeable batteries). A polyphenol oxidase was used to improve the chelating capacity of acetosolv lignin. An increase of hydroxyl and carbonyl groups and an improvement of 110% in the chelating capacity were detected [49]. An interesting approach is the coating of materials such as starch-based films with laccase and lignin, which can be used as oxygen-scavenging active packaging for bread, cheese, meat, and various fruits. Alkali lignin, hydrolytic lignin, and organosolv lignin and lignosulfonates were tested. Organosolv lignin and lignosulfonates achieved the best results for oxygen-scavenging [51].

Additionally, lignin represents a promising renewable source of aromatic chemicals [52]. The most current commercial approaches utilize the lignin

macromolecule, whereas the main potential can be seen in the depolymerization of lignin into aromatics such as vanillin, phenol, toluene, and benzene. The degradation processes are still in the early stages of development. Approaching research aims at increasing yield and selectivity [53]. Besides the degradation processes, the methods for separating mixtures of lignin derived chemicals such as vanillin and syringaldehyde are challenging and still under investigation [54]. One successful approach of utilizing these mixtures directly was made by Fache et al. They functionalized a mix of vanillin, acetovanillon, vanillic acid, *p*-hydroxybenzaldehyde, syringaldehyde, and acetosyringone by oxidation and subsequent glycidylation. The mixtures were polymerized and the epoxy resins obtained displayed remarkably good thermomechanical properties [55].

Together with chemical and physical methods (for further information see reviews [56–58]), there is an interest in eco-efficient biological methods for lignin degradation. For instance, biological processes could be used to generate aromatic chemicals from lignin. However, a deeper understanding of the natural degradation of lignin is needed to develop lignocellulosic biorefineries [52].

4 Lignin Degradation in Nature

In this section, details of fungal lignin degradation are described. First, the fungal degradation mechanisms are explained, including white-rot, brown-rot, and soft-rot decay. The ligninolytic enzymes participating in these processes, such as laccases, peroxidases, peroxygenases, as well as involved accessory enzymes, are also characterized. Second, the lignin-degrading strategies of bacteria are addressed, which were so far underestimated in their ligninolytic capacity. Bacteria degrade lignin to a far lesser extent compared to fungi. However, as their bioengineering potential is much higher, new methods to find lignolytic bacteria are of interest. Herein, some known lignolytic bacterial strains are described, although the enzymatic background is so far less clear. Potentially, DyP-type peroxidases or laccases could be engaged in the bacterial degradation of lignin.

4.1 Fungal Lignin Degradation

Lignocellulosic biomass is mainly degraded by particular fungi belonging to the basidiomycetes. These specialists can be divided into two main classes termed white-rot and brown-rot fungi [59]. Brown-rot fungi consume primarily carbohydrates, resulting in a brown dry rot of brittle consistency. In contrast, white-rot fungi degrade both lignin and carbohydrates and leave soft spongy debris of light appearance [60]. The wood-rotting fungi often co-exist in a similar ecological niche, which leads to several interactions. Synergistic interactions such as cooperative degradation of substrates are found [61, 62]. Synergetic effects in the

production of lignin modifying enzymes were observed when different white-rot fungi species were combined [63, 64]. However, these effects seem to be dependent on the used species, their interaction, and nutritional conditions and environmental factors [64]. The hindrance of further growth of other organisms by occupying a territory and antagonistic effects as nutrition competition can occur. Adverse interactions of fungi may lead to deadlock or replacement [65]. When wood composition and physical properties change during the decaying process, better adapted species replace the prior species [66]. Thus specific patterns of colonialization are found during the decay, partially depending on the wood type [66]. Soft rot fungi often appear as pioneers followed by white-rot and brown-rot fungi [67].

Despite their different decaying mechanisms, further data about nuclear and mitochondrial DNA prove a phylogenetical relation between white-rot and brown-rot fungi [68]. Consequently, the separation of white-rot and brown-rot into two classes is disputed, for the two species *Botryobasidium botryosum* (found in a genetic tree between Auriculariales and Dacrymycetes) and *Jaapia argillacea* (probably a sister group of the Gloeophyllales) as these combine features of both categories (see Fig. 4). Analysis of the genomic sequences showed that both lack class II peroxidases and possess reducing polyketide synthase genes, both typical of brown-rot fungi. However, they are closely related to the white-rot fungus *Phanerochaete chrysosporium* and both strains were able to degrade all polymeric components of wood. Moreover, genes coding for cellobiohydrolases were present, which is characteristic of white-rot fungi [70]. By an investigation of 31 fungal genomes it was suggested that the ancestor of all Agaricomycetes was a white-rot

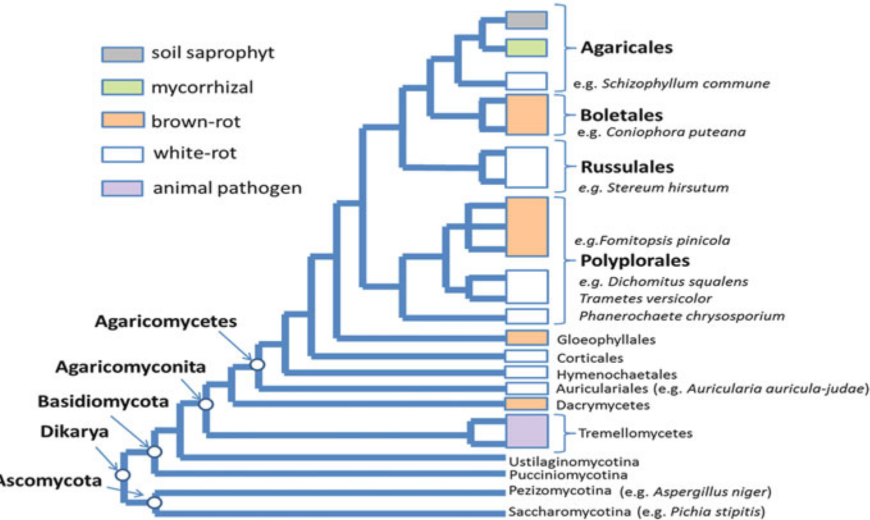

Fig. 4 Overview of Dicarya fungi and illustration of the relationship between white-rot and brown-rot fungi (modified after [69])

fungus owning class II peroxidases, DyP-type peroxidases, and H_2O_2-supplying enzymes. Thus, it was assumed that on the one hand, an expansion of class II peroxidase genes leads to the white-rot orders *Auriculariales*, *Hymenochaetales*, *Corticiales*, *Russulales*, and *Polyporales*. On the other hand, a parallel decline of class II peroxidases was suggested, finally resulting in the brown-rot fungi *Dacryopinax* sp., *Gloeophyllum trabeum*, the Boletales, and the brown-rot species inside the Polyporales (Fig. 4).

By molecular clock analyses the origin of fungal lignin degradation could be dated to the late Carboniferous period. Remarkably, coal formation, which is mainly caused by lignin burial, has strongly decreased since this period. Accordingly, a correlation of both these events was hypothesized [69].

The woody plant cell walls are structured in different layers. The cells themselves are linked by the middle lamella, which possesses the highest concentration of lignin and pectins and builds the outer layer of the cell wall [71]. Then, from the outside to the inside an S1 layer, a thick S2 layer, a thin inner S3 layer, and a bumpy layer consisting of aromatic precursor molecules are attached to the middle lamella. Therein the lignin content decreases in the same order (see Fig. 5) [73].

Rotting fungi colonialize deadwood through hyphal growth. The accession and spreading is enabled by the organization of the tracheids and vessels in the axial direction and in the radial orientation of the xylem ray parenchyma. The joining cells are invaded either by pit apertures or directly through penetration of the cell wall [72].

As already described, there are three modes of lignocellulose degradation: soft rot, brown-rot, and white-rot. White-rot fungi can be further divided on the basis of two types of decay. Some white-rot species consume lignin and structural carbohydrates simultaneously, whereas others selectively degrade lignin and

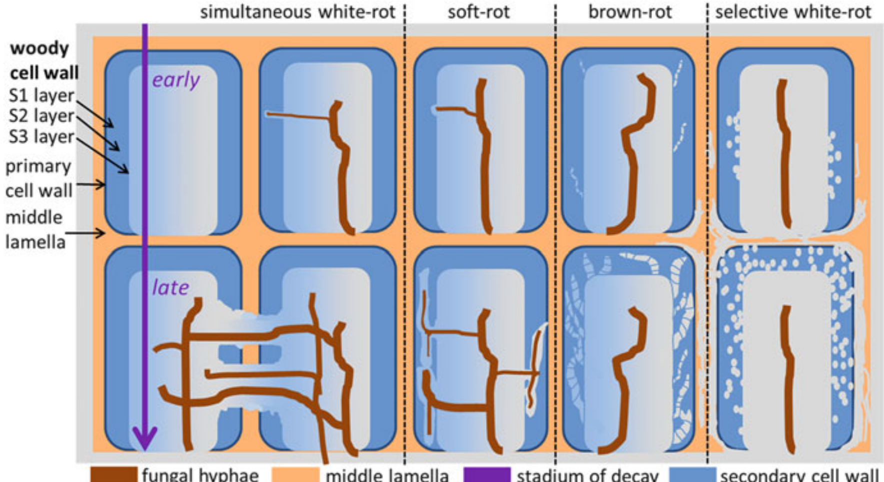

Fig. 5 Modes of fungal wood decay (modified after [72] with permission from Elsevier, Copyright 2007 The British Mycological Society, published by Elsevier Ltd. All rights reserved)

hemicellulose first [74]. These four types of fungal wood decay are illustrated schematically in Fig. 5. The species *Fomitopsis pinicol* (Polyporales) is an example of brown-rot decay. It starts degradation by secreting low molecular weight substances, which diffuse into the S3 layer of the cell wall. At a later stage, enzymes are involved in the excessive breakdown of hemicellulose and cellulose in the whole secondary wall, whereon the cells contract, resulting in numerous cracks in the cell walls. However, the S3 layer stays intact throughout the whole process and a skeleton of modified lignin is preserved. At an early stage of soft-rot decay by *Kretzschmaria deusta* (Ascomycota) the hyphae penetrate the S2 layer. There they branch and grow parallel to the cellulose microfibrils. At the end of the decay process the entire secondary cell wall is perforated by holes with conically formed ends and therefore almost completely broken down. Only the guaiacyl-rich middle lamella remains intact. *Fomes fomentarius* (Polyporales) is a white-rot fungus that causes simultaneous rot. Here, degradation of the cell wall close to the hyphae takes place first. Then hyphae enter the cell wall at right angles to the cell axis. The cell wall is degraded from the lumen toward the outside. Later, the cell wall becomes thinner and holes appear between neighboring cells. At an advanced stage the degradation process is limited by the strong lignified middle lamella. In the other mode of white-rot decay, the so-called selective delignification the middle lamella is also attacked. Herein, low molecular weight substances start the decaying process when diffusing from the hyphae into the secondary cell wall. The degradation of hemicellulose and lignin occurs within the secondary cell wall and even in the middle lamella. In the later stages, the favored degradation of pectin and lignin leads to separation of cells from each other. The compound cellulose is not degraded. This kind of decay is, for example, found in *Heterobasidion annosum* [72], belonging to the Russulales.

White-rot fungi are of special interest for the biotechnological industry because of their ligninolytic enzymes, which could be used in several industrial processes, such as pulp bleaching and decolorization of dyes in waste water [75]. It was shown that the production of ligninolytic enzymes takes place within the secondary metabolism and depends mainly on the limitation of carbon or nitrogen. However, the expression patterns can differ according to the microorganism and the type of enzyme [76]. Besides that, the gene regulation of these enzymes depends on several factors, such as the presence of xenobiotics, the temperature, day length, or metal ion concentration. A better understanding of these molecular mechanisms is needed to develop an efficient production process for ligninolytic enzymes [75].

To degrade lignin, white-rot fungi secrete class II peroxidases, dye-decolorizing peroxidases (DyP-type peroxidases), laccases, and several accessory enzymes such as aryl alcohol oxidases and glyoxal oxidases [77, 78]. In addition, cellobiohydrolases and lytic polysaccharide monooxygenases for the depolymerization of crystalline cellulose and other carbohydrate active enzymes are found [77]. Lignin peroxidases, manganese peroxidase, versatile peroxidases (class II peroxidases), and laccases have been investigated and stated to be involved in lignin degradation [79]. White-rot fungi secrete one or more of these ligninolytic enzymes [80]. More recently, two additional heme peroxidase families, the dye

decolorizing peroxidases (DyPs) and the aromatic peroxygenases, were discovered in the secretome of fungi. The latter catalyze oxyfunctionalization reactions, such as epoxidations or the hydroxylation of aromatic rings and alkyl chains. Furthermore, oxidations of alcohols, aldehydes, and phenols and cleavage of ether bonds were observed. DyPs catalyze the oxidation of aromatics and recalcitrant dyes [79]. The physiological role of these enzymes is not yet fully understood, although the catalytic reactions of DyPs and aromatic peroxygenases seem to be linked to the fungal conversion of lignin [81]. By transcriptome analyses the occurrence of peroxidase expression in different forest soils (oak, beech, spruce, aspen, and sugar maple) was studied. Class II peroxidases were detected in 90% of the tested samples and aromatic peroxygenases were identified in 85%. DyPs were found in 55% of the soil samples. All the peroxidase classes were found in all forest types, with the exception of DyPs which were absent in spruce. Interestingly, within the group of the class II peroxidases, numerous manganese peroxidases but no typical lignin peroxidases or versatile peroxidases were found [79]. Lignin peroxidases were the first ligninolytic enzymes to be discovered, but their essential role in lignin degradation is uncertain as they are not found in all ligninolytic fungi [82]. Additionally, it has been suggested that DyPs may substitute the rarely-found lignin peroxidases in the biodegradation process of non-polyporous white-rot fungi [83].

Non-enzymatic processes are less widespread in white-rot fungi, whereas hydroxyl radicals generated by the Fenton reaction seem to play a major role in the initial stages of polysaccharide degradation in brown-rot fungi. In the Fenton reaction, hydrogen peroxide is reduced by Fe^{2+}, resulting in Fe^{3+}, a hydroxyl ion, and a hydroxyl radical. After the chemical attack, an enzymatic decomposition of pectin and hemicelluloses, and further degradation of cellulose, take place. In contrast to white-rot fungi, the genome of the brown-rot type mostly does not contain genes of cellobiohydrolases, which are important factors in converting crystalline cellulose. In the course of the brown-rot degrading process, the lignin molecule itself is also affected, but is modified rather than degraded [9, 70, 77, 84].

The soft-rot fungi, which are of minor importance, are similar to brown-rot fungi in terms of the chemical processes during the decay [59]. In wet environments soft brownish debris is left by these organisms, which belong primarily to the Ascomycota. In addition, some basidiomycetes and bacteria may cause soft rot as well [60, 85]. Soft rot frequently occurs in environments with extreme conditions, such as high pH and low moisture, where white-rot and brown-rot fungi would be unable to survive [86].

In view of the remarkable differences in the expression pattern of ligninolytic enzymes between diverse fungi [87], all so far well-known enzymes of several species are described below. The diffusion of enzymes into the lignin molecule is limited. Thus a direct attack on lignin is questioned. Accordingly, it was suggested that low molecular weight compounds are required to initiate the oxidative attack on lignin [88]. Thus, besides the features of the ligninolytic enzymes, the mediation of lignin degradation by mediator molecules, as well as other assisting mechanisms, has been considered (see Fig. 6).

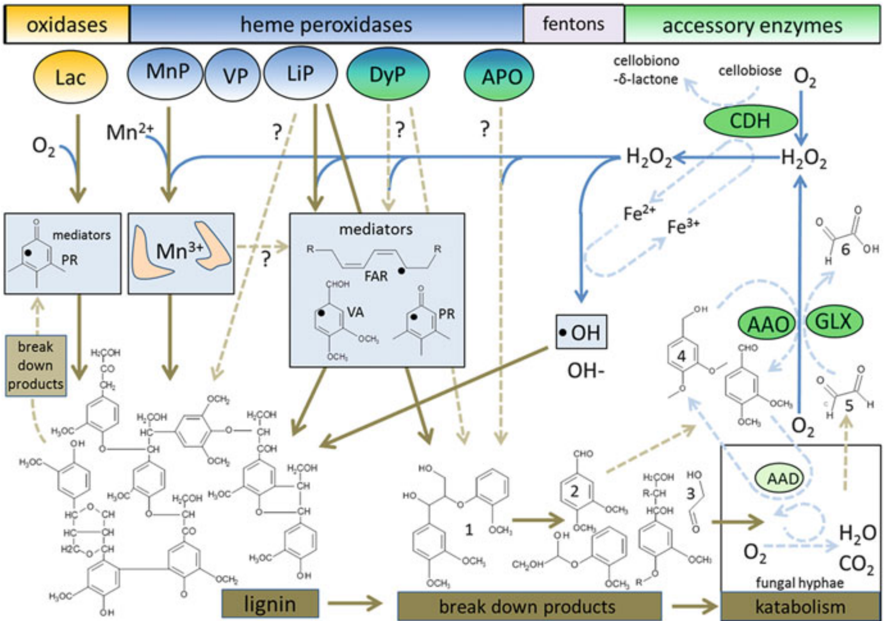

Fig. 6 Lignin degradation mechanism in fungal species. *Lac* laccase, *MnP* manganese peroxidase, *VP* versatile peroxidase, *LiP* lignin peroxidase, *DyP* DyP-type peroxidase, *APO* aromatic peroxigenase, *CDH* cellobiose dehydrogenase, *GLX* glyoxal oxidase, *AAO* arylalcohol oxidase, *AAD* aryl aldehyde/aryl alcohol dehydrogenase, Mn^{3+}-chelators: for example, oxalate; mediator molecules: *PR* phenolic radical, *FR* fatty acid radical, *VA* veratryl alcohol; lignin degradation products: (1) β-aryl-ether model compound, (2) *p*-anisaldehyde, (3) glycolaldehyde, (4) *p*-methoxybenzy alcohol, (5) glyoxal, (6) glyoxylic acid (modified after [60, 88–92])

Details about the ligninolytic enzyme laccases and class II peroxidases are presented in the following section. Facts about accessory enzymes which provide H_2O_2 are specified. Additionally, new enzyme classes, the DyP-type peroxidases and peroxygenases, are described, which are associated with lignin degradation.

4.2 *Fungal Ligninolytic Enzymes*

Herein, fungal enzymes associated with lignin degradation and their mechanisms are described. For visualizing the interaction and function of the enzymes described below, please see Fig. 6.

4.2.1 Laccases

The first laccase was discovered in 1883 in exudates of the Japanese lacquer tree *Rhus vernicifera*. Since that time, laccases have also been identified in numerous basidiomycetes and ascomycetes [93]. Meanwhile, laccases have been isolated from bacteria and even insects and thus occur almost everywhere. Laccases are the largest group within the so-called multi-copper oxidase enzymes [94]. An exact definition of laccases has not been given. Generally, multi-copper oxidases are considered to be laccases if at least some phenol oxidase activity can be measured and the copper ions are present in the correspondent position [95].

The physiological role of laccases is diverse. For example, laccases catalyze the biosynthesis of a spore pigment in the bacterium *B. subtilis* and they are involved in the production of the external cuticle of insects. The biological functions of laccases in plants and fungi apparently include the biosynthesis and degradation of lignin [95]. Moreover, fungal laccases take part in stress defense, morphogenesis, plant pathogen/host interaction, and the detoxification of phenol compounds [93, 96]. Fungal laccases are mostly extracellular monomeric proteins of about 60–70 kDa [94].

Characteristically, the laccases contain four copper ions: one of type-1 (Cu1), one of type-2 (Cu2), and two of type-3 (Cu3). The two Cu3 ions and the Cu2 ion are arranged in a triangle and form the trinuclear copper cluster (TNC) (see Fig. 7)

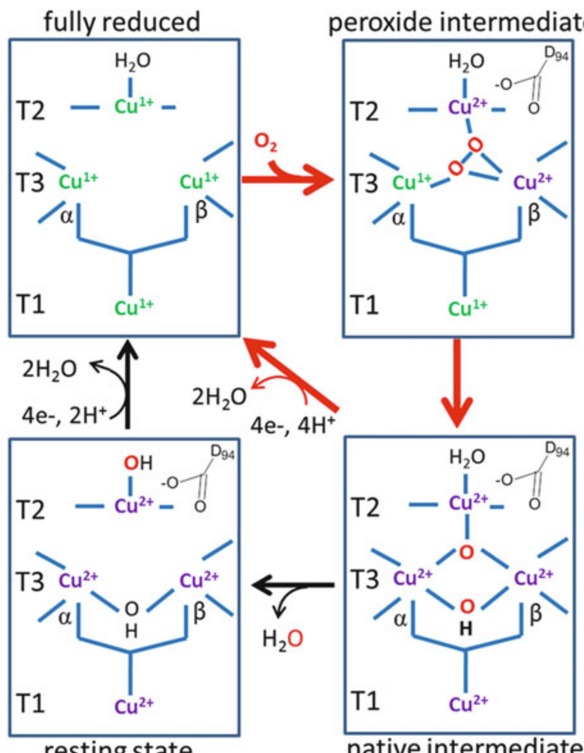

Fig. 7 Catalytic cycle of laccases (modified with permission from [97], Copyright 2010 American Chemical Society)

[94]. A strong absorption at about 600 nm is caused by the Cu1 copper and results in a characteristic blue color of these enzymes [94]. Laccases catalyze the following overall reaction [96]:

$$4 \text{ benzenediols} + O_2 \rightarrow 4 \text{ benzosemiquinones} + 2\, H_2O$$

In the first step the substrate donates four electron equivalents ($4e^-$) and in the native intermediate all four copper ions Cu(II) are reduced to Cu(I). The electrons enter thorough the Cu1(T1) site and are transferred to the Cu2(T2) and the two Cu3 (T3) (TNC). The single steps of the Cu(II) reduction are not fully understood. The TNC is afterward fully reduced, which is required for the following reaction with dioxygen [97]. The dissociation of two water molecules during this reducing step is suggested [94]. The dioxygen is then reduced successively in two two-electron transfer steps [98]. The TNC reacts with dioxygen and forms a peroxide intermediate with the Cu2 and one of the two Cu3 ions. In this step, two electrons are transferred from Cu2 and the β-Cu3 copper on the dioxygen molecule.

An aspartic acid residue (D94), which is close to the β-Cu3 and Cu2 sites seems to support the reaction of the dioxygens with the Cu2 and the β-Cu3 by providing a negative charge. Furthermore the β-Cu3, in comparison to the α-Cu3, lies near a glutamic acid residue. Next, the bond between the dioxygen is cleaved. The reaction is supported by the glutamic acid residue providing a proton. Finally, the enzyme is in the native intermediate state (NI), which is oxidized completely and is catalytically relevant [97]. In the presence of a substrate the catalytic cycle starts again. If not enough substrate equivalents are available, a resting state can occur as well [94].

The laccases possess a broad substrate range. They can directly oxidize polyphenols, diphenols, aminophenols, polyamines, and aryl diamines. They are also able to catalyze the oxidation of inorganic ions [94]. However, most of the laccases are unable to oxidize non-phenolic compounds directly because of their high redox potentials above 1.3 V vs "normal hydrogen electrode" (NHE), whereas the laccases' redox potentials are below 0.8 V [99]. Thus, the participation of laccases in lignin degradation of white-rot fungi was questioned, as more than 80% of the total lignin consists of non-phenolic units. Several discoveries, however, affirmed laccases as important participants of the lignin-degrading system of fungi. Laccases were shown to be able to degrade phenolic lignin substructure model compounds. Moreover, the addition of redox mediators enables laccases to oxidize non-phenolic molecules. Numerous fungal strains do not possess lignin and manganese peroxidases but are nevertheless able to degrade lignin. In addition, laccase-deficient mutant strains were inhibited in lignin degradation [100].

Redox mediators are small molecules which act as electron shuttles. Thus, bulky substrates, which cannot be oxidized directly because of steric hindrances, can be converted as well. Furthermore, the different mechanism of the mediator system allows an oxidation of high redox potential molecules [99]. Redox mediators are either artificial molecules such as 2,2′-azino-di-(3-ethylbenzthiazolin-6-sulfonic acid) (ABTS) or 1-hydroxybenzotriazol (HOBt) or can be natural mediators. The

latter include phenolic molecules derived from the fungal or plant secondary metabolism or lignin degradation products. The effect of lignin-degrading products as mediator is of course difficult to prove when lignin at the same time is used as substrate [100]. However, the molecules syringaldehyde, acetosyringone, vanillin, acetovanillone, methyl vanillate, and *p*-coumaric acid enabled the degradation of recalcitrant dyes by laccase [99].

For the oxidation of non-phenolic substrates by laccase-mediator systems, two different mechanisms are proposed. Although ABTS reacts through an electron transfer (ET) route, the mediator HOBt follows a hydrogen atom transfer (HAT) mechanism. Here, a hydrogen atom is subtracted from the –N–OH mediator and an –N–O· radical is formed [94]. For natural phenolic mediators an analogous mechanism is suggested, which was investigated by using phenol red as model substrate for phenolic compounds. Herein, a hydrogen atom is abstracted and a phenoxyl radical (PhO·) is generated.

Comparing natural with artificial mediators, inactivation of the laccase was reduced using natural mediators, but pulp bleaching was more efficient with the HOBt mediator system [99]. However, in consideration of the natural mediators, laccases gain prominence in the fungal lignin degradation mechanism. Moreover, these mediators are of interest for industrial processes as they can be gained easily from lignocellulose material and are also environmentally friendly [99].

The yellow laccases are an interesting exception within the laccases. These laccases do not show the typical blue color, which is caused by another adsorption spectrum of the Cu1 atom. An explanation for the color shift in earlier reports was the incorporation of a lignin-derived mediator in the catalytic center, whereas more recently a variation of the coordinating sphere of Cu1 was postulated [101]. As yellow laccases have a higher redox potential and were shown to oxidize non-phenolic compounds without mediator, they have a high potential to perform several industrial applications [102].

4.2.2 Class II Peroxidases

Peroxidases can generally be described as a group of enzymes that reduce peroxide and oxidize numerous substrates [103]. Genes of heme peroxidases have been identified in nearly all kingdoms of life. They used to be divided into two main superfamilies. The so-called animal peroxidases (recently termed peroxidase-cyclooxygenases) are primarily found in animals, fungi, and bacteria. The plant peroxidases (now peroxidase-catalase superfamily) mainly occur in bacteria, fungi, and plants (see Fig. 8) [104, 105].

The plant peroxidases superfamily is again subdivided into different classes. Class I includes peroxidases, which are located in eukaryotic organelles, catalase-peroxidases, or bacterial peroxidases. Class III contains the secreted plant heme peroxidases [104, 106]. Prominent examples for class I are the cytochrome c peroxidase and class III includes the horseradish peroxidase [107]. Class II contains the secreted fungal heme peroxidases. These peroxidases are found solely in fungi,

heme peroxidases				
super-family	peroxidase–catalase	peroxidase–chlorite-dismutase	peroxidase–peroxygenase	peroxidase–cyclooxygenase
subfamily	Class II peroxidases	Dyp-type peroxidases	Aromatic peroxygenases	

Fig. 8 Superfamilies of heme peroxidases and selected subfamilies, which are associated with lignin degradation (according to [104])

more precisely in Agaricomycetes (formerly homobasidiomycetes) [103, 108]. Class II mainly consists of the enzyme type manganese peroxidase (MnP), lignin peroxidase (LiP), and versatile peroxidase (VP) [109].

LiP and MnP were discovered in the 1980s and, because of their high redox potential, described as true ligninases. Later the versatile peroxidases, an additional type of class II peroxidases, were discovered. The versatile peroxidases were shown to combine the catalytic features of MnP and LiP, oxidizing Mn^{2+} and veratryl alcohol [60]. These enzymes are classified by molecular models, including data of LiP, VP, and MnP from *Phanerochaete chryosporium* and *Pleurotus eryngii*. There is an exposed tryptophan residue described, which is typical for LiP. Furthermore, a putative Mn^{2+}-oxidation site was found in MnP. Despite this, there are also class II peroxidases known, which lack both of these sites [110]. These so-called generic peroxidases have a low redox potential and are therefore unlikely to participate in lignin degradation [70, 111]. In a study which included 10 genomes of Polyporales, a reconstruction of the ancestral state showed that a generic peroxidase appears to have evolved an Mn^{2+}-oxidation site, which implies that this gene could be the ancestor of all the class II lignin degrading peroxidases. Furthermore, the development of an exposed tryptophan site supposedly led to the first versatile peroxidase. It was also suggested that the loss of Mn^{2+}-oxidation by an early versatile peroxidase is the origin of all lignin peroxidases (LiP) [112]. The three ligninolytic class II enzymes—manganese, lignin, and versatile peroxidase—are specified below.

Manganese Peroxidases

Manganese peroxidases are the most widespread lignin-modifying peroxidases and are secreted by nearly all wood-colonializing fungi [91]. Manganese peroxidases (MnP) are monomeric glycosylated enzymes with a molecular mass of about 30–60 kDa and contain one molecule of heme as iron protoporphyrin XI [88]. The pH optimum of these enzymes lies between 2.5 and 6.8 and the redox potential of MnP is about 1.0–1.2 V (vs normal hydrogen electrode) [88, 113]. MnP catalyzes the following overall reaction:

$$2\ Mn(II) + 2H^+ + H_2O_2 \rightarrow 2\ Mn(III) + 2\ H_2O$$

Interestingly, MnP is the only heme peroxidase, which catalyzes a one-electron Mn^{2+}-oxidation [91]. The Mn^{2+} ion is oxidized at a binding site close to the heme cofactor. Three ambient acid residues mediate the binding of Mn^{2+} by their carboxylates, facilitating a direct transfer of electrons to one heme propionate side chain [60].

The catalytic cycle of MnP starts by the formation of an iron–peroxide complex with hydrogen peroxide binding at the native ferric enzyme. For the cleavage of the O–O bond of the hydrogen peroxide, two electrons are essential. The heme transfers these two electrons, which results in the formation of a Fe^{4+}-oxo-porphyrin-radical complex, called compound I. After receiving two electrons, the O–O-bond is cleaved heterolytically and one molecule of water is excluded. Compound I is afterward reduced to compound II by Mn(II). Compound II is likewise reduced by a second Mn(II), resulting in the generation of another molecule of water and the native enzyme. Compound II is dependent on Mn(II) as electron donor, whereas compound I can in turn be reduced by alternative electron donors such as phenolic compounds [93, 114] (see Fig. 9).

Mn^{3+} is a strong diffusible oxidizer, but is not stable under aqueous conditions. However, chelators such as oxalate and malonate can stabilize Mn^{3+} against dissociation to Mn^{2+} and insoluble Mn^{4+}. The complexed Mn(III) can diffuse into the lignin molecule, where it acts as reactive redox-mediator [76, 82]. The complexing of the chelates, however, lowers the electron potential of Mn^{3+}, resulting in a mild oxidant. MnP is primarily considered to be an enzyme that oxidizes phenolic compounds because the Mn^{3+}-complex oxidizes monomeric and dimeric phenols as well as phenolic lignin model substances. In contrast, it cannot directly attack non-phenolic compounds of lignin. However, it was supposed that MnP is able to oxidize non-phenolic lignin structures in the presence of additional secondary mediators [76, 114]. MnP-derived Mn^{3+} has been shown to oxidize thiols and saturated fatty acids to form secondary mediators such as thiyl or lipid radicals [114, 115].

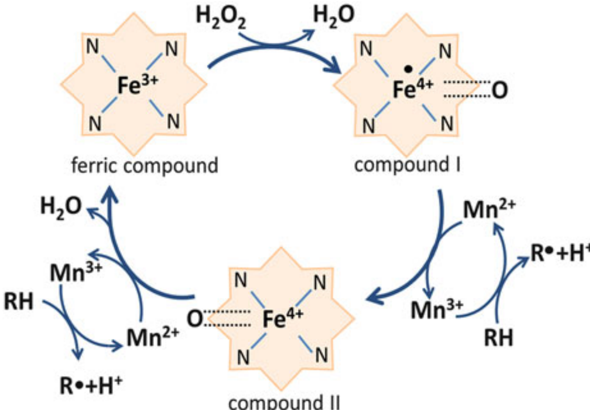

Fig. 9 Catalytic cycle of manganese peroxidase. *Mn* Manganese, *Fe* iron, *RH* organic molecules (modified after [114] with permission from Elsevier, Copyright 2002 Elsevier Science Inc. All rights reserved)

Furthermore, another possible oxidation mechanism of non-phenolic lignin compounds with participation of MnP was suggested. Cellobiose dehydrogenase-generated OH radicals react with non-phenolic lignin structures, thereby introducing hydroxyl groups and enabling Mn^{3+}-chelates to perform a further oxidation step [116].

Lignin Peroxidases

Lignin peroxidases (LiP) are similar to MnP monomeric glycosylated enzymes. Their molecular mass ranges between 35 and 55 kDa and they contain one molecule of heme as iron protoporphyrin XI [85].Their pH optimum is quite low and ranges from 1 to 5. The redox potential of LiP is remarkably high at about 1.4–1.5 V [85, 110]. The overall reaction of LiP is represented by the following equation [93]:

$$\begin{aligned} &1,2\text{-bis}(3,4\text{-dimethoxyphenyl})\ \text{propane-}1,3\text{-diol} + H_2O_2 \\ &\quad \rightarrow 3,4\text{-dimethoxybenzaldehyde} \\ &\qquad + 1\text{-}(3,4\text{-dimethoxyphenyl})\text{ethane-}1,2\text{-diol} + H_2O \end{aligned}$$

Herein, the substrate is a non-phenolic lignin model dimer [117]. The favored substrate for LiP, though, is the non-phenolic monomer veratryl alcohol, whose conversion is used in photometric assays for determination of LiP enzyme activity [96].

The first reaction of the catalytic cycle is the two-electron oxidation of the native ferric enzyme, which results in compound I (see Fig. 10). There, the iron appears as Fe(IV) and a free radical exists on the tetrapyrrole ring. Herein, hydrogen peroxide is reduced and cleaved at the O–O bond and a molecule H_2O is released. Instead of a radical in the tetrapyrrole ring, a tryptophan 171 radical state was suggested for

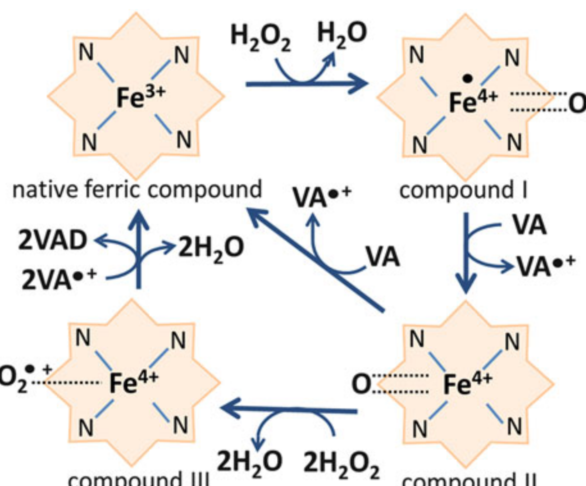

Fig. 10 Catalytic cycle of lignin peroxidase. *VA* veratryl alcohol, *VAD* veratryl aldehyde (modified after [96] with permission of Springer, Copyright Humana Press 2008)

compound I (see below) [96]. Next, two successive one-electron transfer steps of compound I from an electron donating substrate (e.g., veratryl alcohol) take place [91]. First, compound I oxidizes a donor substrate by taking one electron and releases a free-radical substrate and compound II, which still contains an Fe (IV) but no tetrapyrrole radical. Then compound II oxidizes a second donor molecule and again a free-radical substrate is formed. LiP is returned to the native ferric oxidation state, which completes the catalytic cycle [82, 91]. In the absence of an electron donor substrate, compound II can react with H_2O_2, which results in a catalytically inactivate state of LiP (compound III). This ferric-superoxo form can be reset to the native form by oxidation with a veratryl alcohol radical cation or by spontaneous auto oxidation [76]. The oxidation of phenolic compounds by LiP is associated with an assimilation of compound III and thus an inactivation of LiP, because phenolic compounds are unable to reduce compound III to the native state [96].

Although their catalytic cycle is similar to other peroxidases, LiP demonstrates unique features in oxidizing high-redox potential substrates. Structural aspects that distinguish LiP and other peroxidases were found by crystal structure analysis. One of the nitrogen atoms of the proximal histidine residue (see Fig. 12b) forms a hydrogen bond with the iron of heme peroxidases. In LiP this histidine is quite distant from the heme iron, resulting in a significantly longer hydrogen bond. Thereby the electron deficiency of the heme iron is increased, which leads to the higher redox potential of the oxo-ferryl complex [60, 76].

Another unique structural property of LiP is the exposed tryptophan residue (W171), which seems to play a central role in the oxidation of veratryl alcohol and other non-phenolic substrates [115]. The role of the tryptophan residue in veratryl alcohol oxidation was revealed by its substitution with Phe or Ser. The mutant showed no essential residual activity toward veratryl alcohol, whereas unaltered activity with two artificial dye substrates was detected. In addition, a tryptophan residue, which was introduced into a manganese peroxidase, located equivalent to the 171 position of LiP, resulted in an MnP with an oxidation activity for non-phenolic aromatics [121]. Through so-called long-range electron transfer, which is mediated by the exposed tryptophan at the protein surface, even bulky substrates such as lignin can be oxidized directly [76].

Nevertheless, the oxidation efficiency of LiP decreases significantly as the size of the lignin structure increases. For instance, the catalytic efficiency for a lignin model trimer was only around 4% compared to the activity against a monomeric lignin model compound. Synthetic lignins, which consist of about 20 subunits, were still oxidized, but the presence of veratryl alcohol was required [82]. Veratryl alcohol is secreted by ligninolytic fungi together with LiP and its role in the lignin degradation by LiP has been discussed [96]. Regarding the low efficiency of direct LiP attack on large lignin structures, veratryl alcohol (VA) might act as diffusible mediator and oxidize lignin at distant locations. However, the stability of the VA radical is still disputed. Alternatively, VA could be necessary for reducing LiP during slow cleavage processes of bulky lignin structures. Thereby, VA helps rescue LiP from inactivation by avoiding too long phases in an oxidized state.

Furthermore, compound II is not as reactive as compound I and only the latter might be involved in oxidizing methoxylated lignin structures. Veratryl alcohol could be essential to reduce compound II and complete the catalytic cycle. If VA is not a diffusible mediator, the physiological role of LiP may be restricted to the oxidation of smaller lignin breakdown products released by other mechanisms, or their site of action remains solely at the surface of the plant cell walls [82].

Versatile Peroxidase

Versatile peroxidases share typical features of the LiP and MnP, showing a hybrid catalytic function. Consequently, they can directly oxidize Mn^{2+} as well as high redox potential aromatic compounds, including both phenolic and non-phenolic lignin models. Interestingly, different pH optima for Mn^{2+} (pH 5.0) and aromatic substances (pH 3.0) were observed [76]. VP not only combines the substrate spectrum of LiP and MnP, but actually oxidizes reactive black 5. This reactive dye can neither be oxidized by Mn^{3+} tartrate, because of its high redox potential, nor by LiP (without VA) because of a rapid inactivation of LiP [122]. Versatile peroxidases (VP) are known to possess an Mn^{2+}-oxidation site and a tryptophan residue for the oxidation of high redox potential compounds via long-range electron transfer. Moreover, an additional oxidation site for low redox potential substrates (0.6–0.8 V) was described for VP. This site is located at the main heme-access channel [123]. The long-range electron pathway of VP is comparable to the pathway found in LiP, but the tryptophan (W164) occurs as a neutral radical. VP show a tenfold lower catalytic efficiency for veratryl alcohol compared to LiP, but act on Mn^{2+} as efficiently as MnP [91]. The redox potential for VP was determined to be around 1.4–1.5 V, which is equal to LiP [113].

Versatile peroxidases have unique catalytic features, including the oxidation of Mn^{2+}, veratryl alcohol, phenolic and non-phenolic compounds, and high molecular weight compounds, such as dyes (without Mn^{2+}-mediation).Therefore, versatile peroxidases are the most interesting candidates for biotechnological applications amongst basidiomycetes peroxidases [88]. However, the commercial application of VP, and other ligninolytic peroxidases (e.g., in a biocatalytic process for lignin depolymerization), is mainly hampered by their limited availability in large quantities [88, 124].

4.2.3 Aromatic Peroxigenases and DyP-Type Peroxidases Subfamily D

In recent years, two other heme peroxidase families, secreted by saprobic basidiomycetes, have come into focus: The aromatic peroxygenases (APO) and the dye-decolorizing peroxidases (DyP-type peroxidases, DyPs). Lately, a new phylogenetic nomenclature has been suggested. According to Zamocky et al., the DyPs cluster with chlorite dismutases, sharing a common heme binding scaffold, were classified as a peroxidase–chlorite dismutase superfamily [104]. The APO and the

chloroperoxidases (CfuCPO) represent the peroxidase–peroxygenase superfamily [125] (see Fig. 8).

CfuCPO was discovered in the 1960s and oxidizes halides such as Cl- to HOCl. These molecules are, in turn, able to halogenate organic molecules. CfuCPO shows a limited peroxygenase activity with indole or *p*-xylene, but does not act on unactivated carbons, in contrast to APO [126]. The latter catalyze a broad range of reactions such as epoxidations, hydroxylation of aromatic rings and alkyl chains, and ether cleavages, as well as alcohol, aldehyde, and phenol oxidations under consumption of hydrogen peroxide [79]. Following the discovery of these heme thiolate peroxidases, it was obvious that they differ from all so far known peroxidases and are a combination of "classical" heme peroxidases and cytochrome P450 monooxygenases [104].

The first dye-decolorizing peroxidase (DyPs) was discovered in 1995 in the fungus *Geotrichum candidum* by screening for organisms able to decolorize synthetic dyes [125]. DyPs are abundant in many bacterial phyla, in fungi, and, albeit less widespread, in archaea. They can be divided into subfamilies A, B, C, and D [127]. The ancestor of the peroxidase–chlorite dismutase superfamily was most likely a member of the DyP-subfamily A. DyPs probably evolved in thermophilic facultatively anaerobic Firmicutes, which are believed to be of very old origin. One branch that arose from the DyP A group is the subfamilies DyP C and D, whereas a second branch led to the subfamilies of the shortened DyP B and probably further to subfamilies of the chlorite dismutases [104]. However, the hypothesis of a common ancestor for DyPs and chlorite dismutases is disputed by Sugano and Yoshida [128]. DyP C and D cluster quite closely, although DyP C peroxidases are found in proteo-, actino-, and cyanobacteria, and DyP D peroxidases are only present in fungi. An explanation is the development of the DyP D clade by horizontal gene transfer from cyanobacterial ancestors on dikarya fungi. This theory is supported by the lack of DyP-type peroxidases in genomes of ancestral (early dividing) fungi (e.g., Mucorcircinelloides) [104].

The reactions, which are catalyzed by DyP-type peroxidases and aromatic peroxygenases, seem to be relevant for the conversion of lignin in the nature. However, the physiological function of these enzymes is still an open question [81].

Aromatic Peroxygenases

Since the first aromatic peroxygenase was discovered in 2004 in the fungus *Agrocybe aegerita*, two more similar enzymes of the fungi *Coprinellus radians* (ink cap) and *Marasmius rotula* were isolated and characterized. The APO showed molecular weights of 32–46 kDa, were highly glycosylated (up to 40%), and occur extracellularly. The APO catalyzes oxygen transfer reactions at a pH range of 3–10, with a maximum rate at around pH 7 [129]. The sequence homology of APO and chloroperoxidases is only about 30%. However, APO is even less of a homologue to p450s or lignin peroxidases [130]. The prosthetic heme group is linked via iron to

an exposed cysteine and shows a soret band at 445–450 nm, which is comparable to the heme signal of cytochrome p450 monooxygenases [126].

Cytochrome P450 monooxygenases can catalyze several industrial relevant oxy-functionalizing reactions. The requirement of redox equivalents such as NADH, which is a main drawback, can be overcome by adding hydrogen peroxide as a co-substrate. Thereby, a side reaction of p450 enzymes is observed, which is termed “shunt pathway.” Nevertheless, the p450 enzymes still suffer from low stability and moderate turnover numbers. APO could overcome these limitations because of their superior stability and high catalytic efficiencies [126, 130].

A catalytic cycle for APO was suggested, which combines elements of the heme peroxidases with the peroxide cycle and the “shunt pathway” of p450 monooxygenases [129]. At the resting state a water molecule is bound at the ferric heme of the enzyme. The catalytic cycle starts with hydrogen peroxide which reacts with the ferric heme, resulting in a negatively charged ferric peroxo-complex (only shown for chloroperoxidase). After a heterolytical cleavage of the peroxide O–O bond, compound I is built [126]. Depending on the substrate, its binding site and the reaction conditions, compound I can react in a monooxygenase or a peroxidase manner [129]. In the monooxygenase pathway, ferryl oxygen is transferred to the substrate and accepts two electrons [129]. In particular, hydrogen is abstracted from the substrate (e.g., an alkane) by compound I, resulting in a protonated compound II (ferryl hydroxide complex) and a substrate radical. The alkyl radical then recombines with an ·OH-equivalent through a rebound mechanism and builds the corresponding alcohol whilst the ferric enzyme is restored. After association of one water molecule to the catalytic center, the cycle starts again (see Fig. 11b) [126, 131]. However, the oxygenation of aromatic rings and other alkenes seems to involve π-bonds instead of a direct insertion of oxygen into a C–H bond. Thus, a hydrogen abstraction does not take place in this case [126, 129].

In contrast, peroxygenases also catalyze one electron step oxidation of, for example, phenol to a phenoxy radical via the peroxidase pathway. First, an electron

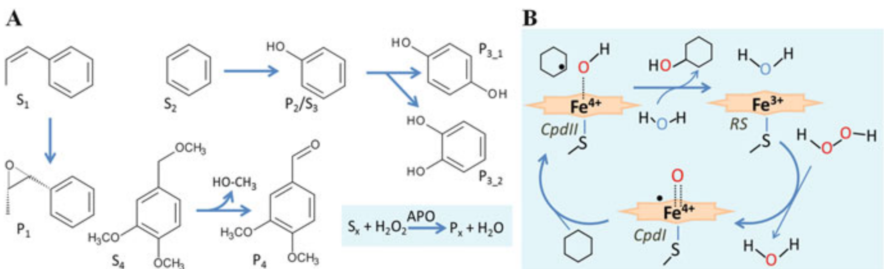

Fig. 11 (**a**) Examples of oxidation reactions of aromatic peroxidases. *S* substrates, *P* products; *S1* *cis-b*-methylstyrene, *P1* (1R,2S)-*cis-b*-methylstyrene oxide, *S2* benzene, *P2*/*S3* phenol, *P3_1* hydroquinone, *P3_2* catechol, *S4* 3,4-dimethoxybenzyl methyl ether, *P4* 3,4-dimethoxybenzaldehyde and methanol (modified after [126]). (**b**) Catalytic cycle of APO. *Substrate* cyclohexane, *Cpd* compound, *RS* resting state (modified after [126] with permission from Elsevier, Copyright 2014 Elsevier Ltd. All rights reserved)

is abstracted and one substrate molecule is oxidized, yielding a radical substrate and compound II. In a second step, another electron is transferred, again releasing a molecule of a radical substrate and the ferric oxygen as a water molecule [129].

Examples for reactions catalyzed by APO are illustrated in Fig. 11a.

DyP-Type Peroxidases Subfamily D

The dye-decolorizing or DyP-type peroxidases (DyPs) of fungi, represented by the subfamily D, were all found in the supernatant of fungi cultures, hinting at an extracellular occurrence of these enzymes [128]. Fungal DyPs show a molecular weight of 43–69 kDa [119]. Furthermore, the most fungal DyPs are glycosylated monomeric proteins, with a glycosylation degree of 8–38% [118, 132, 133]. They exhibit a typical heme soret band at 406 nm and lack the typical heme binding region of other heme peroxidases, which consists of one proximal histidine, one distal histidine and one additional arginine site. However all DyPs share a common heme binding motive GXXDG (glycine, two variable amino acids, aspartic acid, and glycine) [125]. The distal histidine residue is essential as an acid-base catalyst in common heme peroxidases. Interestingly, DyPs do not possess a catalytic active distal histidine but an aspartic acid (see Fig. 12) [120, 125].

In the native conformation, the oxygen atom (OD2) of the aspartic acid residue was shown to be too far away to fulfill its designated role, which is the reception of a proton from the heme-bound H_2O_2. However, Yoshida et al. postulated that in the presence of H_2O_2 the aspartic acid is enabled to move toward the heme molecule by a swinging mechanism [120].

The exact details of the catalytic cycle of DyPs remain unknown. However, a similar mechanism as described for the other well-characterized heme peroxidases is assumed [127]. The cycle starts with the oxidation of the resting enzyme by H_2O_2. Two electron equivalents are transferred and compound I (Fe^{4+}=O and a porphyrin π-cation radical (Por+·)) is built. In two sequential steps the compound I is at first converted to compound II ([Fe^{4+}=O]Por) by reducing a substrate molecule. Then compound II is reduced by another substrate molecule and the resting enzyme state is restored. In contrast to several other investigated heme peroxidases,

Fig. 12 (**a**) Proposed swing mechanism of compound I formation by DyPs. *D171* aspartate 171 (instead of histidine in other peroxidases), *OD2* outer carboxylate oxygen atom of aspartate [120]. (**b**) Heme surrounding in plant heme peroxidases (e.g., MnP) and DyP-type peroxidases; *His* histidine residue, *asp* aspartic acid residue (modified after [107, 120], Copyright 2011 FEBS)

compounds I and II could not be observed universally in DyPs. For the DyP-type peroxidases DyP B of *Rhodococcus jostii* and DyP D of *Bjerka adjusta*, only compound I was detectable. Vice versa, for the peroxidases DyP A of *Rhodococcus jostii*, DyP A of *E. coli*, and DyP A of *Bacillus subtilis*, only compound II was found [134].

DyP-type peroxidases can oxidize bulky substrates that are too large to fit in the active site [127]. Therefore, a long-range electron transfer mechanism, as already described for LiP, was suggested. This mechanism requires an electron pathway from the porphyrin ring to a suitable redox active amino acid (e.g., tyrosine or tryptophan) at the surface of the enzyme [135]. DyP AauDyPI of *Auricularia auricula-judae* was recently expressed in *E. coli*. The heterologous enzyme was investigated by multi-frequency EPR spectroscopy with regard to radical-forming amino acid residues (tyrosine and tryptophan) at the surface of the enzyme. The highest signal contribution was found for tryptophan (W377) [136, 137]. Furthermore, mutants of W377 lacked this radical signal. Additionally, the W377-mutant lost the activity for the bulky substrate reactive blue 19, whereas other substrates (e.g., 2,2′-azino-bis(3-ethylthiazoline-6-sulfonate (ABTS)) were still oxidized [137].

The redox potential of several fungal DyPs was determined by measuring the reacting efficiency against a series of phenolic compounds with increasing redox potential. DyPs showed a relatively high redox potential of around 1.1–1.2 V [83]. The characteristic reaction for DyPs is the oxidation of the anthraquinone dye reactive blue 5 (as reactive black 5 for VP) [125]. However, the fungal DyPs have a wide substrate range including 2,6-dimethoxyphenol and ABTS, as well as the high redox potential dyes Reactive Blue 5 and Reactive Black 5. Veratryl alcohol and non-phenolic lignin model compounds were also oxidized by some fungal DyPs, but no activity against Mn^{2+}-ions was detected [118, 119, 138, 139]. Actually, DyP of *Irpex lacteus* and cellulases acted synergistically on wheat straw, which led to a more efficient hydrolysis and thus an increased glucose yield [118]. Although the optimum of DyPs for the oxidation of phenolic substrates such as 2,6-DMP was found in a range of pH 3.5–4.5, non-phenolic aromatic substrates were converted best under rather acidic conditions (pH 1.4–2.5) [119].

The physiological role of these fungal DyPs is not yet understood, but the catalytic features as well as the secretion under natural conditions (wood cultures) indicate that DyP-type peroxidases might participate in the oxidation of recalcitrant methoxylated aromatics within the lignin polymer. DyPs might take the place of LiPs in species where the latter are absent [83, 125, 127]. The DyP-type peroxidase subfamilies A–C are discussed below.

4.2.4 Accessory Enzymes

Besides the already described ligninases, fungi express "accessory enzymes," which also play an important role in lignin degradation. These oxidases generate H_2O_2, which is required by ligninolytic peroxidases and in the Fenton reaction to

produce reactive hydroxyl radicals (OH·) [88]. H_2O_2-producing enzymes include glyoxal oxidases (belonging to the copper-dependent copper radical oxidases family) and several flavoproteins of the glucose-methanol-choline family. The latter includes the aryl-alcohol oxidases, glucose-1-oxidases, alcohol oxidases, and cellobiose dehydrogenases.

The glyoxal oxidases own a broad specificity and catalyze the oxidation of aldehydes to carboxylic acids, thereby reducing dioxygen to H_2O_2 [140, 141]. The low specificity of this enzyme hampered the determination of the physiological substrate. The enzyme oxidizes, for example, glyoxal, glycolaldehyde, and glyoxalic acid. These molecules could be derived by lignin degradation. It was shown that LiP degraded a β-aryl ether lignin model compound to glycolaldehyde, which is a substrate for the glyoxal oxidase. A further oxidation of glycolaldehyde to oxalate via glyoxal and glyoxylic acid can generate H_2O_2, which is then recycled by LiP. Moreover, glyoxal could be derived by oxidation of linoleic acid, a fungal metabolite, or by degradation of carbohydrates (e.g., sugars) through hydroxyl radicals (OH·) [142]. Glyoxal oxidase seems to be of importance in the lignin degradation process of *P. chrysosporium* (see Fig. 6) [143].

Aryl alcohol oxidases and ligninolytic peroxidases are produced simultaneously in *Bjerkandera adusta* and *Pleurotus*, which hints at participation of these oxidases in the lignin degradation process. The substrates for the aryl alcohol oxidase could be either lignin-derived compounds or fungal aromatic metabolites [89]. The aryl alcohol oxidases generally catalyze the oxidative dehydrogenation of aromatic and aliphatic polyunsaturated alcohols with a primary hydroxyl group and the oxidation of aldehydes to the corresponding acids [89, 140]. Intracellular aryl alcohol and aryl aldehyde dehydrogenases reduce the aldehydes and acids back to alcohols and aldehydes by consumption of redox equivalents (e.g., NADPH). The reduced molecules are secreted and again serve as substrates for the aryl alcohol oxidase, thus building an H_2O_2 generating loop. *p*-Anisaldehyde and the corresponding *p*-methoxybenzyl alcohol seem to be the physiological substrates for the H_2O_2-producing redox cycle in *Pleurotus* species (see Fig. 6) [89].

Brown-rot fungi release methanol during their wood decaying process by demethylation of lignin. The demethylation might be caused by reactive hydroxyl radicals or by an enzymatic process. However, no suitable enzyme has been isolated so far [144, 145]. In *Gloeophyllum trabeum* a methanol oxidase was identified, which is believed to use the lignin-derived methanol to generate H_2O_2. Interestingly, although a signal sequence is missing, the methanol oxidase was located extracellularly [89].

Cellobiose dehydrogenases (CDH) consist of two domains: the *C*-terminal dehydrogenase domain, containing FAD as redox factor and the *N*-terminal cytochrome domain, which is a heme enzyme. Both modules of CDH evolved parallel as fused genes [140]. The suggested biological roles of CDH are manifold. CDH oxidizes cellobiose to cellobiono-δ-lactone rather inefficiently and this might not be a relevant function. The most common biological role of CDH is the production of Fe^{2+} and H_2O_2, which can undergo a Fenton reaction and build a radical hydroxyl (see Fig. 6). The cytochrome subunit perhaps produces reactive

oxygen species directly. The generated reactive oxygen species can attack the lignocellulose matrix [90]. Alternatively, other functions of CDH are possible. The reduction of semiquinones to quinones and the reduction of Mn(IV)O_2 by CDH was observed. Thereby, dissolved manganese is provided for MnP [146]. The formed quinones can be radicalized by laccase or lignolytic peroxidases. These radicals are reduced to semiquinones, whereby Fe^{3+} and H_2O_2 are generated [147].

4.3 Bacterial Lignin Degradation

This section elaborates screening methods for finding new lignolytic bacteria. Moreover, several already known ligninolytic bacteria are described.

Research on lignin degrading organisms has mainly focused on basidiomycetes, especially on white-rot fungi, because of their high ligninolytic activities. In spite of extensive research on lignin degradation, so far no commercial biocatalytic process for lignin depolymerization exists. The challenging protein expression and genetic manipulation in fungi might be one reason. Bacteria might support the progress of industrial lignin utilization. Although the extent of the prokaryotic lignin breakdown is not as complete as in fungi, several bacteria strains have been shown to react on lignin and probably produce small aromatic molecules. It was observed that many soil bacteria, which are able to metabolize aromatic compounds, also show ligninolytic activities. These findings point out the possibility of a reasonable association between lignin degradation and aromatic degradation, considering that lignin is a considerable source for soil-occurring aromatics [124, 148]. The most bacterial lignin degraders known to date belong to the classes actinobacteria, α-proteobacteria, and γ-proteobacteria [52] (see Fig. 13).

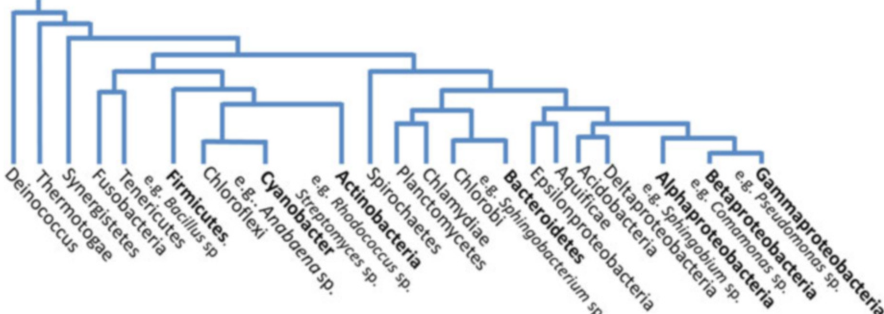

Fig. 13 Phylogenetic tree of bacteria. Bacteria families with members, which are associated with lignin degradation or potential ligninolytic enzymes are shown in bold (modified after [149], adapted with permission from Macmillan Publishers Ltd Macmillan Publishers Ltd, Copyright 2012)

Two different general approaches are being used for the identification of new ligninolytic organisms: culture dependent and culture independent methods.

Screening of new species by culture-dependent methods is carried out by cultivation steps on lignin model compounds, lignin, or lignin-rich waste (e.g., pulping effluent) as carbon source. Two-step screening methods are increasingly used. Herein, a first enrichment by growth on one or several aromatic compounds is followed by a confirmation of the lignin degradation abilities of selected strains through cultivation on lignin polymers.

A proof of lignin degradation by an organism is possible by showing the growth on lignin model substrates as sole carbon source, detecting modifications of lignin model substrates, or identifying known ligninolytic enzymes. Furthermore, polymeric, more or less natural lignins can be used as screening substrates. The chemical features of the lignin are strongly dependent on the extraction method, with dioxane and klason lignin being close to natural lignin and KL, which is significantly modified. Lignocellulose biomass can also be used as screening substrate. The degradation of lignin can be observed by determining the solid acid-precipitable polymeric lignin (APPL). APPL is built with less methoxyl groups and is complexed with bacterial protein. Moreover, feasible methods are established with synthetic ^{14}C-labeled lignin, which is synthesized from ^{14}C-labeled phenols, or ^{14}C-labeled lignin, which is obtained by growing plants on lignin precursor ^{14}C-phenylalanin (lignin (lignocellulose)). The ^{14}C-labeled lignins or lignin model substances are cultivated with ligninolytic species and the degradation rate is indicated by the amount of generated $^{14}CO_2$. Less time-consuming screening assays are based on the conversion of monomeric aromatics or synthetic aromatic dyes. The latter methods are apparently less reliable in validating lignin degradation. The use of aromatic lignin model dimers or tetramers is of intermediate effort and reliability [150, 151].

Currently two novel fast spectrophotometric screening methods have been established. The first screening assay with fluorescein isothiocyanate-labeled lignin gives changes in fluorescence over 10 min when lignin breakdown occurs. The second assay is based on chemically nitrated lignin and detects the release of nitrated phenol breakdown products at 430 nm over 20 min [152, 153].

Culture-independent methods include molecular biological methods and bioinformatics tools. These methods allow a direct analysis of the biodiversity of an environmental probe by 16S rRNA or its enzymatic diversity and functionality by screening on specific enzymes, for example lignolytic peroxidases (metagenomics) and their expression (metatranscriptomics). Herein, species that cannot be cultivated are also considered [151]. Interesting sources for screenings on new ligninolytic species are soils, waste water derived from the paper industry, and decaying wood or straw, as well as guts of wood-eating insects such as termites or beetles [124, 151]. For instance, the metagenome of the gut of a wood-boring beetle *Anoplophora glabripennis* was screened with regard to its microbiome composition and the enzyme functionalities. The microbiome varied between individuals, but Gammaproteobacteria were found to be dominant and Bacilli and Betaproteobacteria were found in equal amounts, although the relative abundances

of Actinobacteria, Alphaproteobacteria, Gammaproteobacteria, and Sphingobacteria were inconsistent [154]. No approved ligninolytic enzymes (e.g., MnP) were identified, but bacterial enzymes such as DyP-type peroxidases, copper oxidases, β-etherases, and glutathione-*S*-transferases, and especially aldo-keto reductases, which are all supposed to be associated with lignin degradation, were described [155].

A considerable amount of ligninolytic bacteria strains were found in the 1970s and 1980s by ^{14}C-labeling methods and the observation of APPL formation during growth on lignocellulose material. For example, soil probes were enriched by cultivation with dioxane lignin as carbon source. *Pseudomonas*, *Xanthomonas*, and *Acinetobacter* strains were isolated and found to change significantly the lignin structure. However, only a small amount (2%) of $^{14}CO_2$ was found after 150 days of degradation of labeled poplar by a *Pseudomonas* strain [156]. Moreover, after enrichment of compost and soil on mineral media with straw, KL or ferulic acid selected strains were incubated with labeled straw and investigated on $^{14}CO_2$ release. A *Streptomyces* strain was found to evaporate 7.5% of the lignin of labeled wheat straw as CO_2 in 15 days, but interestingly was unable to degrade KL or straw. A *Thermonospora* strain, able to degrade KL and ferulic acid, transformed 8.0% lignin to CO_2 [157]. The actinobacteria *Nocardia autotrophica*, *Nocardia corallin, Nocardia opaea*, and others were found to release $^{14}CO_2$ from synthetic dehydropolymer of coniferyl alcohol with about 4–14% in 15 days. Gram-strains as *Pseudomonas testosterone*, *P. putida*, and further *Pseunomonas species* showed a decrease of 0.9–2.2% in 15 days [158]. Strains of the genus *Streptomycetes,* isolated from termites gut produced 5–15% APPL of the initiate lignin, when they were incubated for 3 weeks with corn stover lignin (lignocellulose). The strain *Streptomyces viridosporus* T7A, which was the first strain where APPL was observed, solubilized about 7% of the lignocellulose material. For these strains 6–10% of the ^{14}C labeled lignin (in *Abies concolor* lignocellulose) was found as water-soluble products and only 1–2% as $^{14}CO_2$ after 3 weeks, hinting at a modification but no complete metabolization of lignin in these strains [159]. However, in other publications values of up to 16% of labeled lignin released as $^{14}CO_2$ were reported for *Nocardia, Rhodococcus*, *Arthrobacter*, *Streptomyces*, and *Thermomonospora* strains. The strains *Streptomyces cyaneus* and *Thermonospora mesophila* solubilized up to 30% lignin of lignocellulose materials after about 2 days to form APPL. Herein, bacteria showed higher conversion into CO_2 when extracted lignin and not lignin incorporated in lignocellulose was used [150, 160, 161]. In short, bacteria can solubilize lignin to high proportions and also the metabolization and the release of $^{14}CO_2$ was significant, but rather low compared to white-rot fungi such as *Phanerochaete chrysosporium*, *Sporotrichum pulverulentum*, and *Coriolus versicolor*, which were shown to evolve 30–50% of lignin (lignocellulose) as CO_2 [162, 163].

More recently, numerous bacterial strains were screened on ligninolytic activity with a nitrogen assay and a fluorescence assay. The strains *Streptomyces viridosporus* and *Pseudonocardia autotrophica* were dependent on H_2O_2 for their activity, and activity could only be detected by the fluorescence assay and not by the

nitrogen assay. Via the nitrogen assay, various strains, namely *Rhodococcus* sp., *Acinetobacter* sp., *Arthrobacter globiformis*, *Pseudomonas putida*, *Ochrobactrum pseudogrignonense*, and *Microbacterium oxydans* were found to reveal absorbance signals of around 1–5 milli arbitrary units (mAU). Notably, the three strains *Sphingobacterium*, *Rhodococcus erythropolis*, and *Streptomyces coelicolor* achieved values of 30–70 mAU. For wood-rotting fungi, comparable values of 3–30 mAU were detected, although white-rot fungus *Phanerochaete chrysosporium* exhibited a very high activity (ca. 700 mAU) [152, 153, 164].

Novel strains *Comamonas* sp. and *Pandoraea* sp. isolated from bamboo slips were shown to degrade KL and decolorize KL up to 45% and 40%, respectively. Remarkably, a manganese peroxidase and a laccase activity as well as small molecule degradation products such as cinnamic acid, ferulic acid, 2-hydroxy benzyl alcohol, and vanillyl methyl ketone were detected in culture supernatant of *Pandoraea* sp. [165, 166].

The participation of inducible extracellular proteins in APPL formation of bacteria was observed. Moreover, it was assumed that these proteins could be extracellular peroxidases [159, 167–169]. *Thermonospora mesophila*, *P. autotrophica*, *Streptomyces* sp., and *S. viridosporus* T7A strains were identified as high peroxidase producers in a screening for extracellular peroxidases in actinobacteria [170]. An extracellular peroxidase of *S. viridosporus* T7A was purified and characterized. This peroxidase cleaved lignin model compounds, oxidized monomeric aromatics, and was determined to be a heme peroxidase [171]. However, the gene sequence of this so-called lignin peroxidase of *S. viridosporus* T7A has not been published so far [115]. Members of the new family of the DyP-type peroxidases were associated with lignin degradation in bacteria. Because the extracellular occurrence of these peroxidases has been disputed, a participation in lignin degradation needs to be investigated in detail [148]. Moreover, bacterial laccases have currently come into focus and especially actinobacterial small laccases could be involved in lignin degradation [172]. Both these possibly ligninolytic bacterial enzymes are described in detail in the following section.

4.4 Bacterial Ligninolytic Enzymes

In this section the bacterial laccases and DyP-type peroxidases are addressed. These enzymes might participate in a bacterial lignin decay mechanism.

4.4.1 Bacterial Laccases

Bacterial laccases are not investigated as intensively as fungal and plant laccases. The first bacterial laccase was identified in the plant root associated bacterium *Azospirillum lipoferu* [173]. Prokaryotic laccases are quite diverse and their molecular weight is 28–180 kDa [174]. Whole genome analysis revealed that laccases are

widespread in bacteria and occur, for example, in *Bacillus* sp.*, Escherichia coli, Mycobacterium* sp., and *Pseudomonas* sp. Predictions of the three-dimensional structures suggest that all bacterial and fungal laccases consist of three so-called cupredoxin-like domains, which are mainly formed by β-barrels [173]. In addition to these well-described monomeric three-domain laccases, a new kind of laccase was identified, which is only found in prokaryotes. These laccases have two instead of three domains and are active as trimers. The active site is formed at each of the monomers. So far, bacterial laccases were thought to be intracellular. However, a bioinformatics study revealed that 76% of the bacterial laccases have a signal sequence and thus may be extracellular enzymes [175]. Bacterial laccases, in contrast to fungal laccases, are highly active at high temperatures, high pH, and high chloride and copper ion concentrations, making them compatible with a lot of industrial processes [173, 175]. Moreover, crystal structure analysis revealed a large putative substrate binding pocket in prokaryotic laccases compared to fungal and plant laccases [176]. However, bacterial laccases have low reduction potential T1 Cu, with values usually below 0.5 V, limiting their oxidation of high redox potential substrates [173, 174].

All prokaryotic laccases catalyze the reduction of dioxygen to water, but the substrate specificity and thus the assumed physiological roles differ considerably amongst these enzymes [174]. Generally, laccases oxidize aromatic phenols, amines, and inorganic ions such as [Fe(CN)6]. Some of them show activities against metal ions such as Fe^{2+} and Cu^{+} [174].

Laccase Cot A of *Bacillus subtilis* is assumed to be involved in the production of brown spore pigment of the endospore coat [176].

Laccases of *Escherichia coli* and *Bacillus halodurans* catalyze the conversion of Cu(I) to Cu(II), which is suggested to be a protection mechanism against the toxic effects of Cu(I) [174, 177].

Pseudomonas putida laccase, was shown to oxidize Mn(II) and Mn(III). In prokaryotes the oxidation of Mn(II) leads mostly to the formation of Mn (IV) oxides [178]. It was speculated that these oxides might protect the bacteria, for example, from UV radiation by binding on the cell surface. Another hypothesis is the oxidation of organic matter by Mn(IV) oxides, which can thus be metabolized by the bacteria [179].

A laccase of the γ-proteobacterium JB was assumed to protect the organism from xenobiotic toxicity, because numerous substances such as *p*-toluidine and phthalic acid induced the laccase expression [180].

Finally, two-domain laccases of *Streptomyces* (*S. coelicolor*, *Streptomyces lividans*, *S. viridosporus* T7A) were reported to oxidize a phenolic lignin model substance without mediator, but non-phenolic substances only in the presence of redox mediators. Moreover, wild type strain *S. coelicolor* and a laccase-deficient mutant were incubated with lignocellulose and both were found to produce APPL. Remarkably, the mutant strain produced 33% less APPL, demonstrating an important role of this laccase in the lignin degradation process of *S. coelicolor* [172].

Prokaryotic laccases could be of great interest for future industrial biotechnology because they are easily expressed in industrial host organisms. Basically, an

increase of their redox potential is required. Further studies on the correlation between T1 copper site ligation, reduction potential, and electronic structure may help to overcome this limitation through directed enzyme engineering [174].

4.4.2 DyP-Type Peroxidases Subfamilies A, B, and C

The subfamilies A, B, and C DyP-type peroxidases mainly contain bacterial enzyme sequences (see Fig. 14) [134].

DyP A, the ancestral clade of the whole superfamily, is probably located extracellularly, and thus twin-arginine translocation (TAT) signal sequences are found in these enzyme sequences. In contrast, DyP C and DyP B peroxidases appear to be involved in the intracellular metabolism [104, 127]. DyP B subfamily is rather divergent. Besides its additional branch, which possibly leads to the chlorite dismutases, DyP B peroxidases were also found in fungi and lower eukaryotes (protists or slime molds). DyP B peroxidases probably first evolved in Proteobacteria and afterward spread via horizontal gene transfer amongst the other bacterial phyla and the eukaryotes [104].

DyP C peroxidases cluster close to the fungal DyP D peroxidases and seem to share their high activity. The molecular weights of DyPs were observed to be around 54 kDa, 44–48 kDa, and about 32 kDa for DyP C, A, and B, respectively [181–186]. The quaternary structures of DyPs have been reported to vary from monomers to hexamers [127].

The catalytic cycle for DyP-type peroxidases is already concerned in the above Sect. 4.2.3. For the functionality of fungal DyP-type peroxidases, the essential amino acid residue aspartate of the GXXDG motif was proposed to be essential for the interaction with H_2O_2 and the formation of compound I [127]. This might be true for DyP-type peroxidases of subfamily D, and aspartate seems to be important

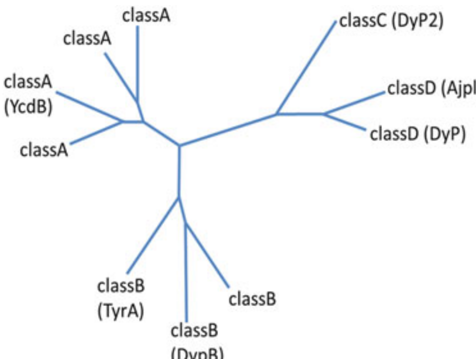

Fig. 14 Phylogenetic tree of the DyP-type peroxidase family members. YcdB (*E. coli*), DyPP2 (*Amycolatopsis* sp.), Ajpl (*Auricularia auricula-judae*), DyP (*Bjerkandera adusta*), TyrA (*Shewanella oneidensis*), DyP B (*Rhodococcus jostii*) (adapted after [128] with permission from Elsevier, Copyright 2015 Elsevier Inc. All rights reserved)

for the peroxidase activity of particular subfamily A members [187, 188]. However, for DyP B of actinobacterium *Rhodococcus jostii* and protobacterium *Pseudomonas putida* and DyP A peroxidase EfeB of *E. coli* this aspartate residue does not appear be essential for the peroxidase activity [189, 190]. Moreover, an arginine residue was shown to be essential in DyP B of *R. jostii*, as a mutant with arginine substituted by leucine had no detectable peroxidase activity [134].

DyPs generally have a wide substrate specificity and a considerable lower pH optimum compared to other plant peroxidases [115]. All DyP-type peroxidases have a peroxidase activity, although for some DyPs, which have inefficient catalytic properties, this activity might not be relevant for their physiological role [134]. For instance, ABTS, which is a general peroxidase substrate, is converted by A- and B-type DyPs three orders of magnitude less efficiently than by C- and D-type DyPs [134]. The exact physiological functions of DyP-type peroxidases are unknown, but some have been proposed [128].

A DyP-type peroxidase of archaebacterium *Halobacterium salinarum* was suggested to be involved in the protection against oxidative stress. The function of DyP B peroxidase of proteobacterium *Francisella tularensis* was also suggested to be involved in the defense of oxidative stress [128, 191].

Studies on YcdB (DyP A) and Yfex (DyP B) of *E. coli* indicate that both these DyP-type peroxidases are able to extract iron from heme and thereby leave the protoporphyrin ring intact [184]. These findings are supported by the genomic context of these enzymes in *E. coli*. The genes efeU and efeO, which code for an iron transporter and an iron uptake component, occur downstream of Yfex and YcdB [134]. A similar function was described for a DyP A and DyP B peroxidase of *Staphylococcus aureus*. The cluster was expressed in a YcdB and Yfex deficient *E. coli* mutant and restored the ability of *E. coli* to use heme as an iron source. DyP A of *S. aureus* showed even less peroxide activity as *E. coli* YcdB [192]. The latter enzymes could be an example of the physiological function for DyPs being independent of the peroxidase activity [128]. In contrast, several members of the DyP-type peroxidases subfamilies B, C, and A were associated with lignin degradation in bacteria.

DyP B from *R. jostii* was shown to cleave a phenolic lignin model compound. In the presence of Mn^{2+}, which is also a substrate for this peroxidase, this activity was increased. Furthermore, an activity against straw lignocellulose could be measured only by adding $MnCl_2$. Nitrated lignin was incubated with *R. jostii* wild type and mutant strains. In the wild type strain, *R. jostii* photometrically measured changes at 430 nm hinted at the evaporation of nitrated phenol compounds. In contrast, in a DyP B-deficient *R. jostii* mutant, no changes were detected. The heterologous DyP B enzyme also showed activity in the nitrate assay [193]. A binding pocket with three glutamate residues and one threonine was proposed for DyP B of *R. jostii*. However, in *Pseudomonas putida* DyP B the same residues are present, but no Mn^{2+}-oxidizing activity was found. Moreover, another Mn^{2+}-oxidizing enzyme (DyP C, *Amycolatopsis* sp.) did not have the same Mn^{2+}-binding motif [193–195]. Engineering of DyP B (N246A) resulted in a 15-fold increase in the catalytic efficiency (kcat/km) against Mn^{2+}. The engineered DyP B was able to

catalyze a manganese-dependent conversion of KL and solvent-extracted fractions of KL. Nevertheless, DyP C of actinobacterium *Amycolatopsis* sp. still oxidizes Mn^{2+} more efficiently within two orders of magnitude [196]. Paradoxically, the ligninolytic peroxidase of *R. jostii* lacks, as do most DyP B peroxidases, a signal sequence for export and consequently is supposed to be an intracellular enzyme. This is in contrast to its suggested function, which is the measured ligninolytic activity in the supernatant of *R. jostii*. It was hypothesized that DyP B might be exported by an unknown mechanism. Downstream of DyP B a gene coding for encapsulin was identified. Encapsulins can form nanocompartments with incorporated DyP B. A higher ligninolytic activity for the complexed DyP B in comparison to free DyP B was measured, but still the export of DyP B remains in question [193, 197].

DyP C of *Amycolatopsis* sp. (formerly *Streptomyces griseus*) was also reported in the context of lignin degradation. As already mentioned, a very high Mn^{2+}-oxidizing activity almost comparable to fungal VP was observed. DyP C was able to degrade high redox synthetic dyes with high catalytic efficiency and was able to cleave a phenolic lignin model substance. Interestingly, an oxidase activity with 4-methoxymandelic acid in the presence of Mn^{2+} was found. Some homolog DyP C peroxidases of other related actinomycetes cluster on operons with other biomass-processing enzymes, though DyP C of *Amycolatopsis* sp. does not [186]. To date, only DyP C of *Amycolatopsis* sp. and DyP C of cyanobacterium *Anabaena* sp. have been characterized. DyP C of *Anabaena* sp. oxidizes guaiacol, pyrogallol, and anthraquinone dyes such as Reactive Blue 5 with high catalytic efficiencies, the latter with 10^7, which is equal to activities of fungal DyPs (*Auricularia auricular).* However, this DyP C peroxidase can neither oxidize veratryl alcohol nor manganese [185, 186]. In general, DyP C peroxidases are found in the neighborhood of various enzymes, such as a doxorubicin resistance gene, halo-acid dehalogenase, or a methyl-accepting chemoreceptor. Overall, the biochemical characterizations and the bioinformatic data indicate that the peroxidase activity of DyP C peroxidases is physiologically relevant and that DyP C peroxidases have diverse biological roles [134].

Just recently two DyP A peroxidases have been characterized, which seem to participate in lignin degradation. DyP A of *Bacillus subtilis* (KCTC2023) was able to cleave a non-phenolic lignin model dimer without mediator and oxidized the high-redox non-phenolic monomer veratryl alcohol with a high efficiency in comparison to other bacterial DyP-type peroxidases. The anthraquinone dyes reactive black 5 and reactive blue 19 were also oxidized. Interestingly, these substrates differed in their temperature optima. Whereas the high redox substrates veratryl alcohol and the lignin model dimer were converted best at 50 °C, the optimum for dye oxidation was 30 °C [198]. The DyP A peroxidase of *Saccharomonospora viridis* was shown to bleach eucalyptus kraft pulp (21.8% reduced kappa number, 2.98% increase in brightness). The enzyme performed well over a broad pH range and had a high temperature stability. The pH optimum of this enzyme was determined with triarylmethane dye brilliant green as a substrate. Astonishingly, in contrast to other DyP-type peroxidases, which work mostly in acid pH, the

optimum of DyP A of *S. viridis* was pH 7. Another interesting observation was the eightfold boost of enzyme activity through the reducing agent β-mercaptoethanol, perhaps by preventing the enzyme from forming S–S linkages [183].

Further bioinformatical and biochemical studies on DyP-type peroxidases could help to clarify their physiological roles. Further research should also help to develop their biotechnological potential, which is expected mainly in processes for modification and degradation of lignin and industrial dyes [134].

4.4.3 Accessory Enzymes

Assuming an analogous mechanism of fungal and bacterial lignin degradation, bacteria also require accessory enzymes, providing H_2O_2 for their extracellular peroxidases. A few H_2O_2-generating enzymes [199, 200] and analogs to fungal accessory enzymes [201, 202] have been described in bacteria, but to the best of our knowledge no publication which deals with their participation in lignin degradation is available.

4.5 Bacterial Catabolism of Lignin Degradation Intermediates

Although bacteria are able to break down lignin, they generally play a more important role in mineralization of oligomeric and monomeric lignin derivatives, which are derived from fungal attack on the lignin macromolecule [203, 204]. Herein, we encounter the degradation pathways of aromatics by bacteria.

Many bacteria strains are known to metabolize lignin-derived aromatic compounds. For example, biphenyl-degrading species are found in several bacterial genera such as *Sphingomonas*, *Burkholderia*, *Rhodococcus*, *Pseudomonas*, *Achromobacter*, *Comamonas*, *Ralstonia*, *Acinetobacter*, and *Bacillus* [124]. The most intensively studied bacterium regarding the aromatics metabolism is the proteobacterium *Sphingobium* sp. SYK-6 [52].

A degradation of biphenyl 5,5-dehydrovanillate is described to proceed via a demethylation step, catalyzed by *O*-demethylase (LigX). The *O*-demethylase is probably a three-component monooxygenase [204]. One of the aromatic rings is cleaved by a dioxygenase (LigZ) and the meta-cleavage compound is converted into 5-carboxyvanillate and 4-carboxy-2-hydroxypentadienoic acid by hydrolase LigY. 5-Carboxyvanillate is decarboxylated by decarboxylase LigW to result in the central metabolite vanillic acid (Fig. 15B). 4-Carboxy-2-hydroxypentadienoic acid is probably hydrated and cleaved by an aldolase to form two molecules of pyruvate [52, 124].

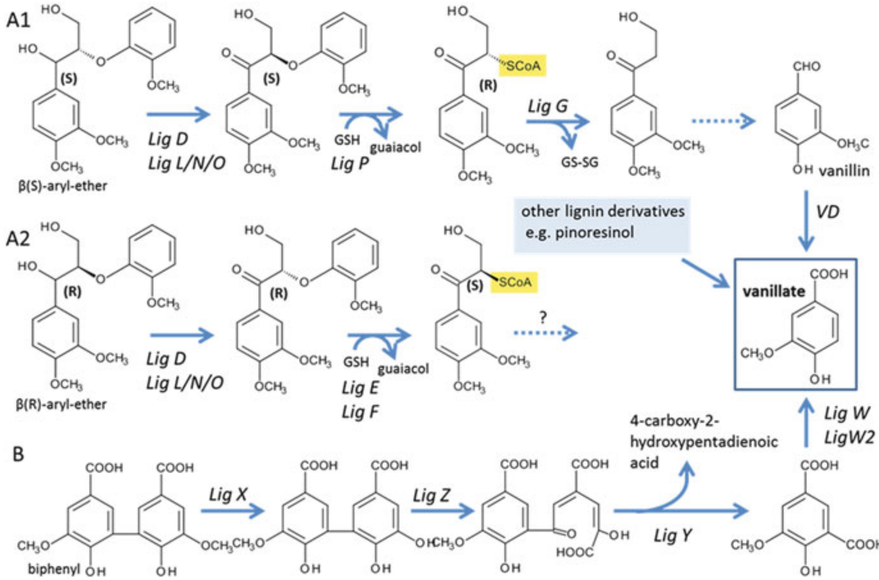

Fig. 15 Degradation pathways for lignin derivatives in *Sphingobium* sp. SYK-6: (**A1**) degradation of (S)-enantiomer β-aryl ether, (**A2**) degradation of (R)-enantiomer β-aryl ether, and (**B**) degradation of biphenyls. Abbreviations: *LigD-O* dehydrogenases, *LigP and LigE/F* glutathione-*S*-transferases, *LigG* β-thioetherase, *VD* vanillin dehydrogenase, *LigX* *O*-demethylase (monooxygenase), *LigZ* dioxygenase, *LigY* hydrolyase, *LigW/W2* decarboxylase (modified after [203, 205] with permission from Taylor & Francis Ltd, Copyright Japan Society for Bioscience and Agrochemistry, Copyright 2014 by The American Society for Biochemistry and Molecular Biology, Inc.)

Sphingobium sp. SYK-6 moreover degrades β-aryl ether compounds such as guaiacylglycerol-β-guaiacyl ether (GGE) or guaiacyl-α-veratrylglycerol (VG). The dehydrogenases LigD, LigL, LigN, and LigO dehydrogenate VG at the α-C a-hydroxyl group to form a ketone. Then a β-*S*-glutathionyl-α-ketothioether is built at the β-ether. Thereby, the cosubstrate glutathione is linked to the β–C and guaiacol is released. Interestingly, this step is stereoselective and catalyzed by different glutathione-*S*-tranferases. LigF only catalyzes β(S)-ether, resulting in a β(R)-thioether, whereas β(R)-ether is converted by LigP and LigE to form a β(S)-thioether. The β-thioetherase LigG seems to be stereoselective, too, and cleaves preferentially the β-(R)-thioether. In this step a second molecule GSH is consumed, producing glutathione disulfide (GS-SG) and β-deoxy-α-veratrylglycerone [205] (Fig. 15A).

In *Rhodococcus jostii* an alternative conversion of the β-aryl ether into a ketone cleavage product was suggested, working with a radical β-elimination mechanism via hydrogen abstraction [152]. The ketone intermediate is then metabolized to vanillic acid. It was assumed that this step might be an oxidation of the hydroxyl

group into carboxylic acid, followed by a C–C cleavage similar to a β-oxidation [52].

Further pathways of other aromatic compounds such as the monomers eugenol and coniferyl alcohol, as well as dimers pinoresinol and di-aryl ether, were described in *Sphingobium* sp. SYK-6 and other species, leading to vanillic acid. Vanillic acid is demethylized by a monooxygenase to form protocatechuic acid [124, 203, 206].

Aerobic degradation pathways include, besides catechol and some other aromatics, protocatechuate as their central intermediate [207]. Protocatechuate may originate from lignin breakdown products, as described above, but also from chlorinated aromatics and other materials [208].

In *Sphingobium* sp. SYK-6, protocatechuic acid is cleaved aerobically by an extradiol dioxygense (protocatechuic acid-4,5-dioxygenase named LigAB), meaning this enzyme cuts the aromatic ring not between the two ring hydroxyl groups, but outside, initiating the meta-cleavage pathway. Other bacteria, for example, *Pseudomonas*, *Acinetobacter*, and *Rhodococcus*, are known to use an alternative designated *ortho*-cleavage pathway, including an aerobic ring cleavage by an intradiol protocatechuate 3,4-dioxygenase as a first step. Moreover, some bacteria, for example, Rhodococcus equii, own a protocatechuic acid 3,4- and a 4,5-dioxygenase.

The meta-pathway continues with oxidation of the dioxygenase cleavage product 4-carboxy-2-hydroxymuconate semialdehyde by a dehydrogenase (LigC), producing 2-pyrone-4,6-dicarboxylate. This molecule is hereupon hydrolyzed by LigI, resulting in 4-oxalomesaconate, which is transformed by a hydratase LigJ and an aldolase LigK into pyruvate and oxaloacetate, which enter the citric acid cycle and are respired (see Fig. 15) [52, 203].

In *Pseudomonas putida*, as an example of the *ortho*-pathway, the cleavage product β-carboxymuconate is transformed by a cycloisomerase into γ-carboxymuconolactone, which is decarboxylated by γ-carboxymuconolactone decarboxylase into β-ketoadipate enol-lactone and then reduced to β-ketoadipate by a hydrolase. The subsequent β-ketoadipate pathway degrades β-ketoadipate to succinyl- and acetyl-CoA via a CoA transferase and a CoA thiolase. Succinyl- and acetyl-CoA can be utilized by the cell in the citric acid cycle or the fatty acid biosynthesis (see Fig. 16) [208].

Catechol may be derived from pollutants (e.g., phenol, toluene, or benzene) and similar sources as protocatechuate. Catechol is also found as central intermediate in bacteria [207, 208]. Its degradation pathway includes mechanisms similar to the protecatechuate pathway and is described here only briefly. Catechol is either cleaved extradiolically to form 2-hydroxymuconic semialdehyde (meta-pathway), which is converted to acetaldehyde and pyruvate, or cleaved intradiolically (*ortho*-pathway) to form *cis,cis*-muconate. This intermediate is, analogous to protocatechuate, cyclo-isomerized to muconolactone, followed by a double bond shift, resulting in the enol-lactone, which is hydrolyzed and enters the corresponding pathway as β-ketoadipate [207, 208].

Fig. 16 *Meta*- and *ortho*-cleavage pathways in bacteria *Sphingobium* sp. SYK-6 and *Pseudomonas putida*. Abbreviations: *P3,4O*, protocatechuic acid-3,4-dioxygenase, *CM* β-carboxy-muconate, *CMLE* β-carboxy-*cis,cis*-muconate lactonizing enzyme (cycloisomerase), *CML* γ-carboxy-muconolactone, *CMD* γ-carboxy-muconolactone decarboxylase, *KAEL* β-ketoadipate enol-lactone, *ELH* β-ketoadipate enol-lactone hydrolase, *KA* β-ketoadipate, *TR* β-ketoadipate succinyl-CoA transferase, *CoA* coenzyme A, *TH* β-ketoadipyl-CoA thiolase, *AC* acetyl, *SC* succinyl, *LigAB* protocatechuic acid 4,5-dioxygenase, *CHMS* 4-carboxy-2-hydroxymuconate-6-semialdehyde, *LigC* CHMS dehydrogenase, *PDC* 2-pyrone-4,6-dicarboxylate, *LigI* PDC hydrolase, *OMA* 4-oxalomesaconate, *LigJ* OMA hydratase, CHA 4-carboxy-2-hydroxy-3-*O*-methylgallate, *LigK* CHA aldolase, *TCA* tricarboxylic acid (modified after [203, 208] with permission from Taylor & Francis Ltd, Copyright Japan Society for Bioscience and Agrochemistry, with permission from Elsevier, Copyright 2012 Elsevier Ltd. All rights reserved)

Further research on bacterial lignin degrading pathways should help not only to clarify the carbon cycle on Earth, but also to identify novel useful tools for the conversion of lignin into building blocks and fine chemicals of industrial interest [203]. Recently, the gene of the vanillin dehydrogenase has been deleted in the ligninolytic bacterium *Rhodococcus jostii*. The mutant strain was grown on wheat straw lignocellulose as a feedstock, which resulted in an accumulation of vanillin in the culture broth [209]. This gives an exemplary approach for biotechnological utilization of lignocellulose and, accordingly, lignin, stimulating further research on bacterial lignin degradation.

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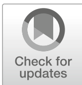
Sustainability Evaluation



Heinz Stichnothe

Abstract The long-term substitution of fossil resources can only be achieved through a bio-based economy, with biorefineries and bio-based products playing a major role. However, it is important to assess the implications of the transition to a bio-based economy. Life cycle-based sustainability assessment is probably the most suitable approach to quantify impacts and to identify trade-offs at multiple levels. The extended utilisation of biomass can cause land use change and affect food security of the most vulnerable people throughout the world. Although this is mainly a political issue and governments should be responsible, the responsibility is shifted to companies producing biofuels and other bio-based products. Organic wastes and lignocellulosic biomass are considered to be the preferred feedstock for the production of bio-based products. However, it is unlikely that a bio-based economy can rely only on organic wastes and lignocellulosic biomass.

It is crucial to identify potential problems related to socio-economic and environmental issues. Currently there are many approaches to the sustainability of bio-based products, both quantitative and qualitative. However, results of different calculation methods are not necessarily comparable and can cause confusion among decision-makers, stakeholders and the public.

Hence, a harmonised, globally agreed approach would be the best solution to secure sustainable biomass/biofuels/bio-based chemicals production and trade, and to avoid indirect effects (e.g. indirect land use change). However, there is still a long way to go.

Generally, the selection of suitable indicators that serve the purpose of sustainability assessment is very context-specific. Therefore, it is recommended to use a flexible and modular approach that can be adapted to various purposes. A conceptual model for the selection of sustainability indicators is provided that facilitates

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identifying suitable sustainability indicators based on relevance and significance in a given context.

Keywords Bio-based product, Bioeconomy, Biorefinery, Food security, LCA

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1 Introduction

Integrated biorefineries and their bio-based products are perceived as a key source for urgently needed innovation for a bio-based economy. Many governments have set up supportive policies for research and development (R&D) in relevant areas, such as biotechnology. An additional advantage of biomass utilisation is that previously unused material flows can be directed towards industrial use, supporting development of innovative refining technologies, increasing resource-efficiency, and reducing environmental burdens. However, the food vs fuel debate has initiated a broad discussion on land competition between the food and animal feed sectors on one hand and energy and material recovery from biomass on the other, as well as the limited availability of land, speculation on raw materials and consequences for biodiversity and its conservation. The environmental, economic and social impacts of industrial biomass use are also dependent on the specific design of the whole value chain, including use, and therefore cannot be assessed to be intrinsically sustainable. It is thus necessary to use a life cycle consideration to assess the sustainability of biorefinery's products.

New biorefinery concepts, designed for use of a wide range of biomass fractions and with minimum (preferably zero) waste generation of a multitude of products for different markets, are not yet state-of-the-art. Demonstration plants are currently emerging in highly integrated process systems, where the greatest possible number of biorefinery products can feed directly into further processes. Integrated biorefineries could manufacture sustainably produced fuels and chemicals.

However, the sustainability implications of integrated biorefineries are still poorly understood.

Most publications regarding the assessment of bio-based products use life cycle assessment (LCA) to evaluate the environmental performance of bio-based products in comparison to their fossil-derived counterparts. Frequently the analyses focus on energy or greenhouse gas (GHG) emissions. Weiss et al. reviewed 44 LCA studies of bio-based products. The authors conducted a meta-analysis and concluded that bio-based products usually provide a GHG emission reduction, but cause increased eutrophication and stratospheric ozone depletion relative to fossil-derived products [1]. These findings are in line with those of Stichnothe and Azapagic as well as with Ekmann and Börjesson, who have also found a trade-off between global warming potential and eutrophication [2, 3]. Adom et al. have compared energy consumption and GHG emissions of eight bio-based chemicals produced either from corn stover-derived sugars or algal-derived glycerol with their fossil counterparts. The authors state that in all cases bio-based products offer a GHG reduction and also lower fossil energy consumption relative to their fossil counterparts [4]. Lammens et al. show that glutamic acid produced from sugar beet vinasse can be used as the building block for *N*-methylpyrrolidone (NMP), *N*-vinylpyrollidone (NVP), acrylonitrile (CAN) and succinonitrile (SCN). A life cycle-based comparison revealed that NMP and NVP derived from glutamic acid have lower environmental impacts than their fossil-derived counterparts. The opposite was true for CAN and SCN, indicating that not all bio-based products have a better environmental performance than their fossil counterparts [5, 6]. Lin et al. compared bio-based *p*-xylene with petrochemical-derived *p*-xylene. The author highlighted the importance of the used feedstock; xylene from starch is identified as less environmental-friendly than petrochemical-derived xylene, whereas oak-derived xylene has an environmental performance comparable to petrochemical-derived xylene [7].

However, it is impossible to compare the results of different publications because the authors used different assumptions, allocation procedures and background data. Despite the plethora of activities related to biorefineries, there is still no agreed framework for sustainability assessment. A common framework would help governments and industry identify, evaluate and support the development of bio-based products which are likely to be most sustainable and thus beneficial [8].

This chapter aims to contribute to a better understanding of the sustainability assessment of integrated biorefineries and bio-based products.

2 Sustainability Development and Sustainability

The term sustainable development is frequently confused with the term sustainability; both terms are closely linked but are not the same. Sustainable development is a process whereas sustainability is a property of something (a policy, a product, a technology, etc.) being sustainable [9].

There is worldwide consensus on the abstract sustainability concept, although there are endless discussions and no agreement about the best way to measure it [10].

Sustainability as a concept is defined as strong or weak sustainability. Supporters of the latter believe that natural capital (well-functioning ecosystems and biogeochemical cycles) can be substituted with other kinds of capital (human, social and manufactured) whilst sustaining human welfare. Opponents of that position reject the assumption of universal capital substitutability based on an evaluation of the nature and functions of natural capital. Advocates of strong sustainability argue that the life supporting functions provided by well-functioning ecosystems and biogeochemical cycles are largely non-substitutable. Strong sustainability therefore demands the maintenance of critical natural capital stocks safely above potential carrying capacity thresholds.

To set the scope we use the Brundtland definition, which is broadly accepted. The Brundtland report "Our common future" expresses the concept of sustainability in the context of 'sustainable development', defining the latter as 'Development that meets the needs of the present without compromising the ability of future generations to meet their own needs' [11].

Within these well-accepted and apparently objective definitions for sustainable development, the central questions of 'sustaining what, for whom, where, and for how long?' remain laden with human values and social choices, which are very context-specific, and therefore the answers differ across time, space and culture [10].

In general, sustainable development is subdivided into three areas or dimensions: environmental, economic and social. Nowadays, a fourth dimension is frequently mentioned: good governance. What is 'good governance'?

> Good governance is about the processes for making and implementing decisions. It's not about making 'correct' decisions, but about the best possible process for making those decisions. Good decision-making processes, and therefore good governance, share several characteristics. All have a positive effect on various aspects of local government including consultation policies and practices, meeting procedures, service quality protocols, councillor and officer conduct, role clarification and good working relationships.[1]

Bad governance is often considered as one of the root causes that hamper sustainable development. Governance is usually beyond the influence sphere of economic operators of biorefineries, and is therefore mentioned here but not further discussed.

The other three dimensions are well-known, but rarely well-understood, and sustainability indicators are used by various institutions at various levels, (e.g. UN, OECD, EU, national governments) as well as by companies and NGOs. Currently, most approaches that measure sustainable development, including triple-bottom-line and PPP (People, Planet, Prosperity), use the weak sustainability concept. This

[1] http://www.goodgovernance.org.au/about-good-governance/what-is-good-governance/.

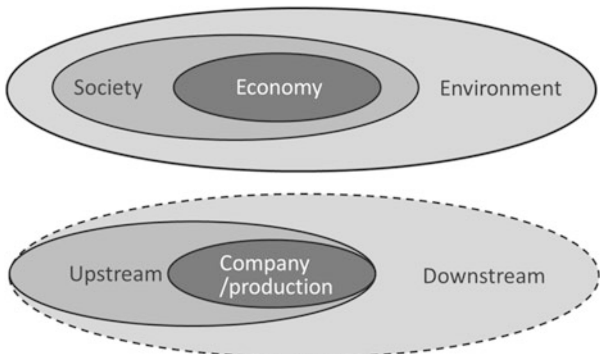

Fig. 1 How policy makers and companies see the world

concept is also preferred by standardisation and certification bodies as it allows the defining of measureable and/or verifiable indicators.

Sustainability means different things to different people and can be used for different purposes. That has caused confusion among involved parties. Sustainability requirements are sometimes considered as trade barriers for developing countries, made responsible for increasing biomass costs, and considered as unfair as the requirements are not equally set for conventional fossil-based products.

The domains and views of companies and policy are different, which is illustrated in Fig. 1.

National governments or regulation bodies set the framework in which companies operate. Companies have full responsibility within their premises. Although companies have some influence on their supplier's activities, depending on their size, they have little or no influence on their customer's activities or the consumer's behaviour and the resulting environmental and socio-economic impacts. However, the deployment of novel biorefineries requires large investments and investors need long-term business perspectives, which are more and more influenced by sustainability-related legislation and public perception [12].

Sustainability demands the integration of multiple knowledge areas, including natural science, health, social science, economic science and policy across the whole life cycle. Successfully engaging diverse disciplines can help identify important sustainability aspects that are overlooked in an isolated disciplinary context [13].

The results of sustainability assessment can be interpreted in various ways by different groups. Moreover, different sustainability assessment methods can provide contrasting results for the same bio-based product, causing confusion among stakeholders and the public. One reason for the confusion is that different stakeholders, such as policy makers, industry managers, NGOs and consumers have a different understanding of what bio-based products and biorefineries are. Additionally, values and aims of the different groups are not necessarily the same. Therefore, clear definitions of biorefineries and bio-based products are needed as well as a transparent and complete description of the purpose and the objectives of each sustainability assessment.

To provide some guidance the most important terms used in this chapter are defined as follows

Biorefinery is a sustainable processing of sustainably produced biomass into a spectrum of marketable products (food, feed materials, chemicals) and energy (fuels, power, heat) modified from Bell et al. [14]. Biorefineries can be further distinguished based on their main product into energy-driven biorefineries and product-driven biorefineries [15].

Various types of biomass are used for the production of bio-based products through many types and sizes of economic operations. Virtually every country in the world produces and consumes some form of bio-based products and/or bioenergy. The characteristics of bio-based products are very heterogeneous, and their production processes depend on several factors, including geographic location, climatic conditions, development level, institutional frameworks and technological issues.

Most biorefineries have deployed multiple technologies and use different integrated processes designs; moreover, they commonly produce a portfolio of bio-based products. The goal of assessing a single biorefinery might be to improve single processing steps or the interaction between multiple processing steps with respect to optimising resource efficiency, including energy demand or reducing emissions or wastes. Another possible goal is to compare different technology set-ups within a given biorefinery concept producing the same products. The results of the assessment differ among different biorefinery concepts and are barely comparable. The sustainability assessment of biorefineries is outlined in VDI 6310, an industrial guidance document for biorefinery operators.

There is no need to reinvent the wheel; hence the sustainability assessment in this chapter focuses on the assessment of bio-based products. The definition of bio-based products is difficult as sometimes people define bio-based products with respect to their functionality, that is biodegradable or on the feedstock origin (bio-based carbon content).

Table 1 demonstrates that biodegradability can be achieved independently from the feedstock, although renewable feedstock does not ensure biodegradability. The same molecules (e.g. ethylene, glycerol, succinic acid) can be derived from fossil-based or renewable feedstock. Hence, the environmental functionality can hardly be used as a criterion for bio-based products. The preferred criterion for bio-based products is the bio-based content, but of which atom(s), C, N, H? It is highly likely that the carbon content is the most relevant, although other bio-based molecules should not be ignored in scientific debates.

Table 1 Feedstock origin and functionality of selected products

Feedstock	Functionality	Example
Renewable	Biodegradable	Polyhydroxyalkanoate (PHA)
Renewable	Non-biodegradable	Polyethylene from sugar
Fossil-based	Non-biodegradable	Polyethylene from crude-oil
Fossil-based	Biodegradable	Polycaprolactone (PCL)

However, there is no agreement on the proportion of bio-based carbon in a bio-based product. It seems reasonable to classify bio-based products based on thresholds, for example 100% bio-based carbon, >50% bio-based carbon and >25% bio-based carbon. Threshold values should be derived from a stakeholder consultation process.

The content of bio-based carbon in the product can be measured by the ^{14}C-method, but that shows only the origin of the carbon in the product. It does not take into account the energy used in production and distribution. Therefore, in addition to a transparent and complete description of the purpose and the objectives of each sustainability assessment, a life cycle-based approach is needed to assess the true (environmental) impacts of any bio-based product.

3 Life Cycle Thinking and Life Cycle-Based Assessment

Life Cycle Thinking (LCT) is about going beyond the traditional focus and production site and manufacturing processes to include environmental, social and economic impacts of a product over its entire life cycle.[2] According to Kloepffer "Life cycle thinking is the prerequisite of any sound sustainability assessment. It does not make any sense at all to improve (environmentally, economically, socially) one part of the system in one country, in one step of the life cycle or in one environmental compartment, if this 'improvement' has negative consequences for other parts of the system which may outweigh the advantages achieved [16]." This includes shifting of burdens in both an intra- and inter-generational context.

LCT means extending the narrow focus beyond production facilities. A life cycle of bio-based products begins with the cultivation of biomass, including all processes needed to provide the necessary ancillaries, such as seeds, fertiliser, pesticides and energy provision. Biomass and energy are then part of production, packaging, distribution, (cascading) use, recycling and recovery or final disposal.

For assessing the environmental sustainability of bio-based products, LCA is frequently used. LCA helps to quantify the environmental burdens related to energy, goods and services, the environmental benefits, the trade-offs and areas for achieving improvements. In LCA, the connections between single processes and process steps in a product system are modelled to quantify the material and energy flows within a product system. LCA is an internationally standardised methodology (ISO 14040 ff. [17, 18] and consists of four steps:

1. Goal and scope definition
2. Life cycle inventory (LCI)
3. Life cycle impact assessment (LCIA)
4. Interpretation

[2] http://www.lifecycleinitiative.org/starting-life-cycle-thinking/what-is-life-cycle-thinking/.

Conducting an LCA is an iterative process; additional information and data gained during the assessment process can require backshift to a previous life cycle stage to include the new data and/or information.

LCI is the collection and analysis of environmental interventions data (e.g. emissions to air and water, waste generation and resource consumption) which are associated with a product throughout its life cycle.

LCIA is the estimation of indicators of the environmental pressures in terms of climate change, summer smog, resource depletion, acidification and human health effects, associated with the environmental interventions attributable to the life cycle of a product.

LCA has been used for many years for decision-supporting purposes in research, policy making and companies. There is a huge amount of information about LCA available from international initiatives and governmental bodies, such as the Life Cycle Initiative, European Platform on LCA, US-EPA. Although the focus on environmentally beneficial approaches is a requirement for environmental and climate protection, the economic potential decides whether a biorefinery concept is developed up to a commercial scale.

Life cycle costing (LCC) is one approach to incorporating a subset of economic considerations into life cycle-oriented assessments. In essence, LCC quantifies costs (related to real money flows) associated with the life cycle of a product that are directly covered by one or more of the actors in the supply chain. Although parallel in many respects to conventional cost accounting, LCC additionally accounts for dimensions such as subsidies and costs related to the use and end-of-life treatment of products, and less tangible costs such as those associated with environmental protection.

Social life cycle assessment (S-LCA) is a relatively new approach to include the third dimension needed for sustainability assessment. S-LCA is a method that can be used to assess the social and sociological aspects of products, their actual and potential positives, as well as negative impacts along the life cycle. Although S-LCA follows the ISO 14040 framework, some aspects differ from traditional LCA. The Life Cycle Initiative provides a guideline for S-LCA of products.

Taken together, these three life cycle-based methodologies (LCA, LCC and social LCA) can contribute strongly to life cycle sustainability assessment (LCSA) [19]:

$$\mathrm{LCSA} = \mathrm{LCA} + \mathrm{LCC} + \mathrm{SLCA}$$

LCSA provides a consistent framework for conducting sustainability assessment, although there are multiple challenges associated with the current status of sustainability assessment. The data used in LCSA should be consistent, quality assured and reflect actual value chains. The methodological choices should reflect a best consensus among stakeholders and should be orientated at the current practise.

The availability of social LCI databases in support of social LCSA modelling is minimal at present, particularly at the process level. Beyond the screening level, the social LCA afforded by the Social Hotspots Database, process-level analyses

requires site level research to characterise accurately the social dimensions of a particular product supply chain.

The available suite of life cycle based-indicators is limited across domains. More importantly, such indicators are invariably linked to products and/or services. Although this is not problematic in the environmental domain, as most environmental impacts can be accounted for in terms of consumption and production, this is less the case for social indicators [20, 21]. However, there are other more important drawbacks. In LCSA, each pillar of sustainability is separately modelled and then results are synthesised in a final decision-analysis step. Although this has the advantage of promoting a strong sustainability perspective, this approach does not consider mutual relations amongst the pillars. Moreover, emissions and environmental impacts might have a mathematical relationship (assumed to be linear), this does not hold for social impacts (e.g. human rights are not divisible). LCSA is an interesting concept that has to be developed further but at its current stage it is operational.

There are different reasons for conducting sustainability assessment. Frequently, sustainability assessment is conducted to support decision making. Figure 2 summarises the rationale for conducting a sustainability assessment.

Sustainability assessment can serve multiple purposes, but each has certain requirements regarding relevant aspects, data quality, methodological choices, and constraints such as data availability, resource restrictions and confidentiality issues. Therefore, a transparent and complete description of the purpose and objectives of each sustainability assessment is necessary. This helps one to decide whether results from different sustainability assessments for the same bio-based product

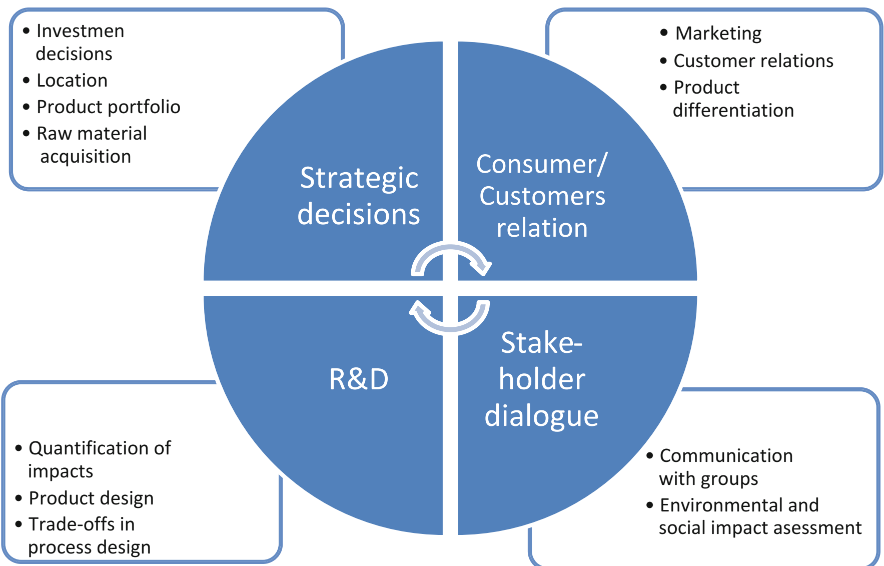

Fig. 2 Decision support by sustainability assessment

Table 2 Important sustainability aspects in the life cycle of bio-based products [2], modified

Environmental	Economic	Social
GHG emissions	Feedstock costs	Human health
Land demand	Investment costs	Labour rights
Land-use change	Subsidies	Working conditions
Biodiversity	Local income generation	Land ownership
Water consumption		Food security
Emissions to water and soil		Rural development

or its fossil-based counterpart are comparable. In a nutshell, results of different studies with diverse purposes are most likely not comparable.

Sustainability assessment must cover all relevant aspects and a sensitivity analysis must be carried out before conclusions can be drawn. Key aspects of sustainability are shown in Table 2. It is important to remember that sustainability assessment is an evolving field and it is unlikely that it can fulfil all requirements from all stakeholders everywhere, as desirable as that might be.

These sustainability aspects are frequently the basis for the development of standards and certification schemes outlined in the next sections.

4 Standards and Certification Systems

Standards are usually a set of principles, criteria and/or indicators that serve as a basis for monitoring and reporting or as reference for assessment. A principle is a fundamental rule; criteria are to be understood as states or requirements linked with the principle, whereas indicators are qualitative or quantitative parameters which are assessed in relation to a criterion. The use of principles, criteria and indicators is the primary method for evaluating performance and compliance with certification schemes. Certification is regarded to be necessary and valuable, leading to the emergence of a number of schemes over the last decade and the acceptance of voluntary schemes to assess compliance with legislation, such as the EU Renewable Energy Directive (RED).

Many countries have set greenhouse gas reduction targets and that has accelerated the development of biofuel production technologies. To evaluate whether biofuels application results in GHG reduction, various assessment frameworks have been developed (Cramer, RFT, etc.) [22]. Moreover, setting GHG reduction targets has also fostered the development of tools (GREET,[3] BIOGRACE,[4] $EnZO_2$,[5] etc.) for the calculation of GHG savings from biofuels. The RED requires that sustainability criteria are fulfilled for biofuels production in order to be counted

[3]https://greet.es.anl.gov/.

[4]http://www.biograce.net/.

[5]https://www.ifeu.de/english/index.php?bereich=nac&seite=ENZO2.

towards the blending obligations [23]. Sustainability criteria for biofuels are defined in the RED and GHG emission savings from biofuels are one core part of these criteria.

The GHG savings is the only quantitative indicator in that legislation. The directive also defines a formula to calculate GHG emission savings. This formula incorporates all life cycle stages from cultivation of the biomass, its harvesting and processing to transport and to storage of the final product. Under the RED, use of this formula when calculating GHG emission savings from biofuels is mandatory. There is no similar GHG requirement for bio-based products and these products are not even considered as GHG saving option in the policy strategies, although they seem to provide more added value in the long term. Additionally, they are more sustainable than the production of bioenergy and biofuels alone. There are various strategies, but there are no distinct policy drivers for the utilization of bio-based chemicals, in direct contrast to the biofuels industry where various national regulations are driving rapid growth [24]. However, increasing production of bio-based chemicals and materials requires changes in the supply chain infrastructure and may also need more efficient socioeconomic and policy frameworks [25], for example an expanded policy that includes bio-based products provides added flexibility without compromising GHG targets [26].

The end-use of biomass from forestry or agriculture is frequently not known as it depends on the market conditions at the point of harvest. To avoid overwhelming bureaucracy and an undue burden to biomass users, a harmonised approach for all biomass utilisation paths is considered to be the best solution. It is equally important to engage all stakeholders across sectors and locations with the purpose of finding common ground where possible, and increasing trust between stakeholders. Unfortunately this has not been achieved so far.

In order to evaluate whether biofuels meet sustainability requirements, numerous biomass biofuel sustainability standards and certification schemes have been developed or implemented by private and public organisations. Schemes are applicable to different feedstock production sectors (forests, agricultural crops), different bioenergy products (wood chips, pellets, ethanol, biodiesel, electricity) and whole or segments of supply chains [27, 28].

Table 3 provides an overview of sustainability indicators used in different schemes for the sustainability assessment of sustainable grown biomass (INRO), biofuels (RedCert) and bio-based materials (ISCC) and also national environmental performance (GBEP) sustainability assessments having goals other than certification, which may use a limited, modified or extended set of indicators.

In general, the selection of suitable indicators that serve the purpose of the assessment is context-specific. Therefore, use of a flexible and modular approach is recommended that can be adapted to various purposes. A conceptual model for the selection of sustainability indicators is shown in Fig. 3.

Indicators for sustainability assessment must encompass three dimensions. Indicators can be quantitative, qualitative or binary, and should address the most pressing issues shown in Table 2. Some criteria and indicators are mandatory, whereas others are only relevant in a specific context. In the first instance, the set

Table 3 Overview of criteria used in of selected standards/certification systems (INRO[a], modified)

	INRO	ISCC Plus	RedCert	GBEP
Ecological criteria				
Protection of natural biotopes (no go areas)	V	V	V	V
Wooded areas	V	V	V	
Areas reserved for nature conservation purposes	V	V	V	
Greenlands with a high degree of biodiversity	V	V	V	
Wetlands	V	V	V	
Peat bogs	V	V	V	
Inclusion of all cultivated areas of an agricultural holding		V		
Sustainable cultivation			V	
Ecological impact assessment and integration of stake-holders in the planning process		V	V	
Soil protection	V	V	V	V
Avoiding soil erosion	V	V	V	
Soil quality: preservation of soil structures and organic matter	V	V	V	
Consideration of crop rotation	V	V	V	
Water protection	V	V	V	V
Ensuring water quality	V	V	V	V
Avoiding contaminant input into rivers and groundwater	V	V	V	
Efficient irrigation and controlled water consumption	V	V	V	
Preservation of natural river courses	V	V	V	
Environmentally compatible use of fertilisers and pesticides	V	V	V	
Controlled use of fertilisers and pesticides (according to demand)	V	V	V	
Environmentally sound storage of chemicals	V	V	V	
Exclusion of internationally prohibited chemicals and agrochemicals	V	V	V	
Sound handling of chemicals and sound disposal of chemicals containers	V	V	V	
Documentation of chemicals use	V	V	V	
Waste management	V	V	Indirect	
Using by-products	V	V	Indirect	
Consideration of waste prevention measures and recycling	V	V		
Environmentally sound waste storage	V	V	V	
Conservation of biodiversity		V	V	V
Greenhouse gas emissions measured in unit: x kg CO_2 eq/kg	V	V	V	
Defining reduction targets				
Social criteria	V	V	V	V
Compliance with the criteria of the ILO core working standards at the operative level	V	V	V	

(continued)

Table 3 (continued)

	INRO	ISCC Plus	RedCert	GBEP
Freedom of association and right to collective bargaining	V	V	V	
No forced labour	V	V	V	
No child labour	V	V	V	
No discrimination	V	V	V	
Further social criteria			V	
Availability of accommodation		V		
Access to drinking water		V		
Safe working conditions		V	V	
Protective clothing		V	V	
Trainings and further education		V		
Adequate remuneration and working contracts		V		V
Backing in case of illness		V		
Possibilities to lodge complaints on the operative level		V		
Possibilities for children to attend primary school		V		
Time recording, recording of overtime, breaks, holidays		V		
Respecting land use rights	V			V
Proof of land use right through producer	V	V		
Safeguarding traditional land use rights	V	V		
Fair contracts with farms and agricultural holdings	V	V		
Backing in case of illness	V			
Cultivation of biomass is not harmful to local food production	V	V		
Economic criteria		V		
Good management practise		V		
Registration of cultivation areas in use		V		
Involvement of subcontractors		V		
Reporting of economic/management indicators		V		
Business habits		V		
CR guidelines for business relations		V		
Anti-corruption and bribery measures		V		
Transparency of payment flows		V		V

[a] http://inro-biomasse.de/en.htm

of criteria and indicators should address the core issues. To be context-specific requires prioritisation and hierarchies that have to be defined to guide the selection of indicator for sustainability assessment. For simple questions, a full sustainability assessment may not be necessary and a streamlined approach using a limited number of indicators is sufficient. The selection of indicators for the assessment should be based on relevance and significance for the question in hand. As a first step, the criteria and indicators for biofuels can be used as the basis for the sustainability evaluation of other bio-based products, as shown in Table 3.

Fig. 3 Conceptual model of indicator selection for sustainability assessment

If the purpose of the assessment is the comparison of two options (technologies or products), all relevant indicators for each option should be considered. The indicators for the comparison should be derived using a consistent approach, consistent system boundaries and relevant data.

However, the sustainability assessment of bio-based chemicals and materials is far more complex than the assessment of biofuels, as explained in the next section [29].

5 Evaluation of Biofuels and Other Bio-Based Products

First generation biofuels are bio-based products. However, they are different from other bio-based products such as bio-based chemicals and materials in many respects as they have a single use phase and no end-of-life option, they are not re-usable or recyclable and, at least as transport fuel, their combustion products cannot be captured. From biomass feedstock, biofuels are converted in a limited number of processes, blended and "ready for use" [8]. Even food and feed products are more complex than biofuels because the associated organic waste or manure can be used as feedstock for biogas or compost production, or organic fertiliser.

For the sustainability assessment of other bio-based products the cultivation of biomass and, in the case of second generation biofuels transport, pre-treatment and processing of biofuels are common. The production of bio-based products is more complex than the production of biofuels. It can involve several processing steps; intermediates can be traded and blended with fossil-based intermediates, and not all processing steps have to occur in the same country. In a circular economy, bio-based products can be reused, recycled, down-cycled and go through different end-of-life options such as conversion to energy or disposal. The latter can differ

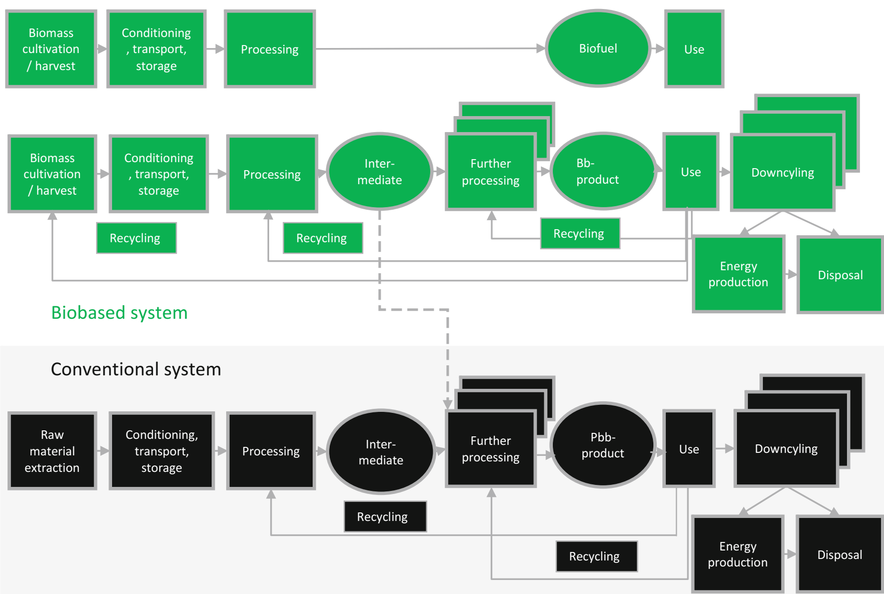

Fig. 4 Comparison of the life cycle of bioenergy, bio-based (Bb) or partly bio-based (Pbb) chemicals/materials

from country to country. Figure 4 illustrates the difference between biofuels and bio-based chemicals and materials.

Biomass provision and early processing steps of bio-based products are equal to those for biofuels, although the processing usually involves more steps and life cycle stages.

The cultivation and provision of biomass is paramount to the analysis and assessment of bio-based products. Cereal crops, oilseeds and sugar beet are grown primarily for utilisation as a food or feedstuff. The use of fertilisers and pesticides is required to achieve the highest possible yield per area, but their applications have been connected to undesirable effects on the environment. During cultivation, lignocellulosic agricultural by-products occur that are not suitable for food production and can be used as feedstock in biorefineries.

The production of crops causes a higher environmental impact per unit area than the production of timber; however, agricultural products can be more easily converted into value added products [30]. Agricultural products are either used directly (e.g. sugar or vegetable oil) or require only minimal conversion (e.g. starch), whereas the conversion of lignocellulosic feedstock requires pre-treatment, which can be either energy or chemical intensive. Although the production of bio-based products from lignocellulosic feedstock is desirable, the conversion technologies have not yet proven to be economically competitive.

6 Land Use

Land use change (LUC) is potentially the most controversial issue associated with the production of bio-based materials [31–33]. LUC has raised considerable concerns as it is frequently correlated with deforestation, animal extinction and peatland destruction [34]. Direct LUC entails conversion of existing land from a current use to the cultivation of agricultural biomass. Feedstock production for a bio-based industry can cause LUC on a global scale [35]. Indirect land use change (iLUC) is associated with the displacement of food production by bioenergy crops, and may cancel or exceed GHG emission mitigation achieved via fossil energy substitution [36].

It is therefore important that potential iLUC effects are accounted for in sustainability assessment. However, tracing such indirect consequences and attributing responsibility through proven causal links is challenging. Indirect LUC emissions are the component with the highest level of uncertainty. Despite this uncertainty, indirect LUC can be of importance when making strategic decisions. If sustainable biomass production for bioenergy or bio-based chemicals replaces intensive agriculture, the effects can range from neutral to positive. Appropriate management of cultivation could improve soil quality and positively affect habitats and the biodiversity of current arable land; reduced tillage, leaving crop residues in place and increasing external input of organic matter can contribute to carbon stock improvement. The impact of LUC depends on factors such as the cultivation methods, type of soil and climatic conditions, and may be positive or negative. LUC can be the most important factor for GHG emissions when it occurs [37].

7 Food Security

According to the FAO "the right to food" voluntary guideline, state governments have obligations to ensure availability of and access to adequate food. Governments should respect existing access to adequate food by not enacting measures that hamper food access, and should protect the right to adequate food by ensuring that enterprises and individuals do not deprive individuals of their access to adequate food. Unfortunately, because not all governments fulfil these obligations, the use of food biomass for other purposes is sometimes blamed for food insecurity [38]. Frequently, food is available but is too costly because of the lack of purchasing power of individuals or poor families. Whether that is regarded as unfair is an individual choice, but the food security issue puts responsibility onto governments and large companies to include this aspect in their decisions. This is particularly relevant for investment decisions and feedstock acquisition.

8 Processing Biomass

The production of bio-based products usually consists of more processing steps than the production of biofuels. However, process data are available to plant operators, although data from novel, not fully optimised processes have high uncertainty. One challenge is when process steps provide more than one product, and impacts (environmental, social and economic) have to be allocated between product and by-products. There are various options to conduct the allocation described in ISO 14040, but the choice for a certain allocation procedure influences the results. Another challenge is to gather or model relevant mass and energy flows in a consistent manner and communicate these data along the supply chain. This is necessary to assess the sustainability implications of the entire production process. There is no one-fits-all approach; it depends on the context in which results of sustainability assessment are used. A trade-off exists between accuracy and comparability of results. The latter requires a harmonised approach.

9 Life Cycle of Products: Time, Consumer Behaviour and Waste Management Infrastructure

In a circular economy, bio-based products can be reused, recycled, down-cycled and have different end-of-life options such as conversion to energy or disposal. The latter can differ from country to country. At the time of production it is not known how a product is used or whether and where it is recycled, down-cycled or disposed. Moreover, neither the technological efficiency at the end of the first use phase (i.e. if recycled or down-cycled) nor the waste management structure at the location, where the product is disposed are known. An additional complexity occurs because of different consumer behaviours. Consumers can collect and dispose used products in a responsible or irresponsible manner. The latter is particular relevant for the possible GHG reduction bio-based products can have. An additional complication arises for bio-based products with an extended service life, because future technology improvements are not known at the time of the assessment.

One possibility to confront those problems is to define scenarios in a modular manner, where the influence of different consumer behaviours, reuse/recycling/down-cycling options and future technologies as well as the waste management infrastructure is defined.

10 Interpreting Results

Given the multitude of criteria and indicators in the sustainability assessment, conflicting results between indicators can occur: When trade-offs arise, it is necessary to prioritise between indicators in a systematic manner to produce meaningful results that support decision makers. Where trade-offs are inevitable, this requires careful reflection on the implications for meeting intra- and inter-generational needs.

Priority needs are often context specific. For example, whereas basic physical needs are, to a large extent, already met for most European citizens; this is not the case in some developing countries. Thus, developmental goals can focus on other non-material needs, not the case in certain developing country contexts [21].

11 Conclusions

Drastically reducing our reliance on fossil resources requires a bio-based economy, and this can only be achieved if biorefineries and bio-based products play a key role in the world market. However, it is important to assess the implications of the transition to a bio-based economy. Life cycle-based sustainability assessment is considered to be the most suitable approach to quantify impacts and identify trade-offs.

The sustainability assessment of bio-based products plays a key role in process development, investment decisions, societal acceptance and funding policies. Several approaches for sustainability assessment of bio-based products have been suggested in recent years [9, 19, 39–41]. These approaches have considered a broad spectrum of relevant sustainability issues to provide decision support for future sustainable bio-based products. Individual approaches have strengths and weaknesses, but have one thing in common; results are rarely directly comparable.

Consistency and transparency are key requirements for communicating sustainability results to stakeholders and the public. Deploying biorefineries requires large financial investments and investors need long-term business perspectives, which are increasingly influenced by sustainability-related legislation and public perception.

A conceptual model is provided that allows for selecting suitable sustainability indicators based on relevance and significance in a given context. For example, using organic waste and lignocellulosic biomass does not pose a risk for food security and consequently food security indicators are neither relevant nor significant for the assessment. In contrast, if food biomass is used for the production of bio-based products, then the impact on food security has to be part of the assessment particularly because it is a crucial issue to public perception. Problems such as food security are in the domain of governments but, when governments fail, this problem is nowadays considered to be the responsibility of companies.

Sustainability assessment does not provide simple answers to complex questions. In-depth sustainability assessment requires substantial resources and the results are not easy to interpret. Decision makers are often reluctant to discuss the outcome of sustainability assessment because the results are not their primary concern and are outside their fields of knowledge. Both often hamper companies to conduct such a kind of assessment. However, there is a risk that important problems are not appropriately identified. Problems may arise at a later point, when solving them might be costly and time-consuming.

Hence, a harmonised globally agreed approach would be the best solution to secure sustainable biomass/biofuels/bio-based chemicals production and trade, and avoid indirect effects (e.g. iLUC). However, there is still a long way to go.

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