Chapter 5
Technological Aspects of Lignocellulose Conversion into Biofuels: Key Challenges and Practical Solutions

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Abstract Biofuels produced from crops have been the driving force in renewable energies since many years. In the first decade of the 21st century, there was a major focus on the debate of food versus fuel. Reports made by various national and international agencies, concluded that the food commodity prices were being impacted by consumption for the production of biofuels. Lignocellulosic biomass is an attractive renewable resource for future fuel. Efficiently and cost-effectively production of bioethanol from various lignocellulosic biomass, not only depends on the development of a suitable pretreatment system but also on other technological aspects with engineered feedstock. The aim of this chapter is to summerize and critically review on existing pretreatment method which is highly efficient due to engineering the feedstock as well as effectively using biocatalytic hydrolysis of various lignocellulosic biomass materials. The success behind this lignocellulosic bioethanol is depend on the modern technological development of pretreatment technologies as well as advanced conversion processes within the line of process intensification strategies.

keywords Lignocellulose · Biofuels · Fermentation · Heterogeneous catalysis Engineered biomass · Biorefinery

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5.1 Lignocellulose Biomass Recalcitrance: Physico-Chemical Characteristics of the Plant Cell Wall

The lignocellulosic biomass is characterized by a natural resistance of the plant cell wall to microbial and enzymatic degradation, due to its rigid and compact structure, known as “biomass recalcitrance” (Himmel et al. 2007). This property is closely related to the chemical and physical features of the plant cell wall, which is a matrix of cross-linked polysaccharide networks, glycosylated proteins, and lignin. Several aspects contribute in building the lignocellulose’s recalcitrance, such as epidermal tissue and chemicals, chemical compositions, physical structure of the cell wall, cellulose structure, and pre-treatment-induced causes (Zhao et al. 2012). In particular, Himmel et al. have provided a list of the natural factors supposed to play a part in constructing the recalcitrance of lignocellulosic feedstocks to chemicals or enzymes, that includes: (i) the epidermal tissue of the plant body, particularly the cuticle and epicuticular waxes; (ii) the arrangement and density of the vascular bundles; (iii) the relative amount of sclerenchymatous (thick wall) tissue; (iv) the degree of lignification; (v) the structural heterogeneity and complexity of cell-wall constituents such as microfibrils and matrix polymers; (vi) the challenges for enzymes acting on an insoluble substrate; and (vii) the inhibitors to subsequent fermentations that exist naturally in cell walls or are generated during conversion processes (Himmel et al. 2007). These chemical and structural characteristics affect liquid penetration and/or enzymes accessibility and activity, resulting in increased conversion costs.

Lignocellulosic biomass is mainly composed of three polymers: cellulose, hemicellulose and lignin along with smaller amounts of pectin, protein, extractives and ash (Bajpai 2016), which do not participate significantly in forming the structure of the material (Harmsen et al. 2010).

Depending on the type of biomass, these polymers are organized in complex non-uniform three-dimensional structures to different degrees and varying relative compositions, as illustrated in Table 5.1, for various lignocellulosic feedstocks.

As can be seen from Table 5.1 cellulose is the major structural component of cell walls, and it provides mechanical strength and chemical stability to plants. Hemicellulose is a copolymer of different C5 and C6 sugars. Lignin is a polymer of aromatic compounds produced through a biosynthetic process that forms a protective layer for the plant walls (Harmsen et al. 2010). Their internal structures will be described in detail in the follwing paragraphs. From a structural point of view, the plant cell wall is a complex matrix typically composed of three types of layers, namely the middle lamella, the primary and the secondary wall (Fig. 5.1), that provide support and strength essential for plant cell survival. The main functions of the cell wall include the conferral of resistance, rigidity and protection to the cell against different biotic or abiotic stresses, but still allowing nutrients, gases and various intercellular signals to reach the plasma membrane (Ochoa-Villarreal et al. 2012).

Primary and secondary cell walls are microfibril-based nanocomposites that differ in the arrangement, mobility and structure of matrix polymers, the higher-order organization of microfibrils into bundles and discrete lamellae, their rheological and

Table 5.1 Composition of representative lignocellulosic feedstocks (Menon and Rao 2012)

Feedstocks	Carbohydrate composition (% dry wt)		
	Cellulose	Hemicellulose	Lignin
Barley hull	34	36	19
Barley straw	36–43	24–33	6.3–9.8
Bamboo	49–50	18–20	23
Banana waste	13	15	14
Corn cob	32.3–45.6	39.8	6.7–13.9
Corn stover	35.1–39.5	20.7–24.6	11.0–19.1
Cotton	85–95	5–15	0
Cotton stalk	31	11	30
Coffee pulp	33.7–36.9	44.2–47.5	15.6–19.1
Douglas fir	35–48	20–22	15–21
Hardwood stems	40–55	24–40	18–25
Rice straw	29.2–34.7	23–25.9	17–19
Rice husk	28.7–35.6	11.96–29.3	15.4–20
Wheat straw	35–39	22–30	12–16
Wheat bran	10.5–14.8	35.5–39.2	8.3–12.5
Grasses	25–40	25–50	10–30
Newspaper	40–55	24–39	18–30
Sugarcane bagasse	25–45	28–32	15–25
Sugarcane tops	35	32	14
Pine	42–49	13–25	23–29
Poplar wood	45–51	25–28	10–21
Olive tree biomass	25.2	15.8	19.1
Jute fibres	45–53	18–21	21–26
Switchgrass	35–40	25–30	15–20
Grasses	25–40	25–50	10–30
Winter rye	29–30	22–26	16.1
Oilseed rape	27.3	20.5	14.2
Softwood stem	45–50	24–40	18–25
Oat straw	31–35	20–26	10–15
Nut shells	25–30	22–28	30–40
Sorghum straw	32–35	24–27	15–21
Tamarind kernel powder	10–15	55–65	–
Water hyacinth	18.2–22.1	48.7–50.1	3.5–5.4

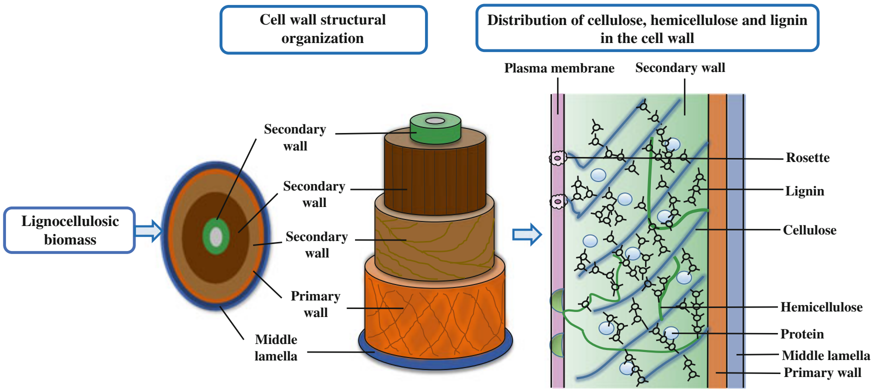


Fig. 5.1 Cell wall structure

mechanical properties, and their roles in the life of the plant (Cosgrove 2012). In the primary wall, the basic structure is a skeleton of cellulose cross-linked with glycans; according to the cross-link types present, there are two types of primary walls: (i) Type I walls that are found in dicotyledonous plants and consist of equal amounts of glucan and xyloglucan embedded in a matrix of pectin; (ii) Type II walls, present in cereals and other grasses, having glucuronoarabinoxylans as their cross-linking glucans, but lacking of pectin and structural proteins (Zhao et al. 2012). The secondary wall usually consists of three sub-layers, which are termed as S1 (outer), S2 (middle), and S3 (inner) lamellae, respectively. The cellulose microfibrils of secondary wall are embedded in lignin, being like steel rods embedded in concrete, but with less rigidity. Cellulose, hemicellulose, and lignin have different distribution in these layers. In wood fibers, it has been found that cellulose concentration is increased from middle lamella to the secondary wall. S2 and S3 lamellaes have the highest cellulose concentration. Hemicellulose has a similar tendency of distribution in the cell wall to cellulose, and most of the hemicellulose distributes in the secondary wall. Lignin is found to be the dominant composition in the outer portion of the compound middle lamellae. The percentage of lignin in the lignocellulosic matrix decreases with increasing distance into the middle lamella; that means that the percentages of lignin in the primary wall and in the S1 layer of the secondary wall are much higher than those in the S2 and S3 sections (Zhao et al. 2012).

5.1.1 Cellulose

Cellulose is a linear homopolymer composed of D-glucopyranose units linked by β-1,4-glycosidic bonds. The chemical formula of cellulose is $(C_6H_{10}O_5)n$; n, called the degree of polymerization (DP), represents the number of glucose groups, ranging from hundreds to thousands or even tens of thousands. In the twentieth century, it

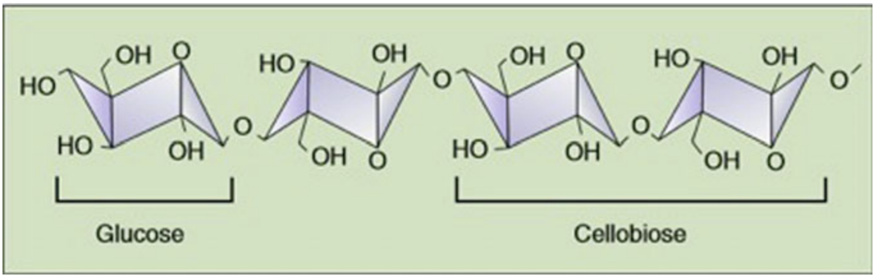


Fig. 5.2 Molecular chain structure of cellulose

was proved that cellulose consists of pure dehydrated repeating units of D-glucoses (as shown in Fig. 5.2), and the repeating unit of the cellulose is called cellobiose (Chen 2014).

The cellulose chains (20–300) are grouped together to form microfibrils, which are bundled together to form cellulose fibers. The long-chain cellulose polymers are linked together by hydrogen and van der Waals bonds, which cause the cellulose to be packed into microfibrils, that in most conditions, are covered by hemicellulose (dry matter accounting for 20–35%) and lignin (dry matter accounting for 5–30%) (Bajpai 2016). Natural cellulose has 10,000 glucose units and the fibril contains approximately 60–80 cellulose molecules. It is insoluble in water, dilute acidic solutions, and dilute alkaline solutions at normal temperatures and is found in both the crystalline and the non-crystalline structure (Harmsen et al. 2010). Indeed, study of the supramolecular structure of natural cellulose showed that the crystalline and non-crystalline phases intertwine to form the cellulose. The noncrystalline phase assumes an amorphous state when tested by X-ray diffraction because most hydroxyl groups on glucose are amorphous. However, large amounts of hydroxyl groups in the crystalline phase form many hydrogen bonds, and these hydrogen bonds construct a huge network that directly contributes the compact crystal structure.

5.1.2 *Hemicellulose*

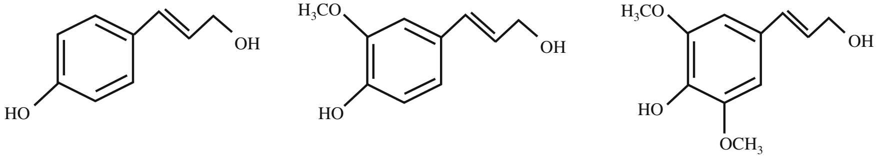
The term hemicellulose is used to represent a family of polysaccharides such as arabino-xylans, gluco-mannans, galactans, etc. that are present in both the primary and the secondary cell walls, and in a small amount also the middle lamella region. They have different composition and structure depending on their source and the extraction method. The most common type of polymers that belongs to the hemicellulose family of polysaccharides is xylan (Harmsen et al. 2010). Xylans are a diverse group of polysaccharides with the common backbone of β-(1,4)- linked xylose residues, with side chains of α-(1,2) linked glucuronic acid and 4-O-methyl glucuronic acid residues. Composition and distribution of the substitutions is wide variable according to the plant cell species. Xylans usually contain many arabinose residues attached to the backbone which are known as arabinoxylans and glucuronoarabinoxylans (Ochoa-Villarreal et al. 2012). The β-(1,4)-linked polysaccharides rich in mannose or with mannose and glucose in a nonrepeating pattern are the glucomannans and galactoglucomannans. Hemicellulose extracted from plants possesses a high degree of polydispersity, polydiversity and polymolecularity (a broad range of size, shape and mass characteristics). However, the degree of polymerization does not exceed the 200 units whereas the minimum limit can be around 150 monomers (Harmsen et al. 2010). Hemicellulose is insoluble in water at low temperature. However, its hydrolysis starts at a temperature lower than that of cellulose, which renders it soluble at elevated temperatures.

5.1.3 Lignin

Lignin is the most complex natural polymer. It is present in the primary cell wall and functions as the cellular glue which provides compressive strength to the plant tissue and the individual fibres, stiffness to the cell wall and resistance against insects and pathogens (Isikgor and Remzi 2015). It is an amorphous three-dimensional polymer with phenylpropane units nonlinearly and randomly linked as the predominant building blocks; the most commonly monomers encountered are p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol (Fig. 5.3).

The coupling modes between each basic unit include “β-O-4, “β-5, “β-1, and so on. Ether bonds in lignin include phenol-ether bonds, alkyl-ether bonds, dialkyl bonds, diaryl ether bonds, and so on. About two thirds to three quarter phenylpropane units of lignin are linked to the adjacent structural units by ether bonds; only a small part is present in the form of free phenolic hydroxyl (Chen 2014).

Lignin is synthesized by polymerization of these components and their ratio varies between different plants, wood tissues and cell wall layers. Dividing higher plants into two categories, hardwood (angiosperm) and softwood (gymnosperm), it has been identified that lignin from softwood is made up of more than 90% of coniferyl alcohol with the remaining being mainly p-coumaryl alcohol units. Contrary to softwoods,



(a) p-coumaryl alcohol (b) Coniferyl alcohol (c) Sinapyl alcohol

Fig. 5.3 Basic structural units of lignin (Srndovic 2011) lignin contained in hardwood is made up of varying ratios of coniferyl and sinapyl alcohol type of units (Harmsen et al. 2010).

5.2 Chemical Interaction Between Components

The types of bonds identified within the lignocellulosic structure are four: ether, ester, carbon-to-carbon and hydrogen bonds. They can be divided into intrapolymer and interpolymer linkages (Table 5.2); the former refer to linkages within the individual components of the lignocellulose, while the latter to the connections among the different components to form complexes.

As shown in Table 5.2 the bonds types linking the molecules in the structure of the lignin are ether bonds and carbon-to-carbon bonds; the ether bonds may occur between allylic and aryl carbon atoms, or between aryl and aryl carbon atoms, or even between two allylic carbon atoms. The total fraction of ether type bonds in the lignin molecule is around 70% of the total bonds between the monomer units. The carbon-to-carbon linkages form the remaining 30% of the total bonds between the units. They can also appear between two aryl carbon atoms or two allylic carbon atoms or between one aryl and one allylic carbon atom (Harmsen et al. 2010). In the cellulose's polymer, the glucose units are connected together by a 1-4 β D-glucosidic bond, that can be considered as an ether bond, since it is in fact the connection of two carbon atoms with an elementary oxygen interfering. The other main type of bond present in the cellulose is the hydrogen bond that is responsible for its crystalline fibrous structure. In fact, every glucosyl ring of cellulose has three active hydroxyls: one primary hydroxyl group and two secondary hydroxyl groups. Thus, cellulose may have a series of chemical reactions related to hydroxyl. However, these hydroxyl groups also can form hydrogen bonds between molecules, which has a pro-

Table 5.2 Different types of bonds identified in the lignocellulose (Harmsen et al. 2010)

Bonds with different components (intrapolymer linkages)	
Ether bond	Lignin, (hemi)cellulose
Carbon to carbon	Lignin
Hydrogen bond	Cellulose
Ester bond	Hemicellulose
Bonds connecting different components (interpolymer linkages)	
Ether bond	Cellulose-lignin Hemicellulose lignin
Ester bond	Hemicellulose-lignin
Hydrogen bond	Cellulose-hemicellulose Hemicellulose-lignin Cellulose-lignin

found influence on the morphology and reactivity of cellulose chains, especially the intermolecular hydrogen bond formed by oxhydryl at C3 and oxygen at an adjacent molecule ring. These hydrogen bonds not only can enforce the linear integrity and rigidity of the cellulose molecule but also make molecule chains range closely to form a highly ordered crystalline region. The accessibility of cellulose refers to the difficulty of the reagents to arrive at the cellulose hydroxyl. In heterogeneous reactions, the accessibility is mainly affected by the ratio of the cellulose crystalline regions to the amorphous regions. The reactivity of cellulose is the reactive capability of the primary hydroxyl and the secondary hydroxyl at the cellulose ring. Generally, because of the smallest steric hindrance, the reactivity of the primary hydroxyl groups is higher than for the secondary hydroxyl groups, so the reactivity of hydroxyl at C6 with a bulky substituting group is higher (Chen 2014). In addition, it was noted that, carboxyl groups are also present in cellulose in a fraction of 1 carboxyl per 100 or 1000 monomer units of glucose. With respect to the structure of the hemicellulose, it can be stated that its molecule is formed mainly by the ether type bonds, such as the fructosic and glucosidic one. The main difference with cellulose is that the hydrogen bonds are absent and that there is significant amount of carboxyl groups. The carboxyl groups can be present as carboxyl or as esters or even as salts in the molecule (Harmsen et al. 2010).

The interpolymer linkages, namely those connecting the different polymers of the lignocellulose complex, can be determined by breaking down the lignocellulose and separating the individual components. Their separation is commonly achieved by methods that cause the alteration of their original structure. Therefore, the results obtained about the connecting linkages between the polymers are not definite. However, it has been identified that there are hydrogen bonds connecting lignin with cellulose and with hemicellulose, respectively and the existence of covalent bonds between lignin and polysaccharides. In particular, it is known that hemicellulose connects to lignin via ester bonds and that there are ether bonds between lignin and the polysaccharides. It is still not clear though whether the ether bonds are formed between lignin and cellulose, or hemicellulose (Harmsen et al. 2010).

5.3 Lignocelluloses Feedstock Biorefinery

Biorefinery represents the sustainable processing of biomass into a spectrum of marketable products and energy, as defined by International Energy Agency (IEA) Bioenergy Task 42 (Van Ree and Van Zeeland 2014; Morais and Bogel-Lukasik 2013). In biorefinery, all the types of biomass feedstocks can be exploited, including products, byproducts, residues and waste provided from different sectors: forestry (wood, logging residues, trees, shrubs and wood residues, sawdust, bark, etc.), agriculture (dedicated crops and residues), aquaculture (algae and seaweeds), industries (process residues and leftovers) and households (municipal solid waste and wastewaters) (De Jong and Jungmeier 2015). The biomass feedstock can be converted into different classes of bio-products, via combinations of different technologies, including mechanical/physical, (bio)chemical and thermochemical processes (de Wild 2015).

Among the possible biomass raw materials, the lignocellulose is one of the most promising feedstock for biorefineries, as the availability of the input material is relatively high and input material prices are low (Uihlein and Schebek 2009). The input material, used in lignocelluloses feedstock (LCF) biorefinery, can be obtained from: forestry residues and wood waste (including residues from harvest operations left in the forest after stem wood removal: branches, foliage, roots, etc.), agricultural residues (e.g. husks, chaff, cobs, bagasse), energy crops (crops specifically bred and cultivated at low-cost, on marginal land not suitable for food crops production), and municipal paper waste (Demirbas 2009).

The LFC biorefinery is classified as “phase III biorefinery”; three different types of biorefinery, known as phase I, II and III, have been described by Kamm and Kamm, and van Dyne et al. The phase I and II biorefinery use only one feedstock such as corn and wheat. The difference is that phase I biorefinery has the capability to produce a single major product by single process, while phase II biorefineries is capable of producing various end-products and has far more processing flexibility (Van Dyne et al. 1999). In Europe, there are many phase I biorefineries producing biodiesel from vegetable oil (rapeseed oil), through transesterification process. The Novamont plant in Italy is, indeed, an example of a phase II biorefinery that use corn starch to produce several chemical products, such as biodegradable polyesters (Origi-Bi) and starch derived thermoplastics (Master-Bi) (Clark and Deswarte 2015). The phase III biorefineries use various types of feedstocks and processing technologies to produce a variety of products (Van Dyne et al. 1999; Clark and Deswarte 2015). In LCF biorefinery, lignocellulosic feedstocks are fractioned into intermediate outputs (cellulose, hemicellulose and lignin) that are further processed into a multitude of products and bioenergy, (such as biofuels, fine chemicals, advanced polymer materials, steam/heat, and electricity), by jointly applying several technological processes (de Jong and Gosselink 2014). A number of commercial technologies are available today for the pretreatment of lignocelluloses all around the world. Some of these technologies have already been commercialized and are well known, whereas others are still at lab scale. The most relevant commercial technologies are given in following Table 5.3:

The general scheme for lignocellulose bioconversion involves multi-step processes. The first step, following feedstock selection, is the lignocellulosic biomass pretreatment that is a necessary upstream process to reduce the size of biomass and to fractionate, solubilize, hydrolyze and separate cellulose, hemicellulose, and lignin components (Capolupo and Faraco 2016). As described before pretreatments methods can be classified into different categories (Table 5.4): physical, physiochemical, chemical, biological, electrical, or a combination of these (Amelio et al. 2016).

Physical (mechanical) pretreatment increases the surface area by reducing the size of the biomass and improves the flow through biorefinery processes (Arens and Liu 2014). The physico-chemical methods requires high temperature and pressure; it is therefore necessary a high control of operating conditions. Steam explosion is the most commonly physico-chemical method used for pretreatment of lignocellulosic

Table 5.3 Pretreatment technologies commercially available

Process	Company	Characteristic
Steam explosion	Beta Renewables	Low xylose yield
		High enzyme loading
Single-stage dilute acid	Abengoa	High xylose yield
		Moderate enzyme loading
Two-stage dilute acid	Poet-DSM	High xylose yield
		Low enzyme loading
Ammonia & Steam	Dupont	Require high enzyme loading

biomass (Verardi et al. 2016). It combines mechanical forces and chemical effects. The mechanical effects cause separation of lignocellulose matrix in individual fibers (hemicelluloses, cellulose and lignin) with minimal loss of material. The chemical effects promote the hydrolysis of acetyl groups included in hemicellulose (Verardi et al. 2015).

Chemical methods remove and/or dislocate hemicelluloses and lignin, loosening the structural of lignocellulosic matrix (Capolupo and Faraco 2016). Biological pretreatment methods use cellulolytic, hemicellulolytic, and ligninolytic systems synthetized from microorganisms, such as fungi, bacteria, and actinomycetes, in order to degrade lignin, cellulose, and hemicellulose (Sindhu et al. 2016). Electrical method, such as pulsed-electric field (PEF) pretreatment, exposes the cellulose content in the biomass through the formation of pores in the cell membrane, allowing the entry of agents necessary to break the cellulose into constituent sugars (Kumar and Sharma 2017).

Following pretreatment, the biomass components (lignin, cellulose, hemicellulose and residues) are subject to a combination mainly of thermochemical and biochemical processes in order to convert lignocellulosic feedstock into valuable products (FitzPatrick et al. 2010). Thermochemical conversion includes processes as combustion, pyrolysis, gasification, and liquefaction. The combustion process, performed at 800–100 °C, allows to transform biomass into energy by oxidation of carbon and hydrogen-rich biomass to CO_2 and H_2O. This method, used for the production of electricity and heat, is similar to fossil-fuel fired power plants and can produce high NO_x emission.

A variety of value-added chemicals can be obtain from main biomass constituents, hemicellulose, cellulose and lignin, by pyrolysis that consists of a thermal degradation, without oxidizing agent, of solid lignocellulosic biomass into gases and liquids (Table 5.5) This thermal decomposition starts at 350–550 °C and goes up to 700–800 °C (de Wild 2015).

Gasification means the conversion of lignocellulosic biomass into a combustible gas mixture, called producer gas, consisting of carbon monoxide (CO), hydrogen (H_2), and traces of methane (CH_4), at 700–1600 °C. After cleaning, producer gas can be used directly as an engine fuel or upgraded to liquid fuels or converted into chem-

Table 5.4 Methods for lignocellulosic biomass pretreatment

		Operating conditions	Advantages	Disadvantages
Physical	Chipping Grinding Milling	Room temperature Energy input <30Kw per ton biomass	Reduces cellulose critallinity	Power consumption higher than inherent biomass energy
Physio-chemical	Steam pretreatment	160–260 °C (0,69–4,83 MPa) for several second (~15 min in the range 200–230 °C)	Causes hemicellulose auto hydrolysis and lignin transformation; cost-effective for hardwoods and agricultural residues	Destruction of a portion of the xylan fraction; incomplete distruption of the lignin-carboydrate matric; generation of compounds inhibitory; less effective for softwoods
	AFEX (Ammonia fiber explosion method)	90 °C for 30 min. 1–2 kg ammonia/kg dry biomass	Increases accessible surface area, removes lignin and hemicellulose;	Do not modify lignin neither hydrolyzes hemicellulose
	ARP (Ammonia recycle percolation method)	150–170 °C for 14 min Fluid velocity 1 cm/min	Increases accessible surface area, removes lignin and hemicellulose;	Do not modify lignin neither hydrolyzes hemicellulose;
	CO2 explosion	4 kg CO_2/kg fiber at 5.62 MPa 160 bar for 90 min at 50 °C under supercritical carbon dioxide	Do not produce inhibitor for downstream processes. Increases accessible surface area, does not cause formation of inhibitory compounds	It is not suitable for biomass with high lignin content (such as woods and nut shells) Does not modify lignin neither hydrolyze hemicelluloses
	Ozonolysis	Room temperature	Reduce lignin content; does not produce toxic residue	Expensive for the ozone required;

(continued)

Table 5.4 (continued)

		Operating conditions	Advantages	Disadvantages
	Wet oxidation	148–200 °C for 30 min	Efficient removal of lignin; Low formation of inhibitors; low energy demand	High cost of oxygen and alkaline catalyst
Chemical	Acid hydrolysis: dilute-acid pretreatment	Type I: T > 160°, continuous-flow process for low solid loading 5–10%,)-Type II: T < 160 °C, batch process for high solid loadings (10–40%)	Hydrolyzes hemicellulose to xylose and other sugar; alters lignin structure	Equipment corrosion; formation of toxic substances
	Alkaline hydrolysis	low temperature; long time high; concentration of the base; For soybean straw: ammonia liquor (10%) for 24 h at room temperature	removes hemicelluloses and lignin; increases accessible surface area	Residual salts in biomass
	Organosolv	150–200 °C with or without addition of catalysts (oxalic, salicylic, acetylsalicylic acid)	Hydrolyzes lignin and hemicelluloses	High costs due to the solvents recovery
Biological		Several fungi (brown-, white- and soft-rot fungi	Degrades lignin and hemicelluloses; low energy requirements	Slow hydrolysis rates
Electrical	Pulsed electrical field in the range of 5–20 kV/cm,	~2000 pulses of 8 kV/cm	Ambient conditions; disrupts plant cells; simple equipment	Process needs more research

Table 5.5 Main chemicals from lignocellulosic biomass pyrolysis (de Wild 2015)

Biomass constituent (thermal degradation range)	Pyrolysis products
Hemicellulose (150–300 °C)	Acetic acid; Furfural
Cellulose (200–400 °C)	Levoglucosan, Hydroxyacetaldehyde,
Lignin (150–600 °C)	2-Methoxyphenols (e.g. guaiacol), 2,6-Dimethoxyphenols (e.g., syringol), Catechols, Phenol, Alkyl phenols, Methanol,
Whole biomass (100–600 °C)	Extractives (e.g., terpenes), Charcoal, Pyrolysis oil, Gases (e.g., CO, CO_2, CH_4)

ical feedstocks by several methods, as biological fermentation or catalytic upgrading through the Fischer-Tropsch process. Hydrothermal liquefaction is the thermochemical conversion of lignocellulosic biomass into liquid fuels, at 280–370 °C and 10–25 MPa, by processing in a hot, pressurized water environment (Rajvanshi 2014).

Biochemical conversion involves breaking down biomass into sugars, which can then be converted into potential fuel blend stocks and other bioproducts, including renewable gasoline, ethanol and other alcohols, and renewable chemical products, through the use of microorganisms and catalysts.

The most common types of biochemical processes are fermentation and anaerobic digestion (Zhao and Bai 2009). Fermentation is one of the oldest technologies in the world, mainly based on bioethanol synthesis from plant biomass. The fermentation process, in the presence of oxygen, is carried out by microorganisms, including bacteria, yeasts, and fungi. The most commonly used microbe is yeast, mainly *S. cerevisiae* (Zhao and Bai 2009). Several fungal species belonging to genera *Fusarium, Rhizopus* (Hahn-Hägerdal et al. 2007), *Monilia* (Gírio 2010), *Neurospora* (Xiros and Christakopoulos 2009), and *Paecilomyces* (Sommer et al. 2004) were able to ferment monomeric sugars. Bacteria used to produce bio-alcohols (ethanol) from fermentable sugars include *Zymomonas mobilis*, *Bacillus macerans*, *Bacillus polymyxa*, *Klebsiella pneumoniae*, *C. acetobutylicum*, *Aeromonas hydrophila*, *Aerobacter sp.*, *Erwinia sp.*, Leuconostoc sp., and *Lactobacillus sp.* (Thatoi et al. 2014). Another bacterial resource is engineering *E. coli* (Srichuwong et al. 2009). Microbial culture types used in fermentation can be classified into five different categories: pure culture, consisting of only one type of organism developed from a single cell (e.g., *S. cerevisiae*); co-culture, containing growths from two distinct cell types (e.g., Aspergillus niger and *S. cerevisiae*); mixed culture, consisting of more than two organisms (*Paenibacillus sp.* and four strains of *Z. mobilis*); immobilized culture; and a co-immobilized culture made by entrapping microorganisms within a given matrix (Thatoi et al. 2014).

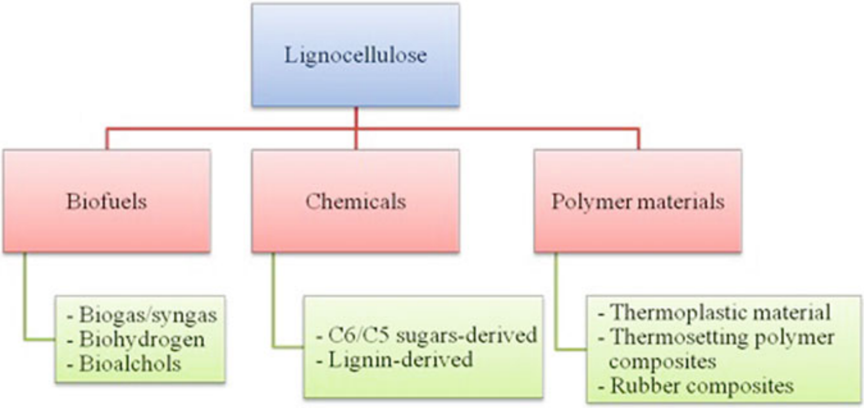


Fig. 5.4 High value bio-products from lignocellulose biomass

Lignocellulosic biomass can be also biochemically degraded by anaerobic digestion. The process is carried out from micro-organisms able to break down organic matter (liquid and solid) in the absence of oxygen and to produce biogas containing mostly methane and carbon dioxide for use as a source of renewable energy. Moreover, anaerobic digestion can be used as an biological pretreatment of lignocellulosic feedstocks, easing the subsequent fractionating of such biomass into its constituent sugars (glucose, galactose, xylose, arabinose, and mannose) and/or short chain fatty acids (acetic, propionic, and butyric acids), which can be further converted into valuable chemicals and biofuels (Surendra et al. 2015).

5.4 High Value Bio-products from Lignocelluloses Feedstock Biorefinery

The products derived from LCF biorefinery, such as biofuels, fine chemicals and advanced polymer materials (Fig. 5.4), might replace petroleum-based products (Cherubini 2010, Cheng and Zhu 2009) The progressive replacement of petroleum refinery oil with lignocellulosic feedstock biorefinery is generally regarded as necessary step for the development of a sustainable industrial society, energy independence, and for the effective management of greenhouse gas emissions (FitzPatrick et al. 2010).

5.5 Biofuels from Lignocelluloses

The conversion of lignocellulose material into several kinds of biofuel, such as biogas/syngas, biohydrogen, and bioalcohols, offers primarily a way to develop renewable and environmental friendly alternatives to substitute fossil fuel with net zero carbon dioxide (CO_2) emission; the CO_2 emitted during fuel combustion is indeed captured during the growth of the feedstock.

Biogas and syngas are complex mixtures composed mainly of methane (CH_4), carbon dioxide (CO_2), hydrogen (H_2), and carbon monoxide (CO) (Awe et al. 2017). The process used to produce the two gasses' mixtures is different: biogas is produced trough anaerobic digestion which involves different groups of facultative or obligatory anaerobic microorganisms (Sárvári Horváth et al. 2016); syngas is created by gasification process that causes the partial combustion of biomass (Samiran et al. 2016).

Biohydrogen production can be obtained with low cost from biomass via hydrolysis and fermentation processes. During combustion process, hydrogen produces only water as its environment-friendly product, receiving widespread attention from researchers in the world (Jiang et al. 2016).

Bio-alcohols, such as bioethanol, biobutanol (or biogasoline), and propanol, can be obtained through the biomass fermentation by the action of aerobic and anaerobic micro-organisms. Today, biological ethanol and butanol are the most commonly produced alcohol fuels: in fact, they can be used directly as substitutes for gasoline, or mixed with gasoline in any ratio (Amelio et al. 2016). The bioconversion process of lignocellulose biomass to ethanol or butanol includes several stages: the pretreatment of feedstock, hydrolysis and fermentation steps, and recovery of products (Verardi et al. 2015). On the contrary, propanol, or isopropyl alcohol, is rarely used as alcohol fuel: it is produced through fermentation of carbohydrates from Escherichia coli to be commonly used as a solvent (Ibrahim 2013).

5.6 Chemicals from Lignocellulose

Besides biofuels production, the lignocellulose biomass holds a great potential for sustainable production of other value-added chemicals. Examples of some chemicals that have been obtained from lignocellulose biomass are given in Fig. 5.5.

The efficient cellulose and hemicellulose depolymerization in hexose (C6) and pentose (C5) sugars is of critical importance for the further development of valuable chemicals. Glucose is the only simple sugar produced by cellulose decomposition. On the other hand, the hemicellulose degradation results in formation of both C6 (glucose, mannose, galactose, and rhamnose), as well as C5 (xylose and arabinose) monosaccharides.

The glucose and xylose can be dehydrated, respectively, into 5-hydroxymethylfurfural (HMF) and furfural (2-furaldehyde), which can further

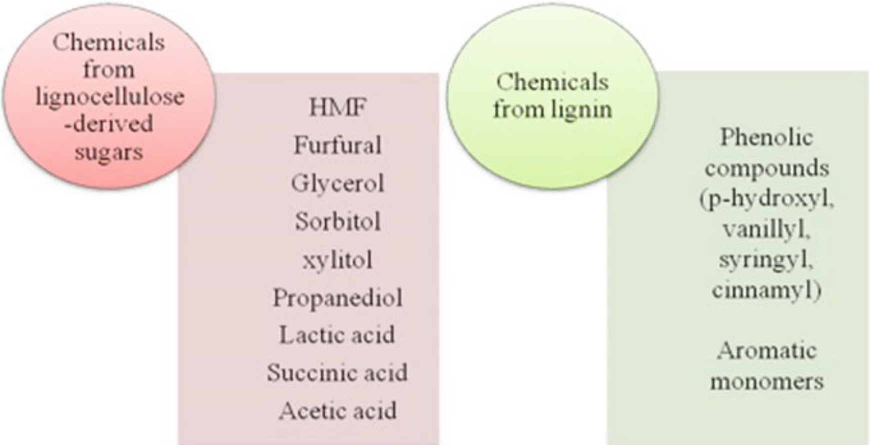


Fig. 5.5 Examples of Chemicals from lignocellulose biomass

be converted into various value-added compounds through feasible chemical transformations (Pereira et al. 2015).

The oxidation of HMF provides an efficient route to synthesis of 2,5-furandicarboxylic acid (FDCA), and 2,5 diformyl-furan (DFF). Currently, FDCA is the most famous HMF derivative: it has attracted much attention recently as potential substitute for terephtalic acid, a petroleum-derived monomer primarily used to produce poly-ethylene-terephthalate (PET) (Han et al. 2017).

Oxidation of HMF to FDCA is a multi-stages process which requires the primary oxidation of HMF to 2,5-diformylfuran (DFF) intermediate, and its sequential oxidation to 5-formyl-2-furancarboxylic acid (FFCA); therefore, FDCA is obtained by further oxidation of FFCA (Zheng et al. 2017).

HMF can also be reduced into 2,5-dihydroxymethylfuran (DHMF), 2,5-dihydroxymethyltetrahydrofuran (DHMTHF), and 2,5-dimethyltetrahydrofuran (DMTHF), or used as intermediate for the production of 2,5-dimethylfuran (DMF), a biofuel with high octane number and energy density that has the potential to replace gasoline directly. DMF is produced by hydrogenation of HMF and subsequent hydrogenolysis.

HMF is very useful also for the production of levulinic acid (LA) by acid rehydration reaction. LA is an important molecule that can be further upgraded in many sectors of industry such as fuel additives, polymer and resin (van Putten et al. 2013).

Furfural can be converted, by hydrogenation, to potential fuel components, such as furfuryl alcohol, 2-methylfuran (MF) and 2-methyltetrahydrofuran (MTHF), and to C4-C5 valuable chemicals, such as valerolactone, pentanediols, cyclopentanone, dicarboxylic acids, butanediol and butyrolactone, by oxidation, hydrogenolysis and decarboxylation processes (Li et al. 2016).

Other chemical compounds, in the form of acids and aldehydes, such as glycerol, sorbitol, xylitol, propanediol, lactic and succinic acid, acetoin or acetic acid, can be produced from lignocellulose biomass-derived sugars (Putro et al. 2016).

The value-added chemicals derived from lignin, via depolymerization or thermal degradation (e.g. oxidation, liquefaction, hydrolysis, hydrocracking, solvolysis and pyrolysis) are phenolic compounds, classified in p-hydroxyl, vanillyl, syringyl and cinnamyl, and aromatic monomers such as benzene, toluene, xylene and hydroxybenzoic acids (Thevenot et al. 2010; Kang et al. 2013; Ma et al. 2015).

5.7 Polymer Materials from Lignocellulose

Lignocellulose biomass can be also used in the preparation of polymer composites materials, using lignin as reinforcement in polymer matrix for making: thermoplastic material, thermosetting polymer composites, and rubber composites (Thakur et al. 2014).

Thermoplastic materials are polymeric materials that can be cooled and heated reversibly without affecting their inherent properties (Wang et al. 2016); several thermoplastic compounds was prepared using lignin as reinforcement, such as: lignin reinforced polystyrene (PS) composites (Barzegari et al. 2012), polydimethylsiloxane-α, ω-diol (PDMS) polymeric matrix-based composites (Thakur et al. 2014), poly(ethylene terephthalate) (PET) matrix-based composites reinforced with lignin (Canetti et al. 2009).

Thermosetting Polymer Composites are polymers that are cured into a solid form and cannot be returned to their original uncured form. Several thermosetting polymer matrix-based composites was prepared using lignin as the reinforcing material, such as: lignin-reinforced epoxy composites (Yin et al. 2012) and lignin-reinforced phenol formaldehyde (PF) polymer composites (Jagur-Grodzinski 2006). Different polymer composite systems were prepared by using rubber as the matrix and lignin as reinforcement, such as: lignin-reinforced styrene-butadiene rubber (SBR)/lignin-LDH (layered double hydroxide) composites (Frigerio et al. 2014), and polymer nanocomposites (Jiang et al. 2013).

Lignin has also been reported to be used as potential reinforcement in foam-based polymer composites, and as a compatibilizer in polymer composites (Thakur et al. 2014).

Finally, the lignin is a promising reinforcement in polymer composites, being biodegradable, CO_2 neutral, abundantly available as industrial waste, low in cost, and environmentally friendly, and having antioxidant, antimicrobial, and stabilizer properties.

5.8 Environmental Impact of Lignocellulose Feedstock Biorefineries

LCF biorefinery should be evaluated for the entire value chain of bio-based products by taking into account environmental, social and economic impacts. For biorefineries, the value chain is classified according to following charcteristics: (i) feedstocks, including production and distribution activities; (ii) conversion processes; (iii) platforms (e.g. intermediate materials used for synthesis of more processed materials and chemicals); and (iv) products obtained after conversion processes from platforms. In particular, LCF biorefieneries may play a major role in reduction of environmental impacts: in tackling climate change by reducing the demand on fossil fuel energy and providing sustainable energy, chemicals and materials. Then, LCF biorefineries are supposed to contribute to a reduction in greenhouse gas. However, biobased products and fuels may also be associated with environmental disadvantages due to, e.g. land use change/intensity or eutrophication of water. These effects also have an impact on biodiversity and ecosystem services. The environmental analysis can be done by life cycle assessment (LCA) methodology which takes into account all the input and output flows occurring along the production chain, from raw material acquisition, to production, use, and end-of-life (Cherubini 2010). This methodology is standardized in the ISO 14040 series by the International Organization of Standardization (ISO) (Mussatto 2016). From various literature data on the environmental impacts of LCF biorefineries, it can be concluded that LCF biorefinery system could be an effective option to mitigate climate change, reduce dependence on fossil fuels and improve cleaner production chains based on local and renewable resources, revitalizing rural areas (Cherubini 2010; Wertz and Bédué 2013; Valdivia et al. 2016; Cheali et al. 2015). The supply of biomass with sustainable practices is a key point to ensure a renewable energy supply to biorefineries. Howevever, an careful environmental evaluation of LCF biorefinery should include several impact categories, for example: the potential consequences due to the competition for food and biomass resources; the impact on use and quality of water; the effects on land use change and soil carbon stocks and fertility of land; the net greenhouse gas balance; impacts on biodiversity and ecosystem services; potential toxicological risks and energy efficiency (De Jong and Jungmeier 2015). Therefore, the determining of all environmental impacts is complex and a certain degree of uncertainty is always present in the final results.

5.9 Conversion Processes

Various conversion processes of lignocelluloses biomass to biofuel are being summarized in following sections giving stress on the engineering of the lignocelluloses materials and mechanism.

5.9.1 First Generation

5.9.1.1 Transesterification

This reaction is used to produce biodiesel or vegetable oil based fatty acid methyl esters (FAME). The product of the reaction is glycerol which is a high value product derived from the oil (Kulkarni et al. 2006; Narwal and Gupta 2013). Transesterification is a reversible reaction and proceeds essentially by mixing the reactant in which the catalyst is a liquid acid or liquid base (called homogeneous catalysis), however in the cases of high free fatty acids (FFA) this process fails that is why solid catalyst is recommended. The reason is that the solid catalysts can simultaneously catalyze the Transesterification of triglycerides and FFA present in biomass to methyl esters (Kulkarni et al. 2006).

5.9.1.2 Ethanol Conversion Process

A wide variety of carbohydrates containing raw materials have been used for production of ethanol by fermentation process. The fermentation process refers to the metabolic conversion of organic substrate by the activity of enzymes secreted by micro-organisms. There are two basic kind of fermentation has been conceptualized, (a) aerobic and (b) anaerobic depending upon oxygen needed in the process or not. There are many micro-organisms capable of providing fermentative changes to both sugars and starches.

5.9.2 Second Generation Biofuel

There are two basic routes for conversion of biomass to liquid biofuels viz. thermo chemical processing and biochemical processing which is described in Fig. 5.6.

- Biochemical—in which enzymes and other micro-organisms are used to convert cellulose and hemicellulose components of the feedstock to sugars prior to their fermentation to produce ethanol;
- Thermochemical—(also known as biomass-to-liquids, BTL), where pyrolysis/gasification technologies produce a synthesis gas ($CO + H_2$) from which a wide range of long carbon chain biofuels, such as synthetic diesel, aviation fuel, or ethanol, can be reformed, based on the Fischer–Tropsch conversion

The clear advantage of thermo-chemical processing is that, it can essentially convert all the organic components of the biomass compared with biochemical processing which focuses mostly on the polysaccharides (Kyung Lee et al. 2015).

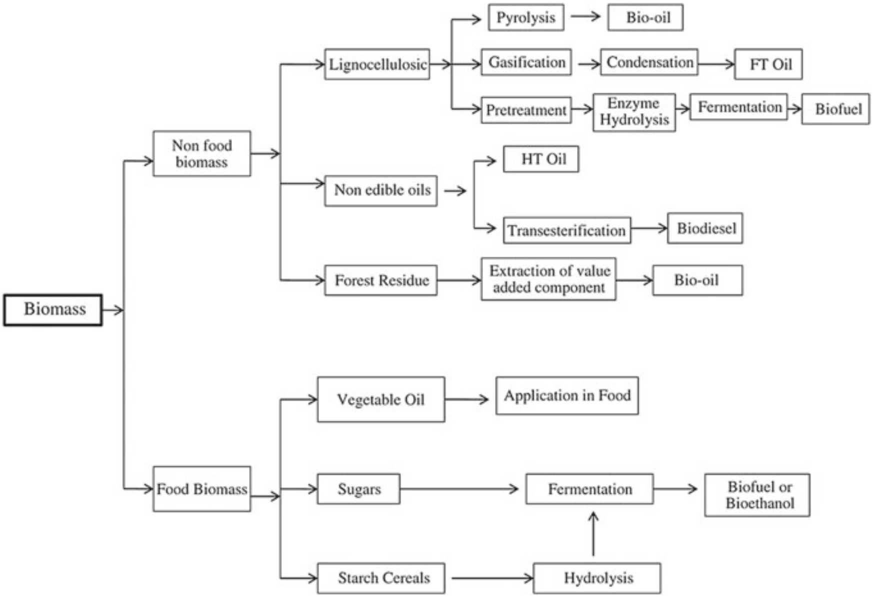


Fig. 5.6 Conversion of biomass to 2nd generation fuels (Chakraborty et al. 2012)

5.9.2.1 Bioethanol from Lignocellulosic Biomass

The ethanol that is produced from lignocelluloses biomass is called bioethanol, which is environmental friendly and renewable (Johnston 2008). It can be used directly in modified spark engines or can be blended with petrol. Ethanol also improves fuel combustion in vehicles hence reduction of emissions. In comparison to petrol ethanol contains only a trace amount of sulphur, so mixing ethanol with petrol helps reduce the sulphur content of fuel, simultaneouly lowering the emission of sulphur oxide which is the major component of acid rain. Sugar and starch can also be fermented to alcohol. This is in-fact the least complex method used in producing ethanol (Kuhad et al. 2011; Chakraborty et al. 2012). With plant biomass it's a different story altogether. Plant biomass consists of cellulose microfibers embedded in hemicelluloses, pectin and lignin. The amount of each component varies among different plant species and parts. Following steps are involved in production of ethanol.

- pretreatment of substrates,
- Saccharification process to release the fermentable sugars from polysaccharides, fermentation of released sugars
- finally distillation step to separate ethanol.

Pretreatment is designed to facilitate in the separation of cellulose, hemicellulose and lignin, so that complex carbohydrate molecules constituting the cellulose and hemicellulose can be broken down by enzyme-catalysed hydrolysis into their constituent simple sugars. The complex structure of cellulose makes it difficult to depolymerise into simple sugars, but once the polymer structure has been broken down, the sugar molecules are simply fermented to ethanol using fermentative microorganisms (Elshaghabee et al. 2016).

Hemicellulose consists of 5-carbon sugars, which although are easily broken down into its constituent sugars such as xylose and pentose, the fermentation process is much more difficult, and requires efficient microorganisms that are able to ferment 5-carbon sugars to ethanol.

Lignin consists of phenols, and for practical purposes is not fermentable, although it can be recovered and utilized as a fuel, providing process heat and electricity for the alcohol (ethanol, butanol) production facility.

The hydrolysis is usually, catalyzed by cellulase enzymes and the fermentation are carried out by yeast or bacteria. The factors that affect the hydrolysis of cellulose include porosity, i.e., accessible surface area of the waste materials, cellulose fiber crystallinity and lignin and hemicellulose content (Kang et al. 2014). The presence of lignin and hemicellulose makes the access of cellulase enzymes to cellulose difficult. The lignin and hemicellulose removal, reduction of cellulose crystallinity and increase of porosity in pretreatment processes can significantly improve the hydrolysis. The cellulose crystallinity can be reduced by a combination of chipping, grinding and milling (Santos et al. 2011). Steam explosion is the most commonly used method for pretreatment of plant biomass (Kang et al. 2014).

Lignin biodegradation could be catalyzed by the peroxidase enzyme with the presence of H_2O_2. Microorganisms such as brown, white and soft rot fungi are used in biological pretreatment processes to degrade lignin and hemicellulose (Cragg et al. 2015). Brown rots mainly attack cellulose, while white and soft rots attack both cellulose and lignin. The white rot fungus Phanerochaete chrysosporium produces lignin-degrading enzymes, lignin peroxidases and manganese-dependent peroxidases, during secondary metabolism in response to carbon or nitrogen limitation (Cragg et al. 2015). Other enzymes including polyphenol oxidases, laccases, H_2O_2 producing enzymes and quinine-reducing enzymes can also degrade lignin. The advantages of biological pretreatment include low energy requirement and mild environmental conditions, but the hydrolysis rate is very low (Santos et al. 2011).

Furfural is an important inhibitor of ethanol production from hemicellulose hydrolysate even at low concentrations. Various bacteria and yeast have been reported to partially transform furfural to either furfural alcohol or furoic acid, or a combination of both (Moysés et al. 2016). A few microbial species such as Neurospora, Monilia, Paecilomyces and Fusarium have been reported to hold the ability to ferment cellulose directly to ethanol by simultaneous saccharification and fermentation (SSF) (Singh et al. 2017). Consolidated bioprocessing (CBP) featuring cellulase production, cellulose hydrolysis and fermentation in one step, is an alternative approach with outstanding potential (Byadgi and Kalburgi 2016). The recombinant strain of E. Coli with the genes from Z. mobilis for the conversion of pyruvate into ethanol has been reported by Olson et al. (2015). A key challenge to commercializing production of fuels and chemicals from cellulosic biomass is higher processing costs (Manochio et al. 2017; Techaparin et al. 2017). Biological conversion opens such low costs production path as it has the potential to achieve a higher yield and the modern tools of biotechnology can improve key process steps.

A range of residual substrates such as sugarcane bagasse, sugarcane molasses and starch has been found suitable for the bioconversion of available carbohydrates in these substrates to produce ethanol (Techaparin et al. 2017; Suryaningsih 2014). A variety of mesophilic and thermophilic microorganisms were employed to optimize the fermentation process, which could be practically viable in different climatic conditions, particularly to reduce the cost of temperature maintenance in large fermenters operating in warmer countries in summer months (Wu et al. 2016).

Sukumaran et al. have recently reported on bioethanol production from the saccharification of wheat bran, a ligno-cellulosic waste (Sukumaran et al. 2009). The cost of cellulase enzymes is a major factor in the enzymatic saccharification of agricultural biomass, which contains lignin. Production cost of cellulases and hence ultimately the cost of ethanol production may be brought down by multifaceted approaches. One important approach is the use of cheaper lignocellulosic substrates for the biosynthesis of the enzyme, and second strategy is the use of cost efficient fermentation process such as solid state or solid substrate fermentation at much cheaper cost.

Whilst bioethanol production has been greatly improved by development of new technologies but there are still challenges that need further improvements in the developed technology to bring forward to commercial scale. These challenges include maintaining a stable performance of the genetically engineered microorganisms and developing more efficient pretreatment technologies for the lignocellulosic biomass and integrating the optimal components into economic ethanol production systems.

5.9.3 Third Generation Biofuel

The conversion technologies for utilizing microalgae biomass can be separated into two basic categories of thermochemical and biochemical conversion (similar to terrestrial biomass). Thermochemical conversion covers the thermal decomposition of organic components to fuel products, such as direct combustion, gasification, thermochemical liquefaction and pyrolysis. The biological process of energy conversion of biomass into other fuels includes anaerobic digestion, alcoholic fermentation and photo biological hydrogen production (Slade and Bauen 2013).

5.10 Advancement in Different Fuels

As mentioned earlier the field of biofuels has seen meteoric change both in the techniques used and the quantities of biofuel produced. Use of food crops was supplemented by agricultural waste and residue and it was hypothesized that microscopic species can be used for further improvement. Transgenic has been used to improve the biofuel crops (Grant 2009). Efficient biotechnical methods to modify the structure of different algal species coupled with photonic techniques has been explored to give high yields. Different designs of bioreactors have been explored which aims at higher growth rate of algae. One such example is found in one of the recent work by Milano et al. 2016 but not only this there had been a thrust on developing different technologies to use biofuel in different forms. Bio-fuel cells both enzyme and microbe based to convert biofuels to electricity has been created and improved. One such advancement in the bio fuel cells is creation of organelle based biofuel cell (Marbelia et al. 2014) which uses mitochondria immobilized on paper instead of complete cells. This kind of fuel cell is more efficient than enzymatic fuel cell and has the efficiency of microbial fuel cell. These and many such other developments have propagated the hope that these biofuels can be used competitively with petroleum based products as well as production of hydrogen in recent days (Sharma 2017).

5.11 Progress in Processing of Lignocellulosics to Biofuels

5.11.1 Pre-treatment

Due to the nonfermentable nature of lignin, biomass is pretreated to separate cellulose, hemicellulose and lignin. Pretreatment is the major step in the successful production of valuable products from lignocellulosic biomass. A suitable pretreatment of biomass is necessary to ensure good yields of sugars from the polysaccharides. Pretreatment disrupts the plant cell wall and improves enzymatic access to the polysaccharides as raw and untreated biomass is usually resistant to enzymatic degradation. A number of biomass pretreatment technologies are available today (Nanda et al. 2014; Putro et al. 2016; Menon and Rao 2012), such as **physical** (comminution by chipping, grinding and milling to reduce biomass particle size; ozonolysis; gamma rays; pulsed electrical field; electron beam; ultrasound and microwave digestion), **chemical** (use of acids, bases and organic solvent in biomass hydrolysis), **thermophysical** (liquid hot water, steam explosion, supercritical water), **thermochemical** (wet oxidation, ammonia recycle percolation, ammonia fiber explosion, supercritical CO_2) and **biological** (enzymatic hydrolysis).

As regard chemical pre-treatments, hydrolysis of lignocellulosic biomass result in undesirable components found in biomass hydrolysates that are inhibitory to fermentation include sugar degradation products (e.g. hydroxymethyl furfural or HMF and levulinic acid), hemicellulose degradation products (e.g. acetic acid, ferulic acid, glucuronic acid and p-coumaric acid) and lignin breakdown products (e.g. syringaldehyde and syringic acid). New pretreatment methods were proposed to be highly efficient and effective for downstream biocatalytic hydrolysis of various lignocellulosic biomass materials, which can accelerate bioethanol commercialization, such as the hydrogen peroxide–acetic acid pretreatment (Wi et al. 2015). Recent advances included acidic treatments to deconstruct biomass in combination with organic solvents in a biphasic system, in order to increase the concentrations of products and the efficiency of downstream processing options (Wettstein et al. 2012). It would be highly desirable if these organic solvents could be produced from biomass, thereby eliminating the need to transport solvents derived from petroleum to the biomass refining site. Recently, ionic liquids are gaining interest in biomass hydrolysis and being attractive alternatives to volatile and unstable organic solvents due to their high thermal stability and nearly absolute nonvolatility (Vancov et al. 2012). Nevertheless, there are several core issues that stand in the way of commercialization, including the relative high cost of the ionic liquids, a lack of knowledge in terms of process considerations for a biorefinery based on these solvents, and scarce information on the co-products of this pre-treatment technology (Klein-Marcuschamer et al. 2011).

Thermophysical and thermochemocal pretreatments often result in the generation of inhibitory byproducts such as furfural, HMF and acetic acid. They have adverse effects on enzymatic hydrolysis and fermentation, consequently several post-treatment steps such as detoxification, neutralization and nutrient supplementation to the hydrolysate medium could curb the inhibitory effects (Nanda et al. 2014).

As regard biological pre-treatments, lignocellulose polysaccharides are hydrolyzed to provide the mono-saccharides used by microbial biocatalysts in fermentation processes.

Synergistic interaction between different enzymes have been investigated in order to design optimal combinations and ratios of enzymes for different lignocellulosic substrates subjected to various pretreatments (Van Dyk and Pletschke 2012). Bio-conversion using enzyme synergy has generally opted for two approaches, individual enzyme combinations or combinations of commercial mixtures. Based on the substrate analysis and identification of sugars, enzymes are selected for hydrolysis of bonds relating to those sugars. Enzymes required for glucose and xylose release are considered the main enzymes, while accessory enzymes are added should those sugars be present. These enzymes are then evaluated for optimal yield and synergy. Once enzyme ratios are optimized, further accessory enzymes can be evaluated for total release of all sugars (see Fig. 5.7a). Finally, commercial mixtures must be selected and characterized to identify the presence of relevant enzyme activities. Ratios of commercial mixtures are optimized based on yield of glucose and xylose. Enzyme activities that are not present in the commercial mixtures must then be added in the form of additional enzymes and evaluated for improved hydrolysis (Fig. 5.7b).

5.11.2 Cellulose and Hemicelluloses Conversion

After biomass pretreatment, cellulose and hemicellulose fractions of the lignocellulosic biomass are converted to various biofuels, while the residue fraction (lignine) is converted via combustion. Hydrotermal, thermochemical, biochemical and chemo-catalytic processes are typically studied to produce biofuels from lignocellulosic biomass. Along with bio-oil, ethanol, butanol and syngas, various value-added co-products including biochar, organic acids, solvents, phenols, aromatic compounds, etc. are also obtained. The most used platform molecules include: (a) levulinic acid

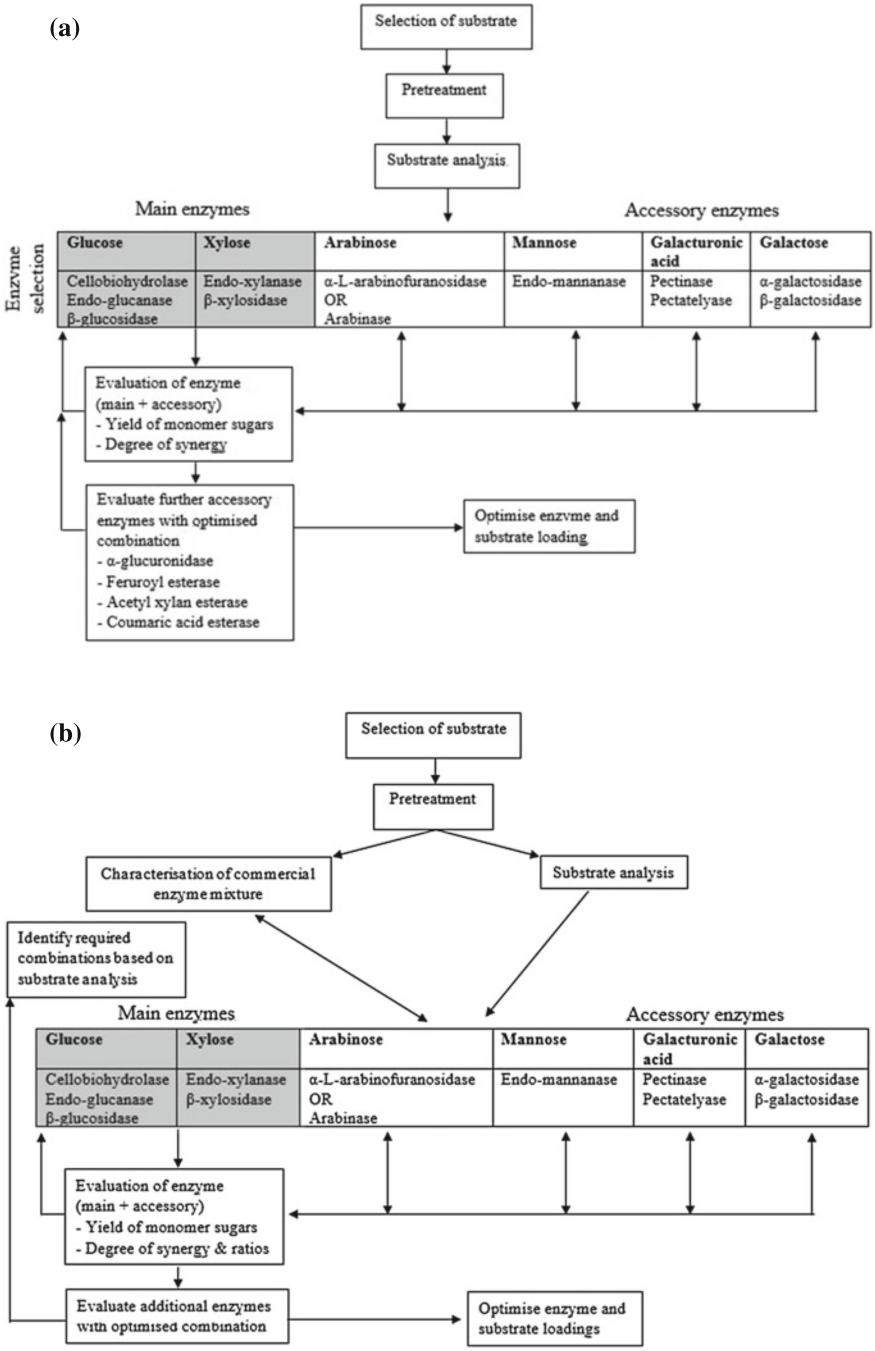


Fig. 5.7 **a** Model for developing optimal combinations with individual enzymes and **b** optimal synergistic combinations with commercial mixtures of enzymes (Van Dyk and Pletschke 2012)

that can be transformed to produce either fuels or additivies for fuels; (b) furan derivatives that can also be transformed into fuels and fuel additives; (c) polyols to produce liquid fuels as well as oxygenated additives; (d) fatty acids for producing diesel and lubricants (Climent et al. 2014).

In **hydrothermal** processes, supercritical water acts as a medium in the biomass conversion to fermentable sugars and H_2-rich syngas. This technology has been found to be promising for the production of H_2 from biomass over last few years, but it has a few limitations for industrial applications (Nanda 2012). **Thermochemical** processes do not require enzymes or microorganisms, they are applicable over a wide range of feedstocks, and they are generally compatible with conventional petroleum processing technologies. However, pre-treatment of the biomass and physical feeding into thermal processing units are challenging. The thermochemical conversion of biomass includes gasification, pyrolysis and liquefaction.

Gasification produces syngas and tar (condensable high molecular weight hydrocarbons produced by incomplete biomass gasification). Syngas is converted to biofuels by using chemical catalysts known as FT process or by using microbial catalysts known as syngas fermentation (Munasinghe and Khanal 2011). The syngas fermentation into ethanol and other bioproducts is considered to be more attractive due to several inherent merits over the biochemical approach and the FT process. In gasification, challenges include minimization of tar formation, syngas cleanup, development of effective catalysts, and integration with Fischer–Tropsch (FT) process. The direct integration of biomass gasification and FT synthesis requires an intermediate gas-cleaning system, because the gaseous stream delivered from the gasifier typically contains a number of contaminants that need to be removed before the FT unit, which is highly sensitive to impurities (Serrano-Ruiz and Dumesic 2011). The utilization of pure oxygen atmosphere, small particle sizes (lower than 1 mm diameter), and a combination of high temperatures, high pressures and low residence times favors the production of syngas versus producer gas (a mixture of CO, H_2, CO_2, CH_4, and N_2 used for heat and electricity production) (Serrano-Ruiz and Dumesic 2011).

Pyrolysis produce bio-oil, gas and char and major challenges include cleanup of the bio-oil and sufficient stabilization of it for practical delivery and use in a petroleum refinery (Hoekman 2009). A new controlled conversion of lignocellulose biomass to bio-jet and diesel fuels by catalytic pyrolysis of biomass into low carbon hydrocarbons coupled with alkylation of aromatics was recently proposed (Zhang et al. 2015).

Finally, liquefaction gives bio-oil and gas as products. Bio-oil results in a complex mixture of volatile organic acids, alcohols, aldehydes, ethers, esters, ketones, and non volatile components. This oil could be upgraded catalytically to yield an organic distillate product which is rich in hydrocarbons and useful chemicals (Naik et al. 2010).

The **biochemical** conversion involves biomass hydrolysis with dilute acids and enzymes to produce monomeric sugars followed by microbial fermentation of the sugars to fuel ethanol and butanol (Balat 2011). Recent studies have aimed to better characterize and understand the mechanisms of cellulase/hemicellulase reactions to design high performance cellulosomes/hemicellulosomes (Gao et al. 2013).

Recent articles reported the identification and characterization of novel xylanases (GH10-XA) and α-glucuronidase (GH67-GA) from *Alicyclobacillus* and *Caldicellulosiruptor* (GH67-GC) (Cobucci-Ponzano et al. 2015). Several authors focused on the current status and advances in cellulase and hemicellulase improvement (Dumon et al. 2012, Behera and Ray 2016, Gao et al. 2011). Recent developments include engineered strains for consolidated bioprocessing for cost-effective production: hydrolytic strains with a recombinant biofuel pathway and engineering of a natural ethanologenic strain by inserting cellulolytic and/or hemicellulolytic potentialities (Amore and Faraco 2012). Although the actual consolidated bioprocessing yields are lower than those of wild fermenting microorganisms on lignocellulose hydrolysates, the concept is promising. The biomass hydrolysates containing monomeric sugars (glucose and xylose) were fermented using *Saccharomyces cerevisiae* and *Clostridium beijerinckii* for ethanol and butanol production, respectively (Nanda et al. 2014). New strains and process intensification are being investigated, including the use of a pervaporation system in order to remove the produced alcohol continuously and increase the yield (Amelio et al. 2016).

Solid-state fermentation technology is expanding with increasing importance for the production of high value-added products, by involving the growth of microorganisms on moist solid substrates in the absence of free flowing water. It has gained considerable attention due to several advantages over submerged fermentation (Behera and Ray 2016). Other authors investigated the rapid bioconversion of lignocellulosic sugars into ethanol using high cell density fermentations with cell recycle by using nine different engineered microbial strains: the results showed that acceptable performance is largely correlated to the specific xylose consumption rate (Sarks et al. 2014).

Bioconversion of lignocellulose by microbial fermentation is typically preceded by an acidic thermochemical pretreatment step designed to facilitate enzymatic hydrolysis of cellulose. Substances formed during the pretreatment of the lignocellulosic feedstock inhibit enzymatic hydrolysis as well as microbial fermentation steps. Conditioning of slurries and hydrolysates can be used to alleviate inhibition problems connected with hydrolytic enzymes and the yeast *Saccharomyces cerevisiae*. Novel developments in the area include chemical in situ detoxification by using reducing agents, and methods that improve the performance of both enzymatic and microbial biocatalysts, such as fermentation technology and microbial resistance to inhibitors (Amelio et al. 2016).

Since lignocellulose conversions carried out at <50 °C have several limitations, thermophilic bacteria and thermostable enzymes were also investigated to overcome the limitations of existing lignocellulosic biomass conversion processes to biofuels (Amelio et al. 2016; Bhalla et al. 2013).

Alternatively, an integrated system including anaerobic digestion and aerobic fungal fermentation was investigated to convert corn stover, animal manure and food wastes into microbial lipids for biodiesel and methane production (Zhong et al. 2015). This novel self-sustaining advanced lignocellulosic biofuel production is based on a combined hydrolysis treating synergistically solid digestate and corn stover (see

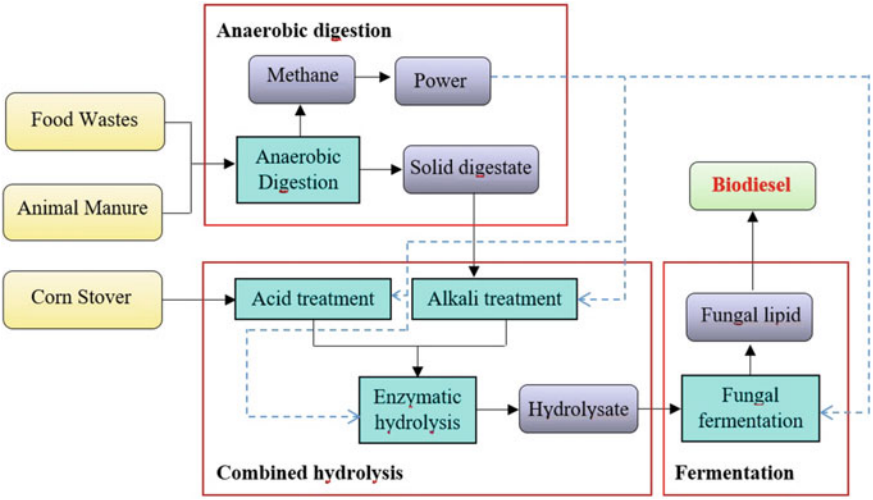


Fig. 5.8 Flowchart of a self-sustaining advanced lignocellulosic biofuel production (Zhong et al. 2015)

Fig. 5.8). Some authors also proposed to link anaerobic digestion and pyrolysis in order to convert lignocellulosic biomass more efficiently (Fabbri and Torri 2016).

In metabolic engineering, significant progress has been made using physical and chemical mutagens to increase production of lignocellulolytic enzymes (Behera and Ray 2016; Chandel and Singh 2011). A wide range of microorganisms are being engineered reflecting the effectiveness of today's gene technology. Successful metabolic engineering strategies are being applied with emphasis on xylose catabolism, inhibitor tolerance, synthetic microbial consortium, and cellulosic oligomer assimilation (Chen and Dou 2016).

In the **chemical or chemo-catalytic** approach, the cellulosic biomass undergoes catalytic hydrolysis, using acids either in aqueous solution or heterogeneous phase. Continued research is necessary to address the use and separation of mineral acids, increase the concentration of product streams, and improve product separations. Mineral acids can be eliminated by the identification of solid acid catalysts easily recoverable from the reaction mixture and recyclable for biomass deconstruction and for upgrading the resulting sugars. Alternatively, effective management and recycle of the mineral acid must be achieved to reduce costs and environmental impact.

Direct transformation of lignocellulosic biomass into 5-hydroxymethylfurfural (HMF)—emerging platform for the next generation plastics and biofuels (Wang et al. 2014)—was carried out using single or combined metal chloride catalysts in DMA–LiCl solvent under microwave-assisted heating or using Sn-Mont catalyst in a tetrahydrofuran (THF)/H_2O–NaCl biphasic system under mild conditions (Wang et al. 2014).

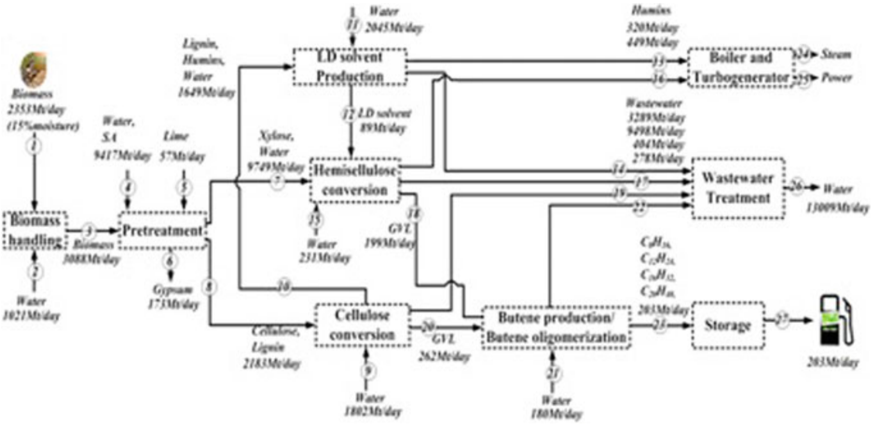


Fig. 5.9 Integrated catalytic process for monophasic conversion of cellulose to butene oligomers (using SBP solvent), biphasic conversion of hemicellulose to butene oligomers (using LD solvent), and monophasic conversion of lignin to LD solvent (Kim and Han 2016)

A novel controllable transformation of lignin into C8–C15 cycloparaffins and aromatics in the jet and diesel fuel range by catalytic depolymerization of lignin using ionic liquid was demonstrated. Ionic liquids may play a role in catalysis, in addition to their role in facilitating the dissolution of cellulose (Zhang and Zhao 2010). However, ionic liquids were not suitable for large scale applications due to their high cost and deactivation by small amounts of water, and their separation from the reaction mixture was still a problem.

An integrated process (see Fig. 5.9) based on a new alkylphenols-based biomass conversion technology was recently developed by Kim and Han (2016) as an economically competitive alternative to current biofuel production approaches. This catalytic production strategy involves separate conversion of hemicellulose and cellulose using 2-sec-butylphenol (SBP) and lignin-derived (LD) alkylphenol solvents, in order to produce liquid hydrocarbon fuels (butane oligomers). Firstly, row biomass is fractionated by dilute sulfuric acid (SA)-catalyzed pretreatment into cellulose and hemicellulose-derived xylose. These two fractions are then converted separately to levulinic acid (LA) using SBP and LD alkylphenol solvents, respectively. Finally, LA is upgraded catalytically to butene oligomers via c-valerolactone (GVL) and butene intermediates.

The proposed strategy has a high biomass-to-fuels yield (34.8 mol%) at low solids concentrations using large volumes of solvents, which are mostly recovered (99%). Energy integration reduced the total heating requirements by 72%.

Hemicellulose and cellulose can be also simultaneously converted in a single reactor, thus eliminating pre-treatments steps to fractionate biomass and simplifying product separation. This process uses gammavalerolactone (GVL) as a solvent, that is also one of the reaction products, over different catalysts (Climent et al. 2014).

Commercial processes for the conversion of biomass to fuels are now based mainly on the production of bioethanol, biodiesel and renewable fuels from gasification and pyrolysis of biomass and hydroprocessing of triglycerides, but there are few commercial processes based on the catalytic approaches. A catalytic process was developed by Avantium (Netherlands) in order to produce furan derivatives such as ethoxymethylfurfural as alternatives to petroleum-derived hydrocarbons. In 2010 Shell and Virent announced the first biogasoline demonstration plant based on Virent's Bioforming® process converting aqueous carbohydrate solutions into mixtures of hydrocarbons by combining aqueous phase reforming using heterogeneous catalysts (Virent modified ZSM-5 zeolite) (Climent et al. 2014).

5.12 Conclusions and Future Trends

Biofuels are a promising short term alternative to petroleum-derived fuels and can be derived from renewable carbon sources to mitigate greenhouse gas emissions. The applicability of biomass as a renewable resource for transportation fuels has been demonstrated by the successful integration of first generation bioethanol and biodiesel into the current infrastructure. However, first generation technologies have drawbacks related to their consequent ethic question food *vs* fuel. A more sustainable biofuels strategy would utilize widely available biomass feedstocks to the largest extent possible, drawing upon non-edible lignocellulosic biomass. A successful lignocellulosic biorefinery can be realized through a combination of different technologies and biomass processing strategies for the flexible production of varied fuel and chemical products.

There are many challenges which need to be addressed to make the syngas fermentation commercially viable in producing biofuels and other value-added products. The yields of the products from syngas fermentation are usually low; hence new recombinant microorganisms with high yields of ethanol are essential for industrial scale fermentation of syngas. Genetic manipulation of microorganisms to amplify solvent production over acetic acid can be considered as a possible option (Nanda et al. 2014).

Bioconversion using enzyme synergy is generally based on two useful approaches, individual enzyme combinations or combinations of commercial mixtures. The use of individual enzymes can lead to a greater understanding of synergy and cooperation between enzymes to degrade a complex substrate, but there is no commercial availability of pure enzymes (mostly the lesser known accessory enzymes) to study interactions between enzymes, as well as there is no characterization of the available enzymes in terms of activity on complex substrates, stability and inhibition in the bioreactor environment. New enzymes and protein engineering are necessary to improve characteristics of enzymes and to provide suitable enzymes for the future.

Instead, the use of commercial enzymes may be a quicker route to commercialization, but it is important that production of these mixtures should be optimised for different substrates with different pretreatments.

In order to perform the biocatalytic conversion of lignocellulose at industrial scale, further aspects need to be addressed. At first, the biocatalysts have to be improved so that higher yields and productivities can be achieved. Moreover, product recovery and the recycling of water as well as biocatalysts have to be considered, since both aspects are essential for industrial processes. Ultimately, the evaluation and model-based synthesis of the complete process chain needs to be performed, since pretreatment, hydrolysis, fermentation, product recovery as well as recycling steps are strongly associated and need to be harmonized (Jäger and Büchs 2012). Further research should be done with respect to hemicellulases and their contribution to lignocellulose degradation, particularly the role of enzymes such as pectinases, mannanases and other accessory enzymes. The role of other proteins and non-hydrolytic enzymes to achieve and enhance complete degradation of lignocellulose requires further investigation (Van Dyk and Pletschke 2012).

New trends in engineering synthetic microbial consortia and direct use of cellulosic or hemicellulosic oligomers are promising potential future directions for research and development. Strain improvement for enhanced cellulases biosynthesis using mutagenesis, metabolic engineering and genomics approaches, should be used for the lignocellulosic bioconversion processes. Recombinant DNA technology and protein engineering are also being used as a powerful modern approach for efficient lignocellulosic bioconversion by improving various aspects of lignocellulolytic enzymes such as production, specific activity, pH and temperature stability, or by producing novel proteins/enzymes with altered properties (Kumar et al. 2008).

Moreover, degrading the recalcitrant part of the lignocellulosic biomass (chitin for example) remains a challenge. Further challenges concern the combination of pretreatment and hydrolysis at high solids loadings to make energetic molecule production economically viable. However, efforts need to be continued to overcome biological bottlenecks and transfer limitations, crucial steps to optimize processes with high solids lignocellulosic materials (Alfenore and Molina-Jouve 2016).

In order to compete with the cost of petroleum fuels, the cost of biofuel processing should be kept as low as possible using energy efficient technologies and using less water. Producing as many co-products as possible in a biorefinery will help to reduce the cost of biofuel production. It is important that a biorefinery should be established in an appropriate location that has good water resources, access to feedstocks, and energy that is needed to process the feedstock. Several studies are in progress to enhance carbohydrate release from lignocellulose by combinations of physical and physicochemical pretreatments; combine pretreatments and hydrolysis to improve yields at high solids loadings; maximize and accelerate the conversion of sugar monomers into the final products by improving enzyme activities for separate hydrolysis and fermentation processes; improve the fermentation performances by ensuring nutritional complementation of both liquid and gaseous media, optimizing mass transfer with new configurations of bioreactor, and developing engineered strains to better understand the metabolic pathways involved in biofuel synthesis to develop over-producing engineered strains with increased inhibitor resistance; develop a cleanup system to remove inhibitors present in both the substrate (liquid or gas) and the fermented broth for purifying energetic molecules.

In conclusion, the success of a biorefinery concept depends on the development of energetically efficient processes to convert lignocelluloses biomass directly into biofuels. For this reason, the research should focus on:

- in-depth understanding of the mechanism of conversion of lignocellulosic biomass by heterogeneous catalysis and of the interrelationship among the feedstock, the catalyst, the reaction conditions and the product distribution;
- design and preparation of multifunctional catalysts for highly active and selective conversion of lignocellulosic biomass;
- development in biological and genetic fields;
- development of strategies for the production of flexible chemical platform molecules, such as levulinic acid and g-valerolactone;
- applications for the production of speciality chemicals and hydrocarbon fuels;
- technologies for biomass deconstruction, such as fast pyrolysis;
- methods for the synergistic coupling of hydrolytic and thermochemical processes into an integrated biorefinery;
- strategies for lignin utilization.

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