1

INTEGRATED BIOREFINERY FOR SUSTAINABLE PRODUCTION OF FUELS, CHEMICALS, AND POLYMERS

SHANG-TIAN YANG AND MINGRUI YU

1.1 INTRODUCTION

A biorefinery is a manufacturing facility that uses biomass as feedstock to produce fuels, power, and chemicals. It is analogous to today’s petroleum refineries, which use petroleum-based feedstocks, mainly oil and natural gas, to produce multiple fuels, commodity chemicals, industrial products, and commercial goods. Biomass includes any organic matter that is available on a renewable or recurring basis. Because it is renewable and abundant, biomass has the potential to replace fossil fuels and petrochemicals. Since the initial pushes by the White House (Executive Order 13101/13134, Developing and Promoting Biobased Products and Bioenergy) in August 1999 and the U.S. Congress (Biomass Research and Development Act) in June 2000, there have been significant industrial developments of various biorefinery systems in the last decade. The U.S. Department of Energy (DOE) and the Department of Agriculture envisioned that biomass will provide 5% of power (heat and electricity), 20% of liquid transportation fuels (ethanol and biodiesels), and 25% of industrial products (chemicals and materials) by 2030, representing 30% of the current U.S. petroleum consumption (Perlack et al., 2005). The commercialization of biomass-based biorefinery is largely dependent on the exploitation of full utilization of biomass components. By producing multiple products, a biorefinery can take advantage of the multiple components in biomass and intermediates and products that can be derived from them, maximizing the value derived from the feedstock while minimizing the wastes. A biorefinery might produce one or several low-volume but high-value products, such as functional food ingredients and pharmaceuticals, and low-value but high-volume liquid transportation fuels, such as bioethanol and biodiesel, while generating process heat (steam) and electricity for its own use and perhaps enough for sale.

Various types of biorefineries, including whole crop, lignocellulosic, and green biorefineries, have been proposed or are being developed (Kamm and Kamm, 2004a,b; Schlosser and Blahušiak, 2011). Historically and presently, corn and soybean are the two largest biomass resources for industrial bioproducts in the United States, and sugarcane is the main biomass resource in Brazil and India. As the oil price continued to rise in the last 10 years, these traditional agricultural crops have been increasingly used to produce fuel ethanol and biodiesel. Wheat, rice, and other grains are the main staple food in Europe, Asia, and other parts of the world. Koutinas et al. (2007) proposed a wheat- and rapeseed-based biorefinery to produce biofuels, biodegradable plastics, and platform chemicals. However, the uses of these traditional crops in biofuel and chemical production have generated serious “food versus fuel” controversial worldwide. Meanwhile, there are abundant agricultural residues and food processing wastes generated in the current agricultural and food industries that have little use but can be converted to higher-value fuels and chemicals. For example, a straw-based...
biorefinery was developed to produce high-value wax products using a supercritical CO₂ extraction technology along with a number of chemicals and energy (Fabien et al., 2007). Therefore, the traditional agricultural processing industry should incorporate the integrated biorefinery concept to minimize the negative impact of biofuel production on food supply while maximizing its revenues.

In addition, there is plenty of forestry woody biomass available as wastes from the paper and pulp industry (Gregg et al., 1998; Pu et al., 2008). Plant biomass contains no or little starch/sugar but is abundant in cellulose and hemicellulose, which can be used for the production of second-generation or cellulosic biofuels and chemicals. Lignocellulosic biomass is well-suited feedstock for renewable bioenergy production because of its low cost, large-scale availability, and environmentally benign production. Particularly bioenergy production and utilization cycles based on lignocellulosic biomass have near-zero greenhouse gas emission (Baral and Bakshi, 2010). DOE has estimated that 1.2 billion dry tons of cellulosic biomass, including agricultural crop residues, dedicated energy crops and trees, and logging and wood processing residues, are available for bioenergy production (Bozell and Petersen, 2010; Perlack et al., 2005). This biomass is equivalent to 21 billion GJ of energy or 21% of the U.S. energy consumption. The global bioenergy potentials of plant biomass are also huge (Offermann et al., 2011). In addition, there have been extensive research efforts in developing new “energy” and “oil” crops as nonfood feedstocks for biorefineries, which are discussed in detail in Chapters 2–4.

Green biorefining is to process wet green biomass such as grass, lucerne, and algae to separate green juice and press cake rich in fiber (Kamm et al., 2010; Mandl, 2010). The green juice is then further converted to fuels and chemicals, while the press cake can be utilized as insulation materials or burned to produce energy. Although the green biorefinery concept has been developed in Europe, it is not as popular as the other two types of biorefineries in the United States. Furthermore, aquacultures including microalgae and marine algae, which can use sunlight and fix CO₂ to produce biofuels, represent another type of biorefinery that can also greatly reduce greenhouse gas emission (Jeong and Park, 2010; Lee, 2011).

In this chapter, we first provide an overview on the current status in the utilization of all components of corn and soybeans to produce various products (corn- and soybean-based biorefineries), illustrating the concept of a whole-crop biorefinery. A similar concept in sugarcane biorefinery is also briefly reviewed. Then, we review the recent developments in the utilization of lignocellulosic biomass to produce biofuels and chemicals (lignocellulosic biorefinery). Finally, a brief discussion on the algae biorefinery using sunlight and CO₂ for fuel and chemical production is provided. Detailed discussions on the different biorefinery feedstocks, bioconversion technologies including the hydrolytic enzymes used in feedstock hydrolysis, and fermentation and separation processes for different bioproducts (fuels, chemicals, and polymers) are given in the various chapters in this book.

1.2 BIOREFINERIES USING CORN, SOYBEANS, AND SUGARCANE

Current commercial biorefineries are using traditional sugar- and starch-based feedstocks such as corn, soybeans, and sugarcane to produce value-added products for food and feed applications, and fuel ethanol and specialty chemicals. These first-generation biorefineries provide good examples of how the traditional agricultural processing companies (e.g., Cargill, ADM, Tate & Lyle) have operated in the past several decades and are gradually transforming into a fully integrated biorefinery industry with an expanded product portfolio with more fuels and chemicals, often partnering with large chemical (e.g., DuPont, Dow Chemical) and oil companies (e.g., Shell, British Petroleum). Almost all of the current biofuels (mainly ethanol, butanol, and biodiesel) and bio-based chemicals (lactic acid, itaconic acid, 1,3-propanediol [1,3-PDO], etc.) are produced in this type of biorefineries, which are discussed in this section.

1.2.1 Corn Refinery

About 12.4 billion bushels or 316 million metric tons of corn are produced annually in the United States, accounting for ∼38% of world corn production in 2011. More than 40% or ∼5 billion bushels of corn produced in the United States were used to produce ∼14 billion gallons of fuel ethanol in 2011. In addition, about 1.7 billion bushels of corn are used in corn refining by wet milling for various industrial products. In addition to corn oil, starch, and feed products, various bioproducts including fuel ethanol, organic acids (mainly citric, lactic, and itaconic acids), amino acids (e.g., lysine, threonine), and biopolymers such as xanthan gum and polyhydroxalkanoates (PHAs) are currently produced by microbial fermentation in corn refinery (Beval and Franse, 2006). In addition, new processes to produce butanol, 1,3-PDO (Bio-PDO), and other platform chemicals such as succinic acid, 3-hydroxypropionic acid, adipic acid, and acrylic acid that can be converted to various polymers (plastics) have also been developed.
BIOREFINERIES USING CORN, SOYBEANS, AND SUGARCANE

3

The lipoprotein and oil in the corn fiber are enriched by the treatment described before and can be extracted with high yields (Kalman et al., 2006). In addition, purified corn fiber can be blended with starch acetate and extruded to produce biodegradable packaging foam (starch acetate–corn fiber foam) (Ganjyal et al., 2004).

Corn gluten meal consists of proteins (∼60%) and hydrophobic amino acids (∼10% leucine), with the

Table 1.1. Major Components in Corn Grains and Products Derived from Them

<table>
<thead>
<tr>
<th>Components</th>
<th>wt % (Dry Basis)</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch</td>
<td>~72</td>
<td>Native and modified starch, dextrins, high-fructose corn syrups, dextrose, ethanol, various chemicals, and biopolymers</td>
</tr>
<tr>
<td>Protein</td>
<td>~10</td>
<td>Corn gluten feed and meal, biopolymers, fermentation feedstock</td>
</tr>
<tr>
<td>Oil (from germ)</td>
<td>~5</td>
<td>Corn oil</td>
</tr>
<tr>
<td>Fiber (from hull)</td>
<td>~13</td>
<td>Feed products</td>
</tr>
</tbody>
</table>

2002; Winkelhausen and Kuzmanova, 1998). The lipoprotein and oil in the corn fiber are enriched by the treatment described before and can be extracted with high yields (Kalman et al., 2006). In addition, purified corn fiber can be blended with starch acetate and extruded to produce biodegradable packaging foam (starch acetate–corn fiber foam) (Ganjyal et al., 2004). The applications of biofibers, including corn fiber and wheat straw, have been reviewed by Reddy and Yang (2005).

Corn gluten meal consists of proteins (∼60%) and hydrophobic amino acids (∼10% leucine), with the
remaining components mainly being moisture, fiber, and lipids. Corn gluten can be used for animal feed, food, pharmaceuticals, and industrial products (Shukla and Cheryan, 2001). Gluten hydrolyzed by proteases to soluble corn gluten hydrolysates containing angiotensin I converting enzyme inhibitor can be used as a physiologically functional food material (Apar and Ozbeke, 2007; Kim et al., 2004). Value-added biodegradable high-performance engineering plastics and composites can be produced using corn gluten by plasticizing with glycerol/ethanol and blending with commercial polymers (Aithani and Mohanty, 2006; Jerez et al., 2005; Samarasinghe et al., 2008). Gliadins extracted from gluten have been investigated to produce nanosized colloidal carriers that can ensure a controlled and targeted drug delivery (Orecchioni et al., 2006). Corn gluten was also used as substrate in solid-state fermentation to produce enzymes (Tanyildizi et al., 2007). Corn proteins extracted from gluten can be used to produce protein-based films and coatings in the food industry (Gennadios, 2002). A tasteless and odorless corn protein isolate with high nutritional values can be extracted as a high-value product from corn germ and used in food and beverage industries.

CSL containing approximately 47% protein (Thomsen, 2005) has been widely used as a nutrient and nitrogen source in fermentation to produce protease (De Azeredo et al., 2006), lactic acid (Agarwal et al., 2008), and ethanol (Amartey and Jeffries, 1994). Dextrose can be easily converted by fermentation into various chemicals, proteins, and biofuels (mainly ethanol and butanol). The fermentation-produced chemicals can be used in foods, detergents, and plastics. Butanol is also used as a solvent and can be converted to other chemicals and jet fuels. The expanded corn refinery plant may also include chemical conversion of glucose to sorbitol via hydrogenation (Castoldi et al., 2007; Perrard et al., 2007), production of industrial enzymes for the conversion of starch to maltodextrins and high-fructose corn syrup (HFCS), and an on-site cogeneration system providing electricity and steam for various processes (Moore et al., 2005). The distiller’s grains, a by-product from ethanol and acetone–butanol–ethanol fermentations, can be used as animal feed or sent to anaerobic digesters for biogas generation (Zverlov et al., 2006).

Lactic acid can be converted to polyactic acid and used as bioplastics for packaging and textile fibers (Gupta et al., 2007). Lactic acid and ethanol can react to form ethyl lactate ester, which can be used as an industrial “green” solvent, replacing the petroleum-based solvents currently used in the semiconductor industry. In addition, 1,3-PDO and succinic acid are chemical building blocks that can be produced from corn dextrose (Du et al., 2007; Nakamura and Whited, 2003). High-value biopolymers such as PHAs (Park et al., 2005; Reddy et al., 2003) and poly-γ-glutamate (PGA) (Ashiuchi and Misono, 2002; Shih and Van, 2000; Sung et al., 2005) can also be produced in corn biorefinery (Yu et al., 2006). Improved production of platform chemicals and biofuels could be achieved by engineering the microorganisms. A genetically engineered Escherichia coli was developed by DuPont to produce high-level 1,3-PDO (up to 130 g/L) from glucose (Emptage et al., 2003; Kurian, 2005; Westervelt, 2004). Using global transcription machinery engineering (gTME), Alper et al. (2006) developed a Saccharomyces cerevisiae strain with improved tolerance to high concentrations of glucose and ethanol to produce high-level ethanol from high-concentration glucose. Corn stover and cob can also be used in fermentation to produce chemicals and biofuels after pretreatment and enzymatic hydrolysis, which will be discussed in the part of lignocellulosic biorefinery.

1.2.2 Soybean Biorefinery

In 2011, the United States produced 3.056 billion bushels (83.18 million metric tons) of soybeans, which is about 33% of soybeans and 56% of oilseed produced worldwide. More than 50% of soybeans (∼44 million metric tons) are processed to produce vegetable oils (8.4 million metric tons) and soybean meal (35.6 million metric tons). Due to the rising oil price, biodiesel production in the United States, which is mainly from soybean oil, has increased rapidly over the last 20 years, from 0.5 million gallons in 1999 to 75 million gallons in 2005, 690 million gallons in 2008, and 1.07 billion gallons in 2011. Current U.S. legislation requires its use to increase to 2 billion gallons in 2015. Brazil and Argentina together account for ∼48% of world soybeans and 16% biodiesel production. Europe and other countries account for more than 50% of world biodiesel production, which is mainly from rapeseed, sunflower seed, cottonseed, and palm oils, and has also increased rapidly in the last 10 years.

Table 1.2 shows and compares different methods for biodiesel production from soybean oil, including non-catalytic process (supercritical alcohol technology) and catalytic processes using alkali, acid, and enzyme as catalysts (Al-Zuhair, 2007; Behzadi and Farid, 2007; Demirbas, 2005). In general, the alkali process is the most efficient of all processes and has a high reaction rate (Marchetti et al., 2007). It is the only process currently used in biodiesel production at an industrial scale. However, enzymatic and supercritical processes are more environmentally friendly and have also shown promising applications, although further optimization
TABLE 1.2. Comparison of Different Technologies for Biodiesel Production from Soybean Oil

<table>
<thead>
<tr>
<th></th>
<th>Alkali Catalysis</th>
<th>Acid Catalysis</th>
<th>Enzyme Catalysis</th>
<th>Supercritical Alcohol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>60−70</td>
<td>55−80</td>
<td>30−40</td>
<td>250–350</td>
</tr>
<tr>
<td>Reaction time (minutes)</td>
<td>60−360</td>
<td>3000–4200</td>
<td>600−3000</td>
<td>7−15</td>
</tr>
<tr>
<td>Ester yield (%)</td>
<td>&gt;95%</td>
<td>90−98%</td>
<td>90−98%</td>
<td>98%</td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>Saponified products</td>
<td>Esters</td>
<td>Esters</td>
<td>Esters</td>
</tr>
<tr>
<td>Water interference</td>
<td>Yes</td>
<td>Yes</td>
<td>Maybe</td>
<td>No</td>
</tr>
<tr>
<td>Glycerol recovery</td>
<td>Difficult</td>
<td>Difficult</td>
<td>Easy</td>
<td>Easy</td>
</tr>
<tr>
<td>Product purification</td>
<td>Repeated washing</td>
<td>Repeated washing</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Production cost</td>
<td>Low</td>
<td>Low</td>
<td>High</td>
<td>Medium</td>
</tr>
</tbody>
</table>

Figure 1.2 Integrated soybean biorefinery for biodiesel production from soybean oil and chemical production from glycerol and other soybean by-products. SPC, soy protein concentrate; SPI, soy protein isolate; PHA, polyhydroxyalkanoate; PHB, poly(3-hydroxybutyric acid); 1,3-PDO, 1,3-propanediol.

of these processes, such as continuous operation, and scale up and economic evaluations are needed.

In general, biodiesel production from vegetable oils and methanol (or ethanol) via transesterification is highly efficient and provides significant environmental benefits as compared with fossil fuels. However, large amounts of meal cake and glycerol by-products are generated from biodiesel production. Haas et al. (2006) analyzed that the degummed soybean oil contributed 88% of the overall biodiesel production cost. To maximize process economics and minimize wastes, soybean- and other oilseed-based biorefineries should integrate biodiesel production with the conversion of meal cake, glycerol, and other residues into additional value-added products. Figure 1.2 shows the integrated bioprocessing scheme for the exploitation of all components in soybeans. Table 1.3 shows the main components of soybeans and the products derived from them.

About 10% (w/w) of glycerol is generated in biodiesel production. It was estimated that 37 billion gallons of biodiesel would be produced annually by 2016, generating about 4 billion gallons or 38.85 billion pounds of glycerol (Anand and Saxena, 2012). The large amounts of glycerol produced in the biodiesel industry has surpassed the market demand and driven down the crude

TABLE 1.3. Major Components of Soybeans and Products Derived from Them

<table>
<thead>
<tr>
<th>Components</th>
<th>wt % (Dry Basis)</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil</td>
<td>~21</td>
<td>Biodiesel and glycerol, which can be converted to various chemicals</td>
</tr>
<tr>
<td>Protein</td>
<td>~40</td>
<td>Food or feed products, pharmaceuticals, adhesives, plastics and coating</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>~34</td>
<td>Chemicals and biofuels</td>
</tr>
<tr>
<td>Fiber</td>
<td>~5</td>
<td>Feed products</td>
</tr>
</tbody>
</table>
glycerol price to $0.05 per pound from $0.25 per pound before the expansion of biodiesel production (Yang et al., 2012). Therefore, biodiesel biorefinery should also convert crude glycerol to value-added products (Almeida et al., 2012). Many microorganisms can use glycerol as carbon source to produce various chemicals that in turn can be used as either end products or precursors for other chemicals (Koutinas et al., 2007; Yazdani and Gonzalez, 2007). For example, both pure and crude glycerol present in biodiesel wastes can be used for the production of PHA (Bormann and Roth, 1999; Eggink et al., 1994) and 3-hydroxypropionaldehyde (Doleyres et al., 2005; Vancauwenberge et al., 1990). The production of 1,3-PDO from glycerol has also been widely studied using Clostridium butyricum (Papanikolaou et al., 2000), Klebsiella pneumoniae (Liu et al., 2007), and E. coli (Dharmadi et al., 2006). Compared with glucose, glycerol as carbon source in succinic acid fermentation can give a higher product yield and concentration with lower production of the by-product acetic acid (Lee et al., 2001). A higher product yield and lower by-product formation from glycerol compared with glucose were also obtained in propionic acid fermentation (Barbirato et al., 1997; Dishisha et al., 2012). Other products that can be biologically produced from glycerol include 2,3-butanediol, n-butanol, dihydroxyacetone (DHA), glyceraldehyde, citric acid, oxalic acid, lactic acid, and polyols (mannitol, arabitol, and erythritol). Some of the glycerol fermentations have a relatively high product titer (>100 g/L), productivity (>1 g/L h), and yield (>0.7 g/g). More details can be found in a recent review article by Almeida et al. (2012). Converting the abundant and low-cost glycerol generated in the biodiesel industry to higher-value products by fermentation represents a promising route to achieve economic viability by offsetting the relatively high cost of soybeans and other oilseeds.

Another major by-product from soybeans refinery is soybean meal, which accounts for about 80% of the quantity and about two-thirds of the value of soybean. Despite considerable public and commercial interests in soybean products as food, the proportion of soy protein consumed directly in human nutrition and other industrial uses is relatively small. The bulk of soybean meal (48% protein) is used in high-protein animal feeds (more than 40% of protein content) in meat and egg production industries (Berk, 1992). Soybean meal can be enzymatically converted into a nutrient supplement for fermentation (Lee et al., 2007). Some value-added products can be produced using soybean meal in solid-state or submerged fermentation. Lipopeptides and PGA have been produced by solid-state fermentation of Bacillus subtilis using soybean and sweet potato residues (Wang et al., 2008). Lipase can be produced using soybean meal and soybean oil in submerged fermentation (He and Tan, 2006).

Soybean meal can also be processed to soybean protein concentrate and soybean protein isolates, which can be used in the food industry. Table 1.4 shows the compositions of soybean meal, soybean protein concentrates, and soybean protein isolates. Soybean protein concentrates, which contain more than 60% of proteins, can be produced from soybean meal or flour by leaching with moist heat/water, alcohol (20–80% concentration), or dilute mineral acid (usually hydrochloric acid) to remove the soluble carbohydrates and salts. Soybean protein isolates with more than 90% of protein content can be produced from defatted soy flour by first dissolving the soy protein in an alkaline water (pH 9) to remove the insoluble material, and the protein in the supernatant is then precipitated after acidifying the solution to the isoelectric point (pH 4–5) of soy protein (Erickson, 1995). Soybean protein concentrate is widely used as functional or nutritional ingredient in a wide variety of food products, mainly in baked foods, breakfast cereals, and in some meat products. Soybean protein isolates are mainly used to improve the texture of meat products, but are also used to increase protein content and to enhance flavor, and as an emulsifier. Some industrial products such as soybean-based plastics, adhesives, and coatings can be produced from soybean protein concentrates and soybean protein isolates (Kumar et al., 2002).

High-value nutritional products can be produced from soybean refining. For example, isoflavones, which work in conjunction with some peptides and proteins to protect against cancer, cardiovascular disease, and osteoporosis (Omoni and Aluko, 2005), can be extracted from soybean meal by various methods, including aqueous alcohol, superheated water, and supercritical fluid extraction (Choi et al., 2004; Rostagno et al., 2002). Lecithin can be produced in the process of soybean oil degumming. Soybean lecithin can be used in food, feeds, and pharmaceuticals as emulsifier and antioxidant (Szuhaj, 1989). The construction industry is interested in using soybean lecithin as a modifier, fluidizing agent, and long-term waterproofing compound. Soybean lecithin can be added to Portland cement to form a latex cement which is used as waterproofing compound.

### Table 1.4. Composition of Different Soybean Protein Products (wt %)

<table>
<thead>
<tr>
<th>Products (wt %)</th>
<th>Soybean Meal (%)</th>
<th>Soybean Protein Concentrate (%)</th>
<th>Soybean Protein Isolate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>48</td>
<td>64</td>
<td>92</td>
</tr>
<tr>
<td>Fat</td>
<td>0.3</td>
<td>0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Fiber</td>
<td>3.0</td>
<td>4.5</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>30</td>
<td>15</td>
<td>–</td>
</tr>
<tr>
<td>Ash</td>
<td>7</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Moisture</td>
<td>10</td>
<td>10</td>
<td>&lt;5</td>
</tr>
</tbody>
</table>

[6] INTEGRATED BIOREFINERY FOR SUSTAINABLE PRODUCTION OF FUELS, CHEMICALS, AND POLYMERS
in the production of medium-density fiberboards using renewable biomass such as soybean and wheat straws (Ye et al., 2007). The soybean straw can also be used to produce chemicals and biofuels as discussed in lignocellulosic biorefinery.

1.2.3 Sugarcane Biorefinery

Brazil is the largest worldwide producer of sugar and sugarcane-based ethanol (Brehmer and Sanders, 2009). In 2011, Brazil had approximately 3.6 million hectares of land in sugarcane production, and produced ~5.6 billion gallons of ethanol from cane sugar. The cost of ethanol production from sugarcane is US$30–35 per barrel of oil equivalent, much lower than the cost of US$50–55 per barrel based on corn (Nass et al., 2007). Sugarcane contains about 70–75% water, 11–16% sucrose, and 10–16% fiber. Sugarcane processing begins with the extraction of cane juice by mill tandems, leaving behind bagasse, the fibrous material that is sent to the lignocellulosic processing to produce ethanol or chemicals, or sent to the boiler house to generate electricity or steam. Most of the sugar juice is used to produce sugar by purification and crystallization. The molasses by-product from sugar processing and some of the sugar juice are used to produce ethanol.

Sugar, fuel ethanol, and bagasse are the main products in the sugarcane biorefinery (Fig. 1.3). Sugar can also be converted into valuable chemicals such as poly(3-hydroxybutyric acid) (PHB) by fermentation. Integrated production of PHB, sugar, ethanol, and energy was proposed by Nonato et al. (2001). Bagasse, the lignocellulosic waste or by-product from sugar extraction, is usually burned to generate steam and electricity. In an integrated sugarcane biorefinery, bagasse can be treated to release more sugars that can be further converted to fuels and chemicals to generate more values (Brumbley et al., 2007; Nel, 2010). Also, an integrated first- and second-generation ethanol production plant would have better economic returns compared with the stand-alone plant (Dias et al., 2012).

1.3 LIGNOCELLULOSIC BIOREFINERY

Today's bioethanol and biodiesel represent the first-generation biofuels produced from readily processable bioresources such as sucrose, starch, and plant oils from grains. Recent research attention has shifted toward the next-generation biofuels from lignocellulosic biomass such as agricultural residues (e.g., corn stovers, corn fiber, and wheat straw), woody biomass, and municipal solid wastes (Ragauskas et al., 2006; Sun and Cheng, 2002). More than 1.3 billion dry tons of plant biomass is produced annually in the United States, which could be redirected to biofuel production, enough to address approximately one-third of the current demand for transportation fuels in the United States (Perlack et al., 2005; Tilman et al., 2006). The corn stover, cob, and fiber, and soybean straw from corn and soybean biorefineries can be used as feedstocks in lignocellulosic biorefinery to produce ethanol or chemicals. Lignocellulose consists of three major components: cellulose, hemicellulose, and lignin. However, their compositions vary greatly, depending on the type of plant, cultivation conditions, and the age of the plant (see Table 1.5). In general, lignocellulosic biomass contains cellulose (39–49%, w/w), hemicellulose (21–25%, w/w), and lignin (20–28%, w/w) as major components and proteins and minerals (4–12%, w/w) as minor components, depending on its source (Friedl, 2012).

Plant biomass has evolved complex structural and chemical mechanisms for resisting assault on its structural sugars from the microorganisms and animals (Himmel et al., 2007). The crystalline cellulose core of cell wall microfibrils is highly resistant to chemical and biological hydrolysis because of its structure, in which chains of cellooligomers are precisely arranged (Nishiyama et al., 2002). Moreover, the coating of lignin and amorphous cellulose and hemicellulose also restricts the catalyst access to the crystalline cellulose cores of microfibrils (Ding and Himmel, 2006). The hydrolysis of lignocelluloses to fermentable sugars remains the greatest challenge in the development of economical plant biomass feedstock for the biorefinery industry.

Current lignocellulosic biorefinery generally involves three processes: (1) production of cellulases, (2) hydrolysis of cellulose and hemicellulose, and (3) fermentation of hexose and pentose sugars. To reduce costs, the last two processes can be combined into simultaneous saccharification and fermentation (SSF) (Qureshi et al., 2008) and simultaneous saccharification and cofermentation (SCSF) (Lynd et al., 2002, 2005; Ohgren et al.,
INTEGRATED BIOREFINERY FOR SUSTAINABLE PRODUCTION OF FUELS, CHEMICALS, AND POLYMERS

To date, pretreatments and enzymatic hydrolysis of lignocellulosics is still a major obstacle in lignocellulosic biorefinery largely due to the plant’s complicated cell wall structure and the crystalline structure of cellulose (Friedl, 2012; Jørgensen et al., 2007). The goal of pretreatment is to alter or remove structural and compositional impediments to hydrolysis in order to improve the enzymatic hydrolysis rate and increase the yield of fermentable sugars from cellulose and hemicellulose (Wyman et al., 2005a). The choice of pretreatment technology must take into account sugar-release patterns and solid concentrations for each pretreatment in conjunction with their compatibility with the overall process, feedstock, enzymes, and organisms to be applied. A successful pretreatment must meet the following requirements (Van Walsum et al., 1996; Wyman et al., 2005b):

1. Improve sugar yield or the ability to subsequently release sugars by hydrolysis,
2. Avoid degradation or loss of carbohydrate,
3. Avoid formation of by-products inhibitory to subsequent hydrolysis and fermentation processes,
4. Be cost effective.

About 18% of the total projected cost for biological production of cellulosic ethanol can be attributed to pretreatment, more than for any other single step (Aden et al., 2002). Pretreatment has the significant implication on the extent which the carbohydrates of cellulose and hemicellulose can be converted to bioethanol. Cost-efficient pretreatment is a challenge of lignocellulosic biofuel technology research and development (Hamelinck et al., 2005).

Many low-cost pretreatment technologies have been developed to realize high sugar yields from both cellu-

treated and hydrolyzed to simple sugars in order to be converted to chemicals and biofuels by microorganisms.

2006). Nevertheless, extensive energy-intensive thermo-
chemical pretreatments and the requirement of relatively expensive cellulases for the hydrolysis of cellulose to fermentable sugars are impeding the development of lignocellulosic ethanol and other biofuels. Compared with these biorefinery processes with multiple procedures, consolidated bioprocessing (CBP), which combines cellulase production, cellulose hydrolysis, and fermentation in one bioreactor (Olson et al., 2011; Yuan et al., 2012), has the greatest potential in reducing the overall production cost of lignocellulosic biofuels. It has been estimated that CBP can reduce the production cost of cellulosic ethanol by ~70% compared with the SSF process (Lynd et al., 2005). However, CBP is mainly applied to ethanol production from cellulose using cellulolytic bacteria such as Clostridium cellulolyticum, Clostridium thermocellum, Thermoanaerobacterium thermosaccharolyticum, and Thermoanaerobacterium saccharolyticum (Jennert et al., 2000; Klapatch et al., 1996; Mai et al., 1997; Tyurin et al., 2004). To date, no microbe has been engineered to produce n-butanol or other chemicals directly from cellulose at a meaningful quantity for industrial application.

In general, today’s lignocellulosic biorefinery comprises three main sections to convert lignocellulose into biofuels: thermochemical pretreatment, enzymatic hydrolysis, and sugar fermentation to fuels. These are discussed in the following sections.

### 1.3.1 Pretreatment

Lignocellulosic biomass is difficult to use directly as substrate in fermentation and usually has to be pre-
lose and hemicellulose. They can be categorized as (1) biological pretreatment; (2) chemical pretreatment such as dilute acid, alkali, lime, and ammonia fiber explosion (AFEX); (3) physical pretreatment such as milling; and (4) thermal processes such as steam and hot water pretreatment (Mosier et al., 2005; Yang and Wyman, 2008). Table 1.6 lists and compares commonly used pretreatment methods. Other methods using ozone, organic solvents, ionic liquids (ILs), and supercritical CO₂ have also been studied. More details can be found in a recent review article (Kumar et al., 2009) and Chapter 6 in this book.

Although various pretreatment methods have been developed over the years, only a few have achieved high sugar yields with low costs, and all of them rely on chemical addition. Dilute acid pretreatment with H₂SO₄ is the most often used method in the industry, but it usually generates some toxic by-products, mainly from the degradation of sugars and lignin (see Table 1.7), which need to be removed before fermentation (Ezeji and Blaschek, 2008). More recently, ILs, which are environmentally friendly solvents, were studied for pretreatment and hydrolysis of cellulose. Some ILs are effective in dissolving crystalline cellulose and biomass under mild conditions, resulting in polysaccharides that can be readily hydrolyzed by cellulases (Sun et al., 2009; Zavrel et al., 2009). However, it is necessary to also recover the sugars released and dissolved in the ILs and wash the treated biomass to remove the residual salts from ILs that may inhibit the commercial cellulase enzymes (Brennan et al., 2010; Zhao et al., 2008). In general, all pretreatment technologies need to be tuned to the

### Table 1.6. Some Pretreatment Methods for Lignocellulosic Biomass

<table>
<thead>
<tr>
<th>Pretreatment Methods</th>
<th>Operating Conditions</th>
<th>Advantages and Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steam explosion</td>
<td>Uses steam at 210–290°C, 20–50 bar for 2 minutes, followed by sudden pressure release</td>
<td>Simple and high downstream enzymatic efficiency. High energy requirement. Low xylan yield of 45–65%, and less efficient for soft wood</td>
</tr>
<tr>
<td>Liquid hot water</td>
<td>Uses compressed hot water 200–230°C for up to 15 minutes</td>
<td>High xylan yield of 88–98% and high downstream enzymatic efficiency</td>
</tr>
<tr>
<td>Ammonia fiber explosion (AFEX)</td>
<td>Uses liquid ammonia (5–15%) and steam explosion (160–180°C) for 10–30 minutes</td>
<td>High energy requirement. Simple and high downstream enzymatic efficiency. High cost of ammonia and less efficient for high-content lignin biomass</td>
</tr>
<tr>
<td>Acid hydrolysis</td>
<td>Uses 0.5–1.5% H₂SO₄ or HCl at 160–220°C for several minutes</td>
<td>High xylan yield of 75–90%, current industrial method. Requires neutralization before hydrolysis and generates some toxic by-products</td>
</tr>
<tr>
<td>Alkali hydrolysis</td>
<td>Uses lime or NaOH at lower temperatures and pressures for a longer time (hours)</td>
<td>High downstream enzymatic efficiency. Long time</td>
</tr>
<tr>
<td>Biological pretreatment</td>
<td>Uses fungi for several days</td>
<td>Simple and low energy requirement. Low yield and long time</td>
</tr>
</tbody>
</table>

### Table 1.7. Some Potential Inhibitors from Lignocellulose after Thermochemical Pretreatment

<table>
<thead>
<tr>
<th>Sugar Degradation Products</th>
<th>Lignin Degradation Products</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image-url" alt="Furfural" /></td>
<td><img src="image-url" alt="p-Coumaric acid" /></td>
</tr>
<tr>
<td><img src="image-url" alt="Hydroxy methyl furfural (HMF)" /></td>
<td><img src="image-url" alt="Ferulic acid" /></td>
</tr>
<tr>
<td><img src="image-url" alt="Glucuronic acid" /></td>
<td><img src="image-url" alt="Vanillin" /></td>
</tr>
<tr>
<td><img src="image-url" alt="Formic acid" /></td>
<td><img src="image-url" alt="Hydroquinone" /></td>
</tr>
<tr>
<td><img src="image-url" alt="Levulinic acid" /></td>
<td><img src="image-url" alt="Syringaldehyde" /></td>
</tr>
</tbody>
</table>
unique characteristics of a specific type of biomass in order to minimize the process cost.

### 1.3.2 Cellulose Hydrolysis and Saccharification

Following pretreatment, lignocellulose can be converted into fermentable sugars via reactions catalyzed by cellulases, which consist of three enzymes: endoglucanase (endo-1,4-β-glucanase, EG, EC 3.2.1.4), cellobiohydrolase (exo-1,4-β-glucanase, CBH, EC 3.2.1.91), and cellobiase (β-glucosidase, EC 3.2.1.21) (Demain et al., 2005). EG hydrolyze internal bonds, while CBH work from the existing ends of cellulose, releasing cellobiose molecules, which are further broken down into two glucose molecules by β-glucosidase. Various factors can inhibit cellulase activities, including hemicellulose, lignin, some enzyme inhibitors formed in the pretreatment process, and the end product glucose. Extensive research and development efforts have been done aiming at improving the performance of cellulases. Genetic and protein engineering of individual cellulase was applied to improve the thermostability and the tolerance to end products (mainly glucose) and increase the enzyme specific activity (Zhang et al., 2006). Hemicellulases (e.g., xylanase), noncatalytic proteins (mainly expansins and swollenins), nonionic surfactants (Tween 20 and 80), and polyethylene glycol (PEG) were also used to improve the cellulase performance and increase cellulose hydrolysis (Balat et al., 2008; Jørgensen et al., 2007). Recycling of cellulases was also used to reduce the enzyme costs for cellulose hydrolysis (Gregg et al., 1998; Lee et al., 1995; Ramos et al., 1993; Singh et al., 1991). Although extensive research studies over the past decade have decreased the cost of cellulase by greater than 10-fold, enzymatic hydrolysis remains an expensive component in the biocconversion of lignocellulose to bioethanol and other chemicals (Greer, 2005). For example, the enzyme in the saccharification step accounts for ~16% of the total cost for ethanol production, while the fermentation cost accounts for 15%. Therefore, it is important to further reduce the hydrolysis and enzyme costs using advanced technologies in enzyme production, cellulose hydrolysis, and fermentation (Solange et al., 2010).

### 1.3.3 Fermentation

The fermentation of lignocellulose-derived sugars to biofuels has been extensively studied using metabolic engineering to improve the inhibitor tolerance and product titer, yield, and productivity (Stephanopoulos, 2007; Zaldivar et al., 2001). Many microorganisms, including *E. coli*, *Klebsiella oxytoca*, *S. cerevisiae*, and *Zymomonas mobilis*, have been engineered to use both glucose and pentoses present in the lignocellulosic biomass hydrolysates for ethanol production (Lin and Tanaka, 2006), achieving a final titer of >40 g/L, yield of >0.42 g/g, and productivity of >0.7 g/L h (Agrawal et al., 2011; Lau et al., 2010; Ohta et al., 1991a, b). However, the production cost of lignocellulosic ethanol is still too high to be economical.

Three different bioprocesses have been developed to convert lignocellulose into biofuels: separate hydrolysis and fermentation (SHF), SSF, and CBP (Olson et al., 2011; Yuan et al., 2012). Enzymatic hydrolysis of cellulose to sugars and fermentation of sugars to biofuels are processed in two stages in SHF, while SSF integrates cellulose hydrolysis and fermentation into one step. CBP integrates the production of saccharolytic enzymes (cellulases and hemicellulases), the hydrolysis of carbohydrate components to sugars and fermentation of hexose and pentose sugars to the final product into one step (Fig. 1.4). Because of process integration, CBP offers the potential of lower cost and higher efficiency.

**Figure 1.4** General bioprocess flow sheet in lignocellulosic biorefinery for fuel and chemical production. The consolidated bioprocess (CBP) combines enzyme production, hydrolysis, and fermentation in one operation step as indicated by the dashed box.
than SSF and SHF. The estimated ethanol production cost for CBP is fourfold lower than that for SSF (Lynd et al., 2005).

Because of the potential advantages, recent research efforts have focused on CBP to further improve its production efficiency and decrease costs. No known native microorganisms can both efficiently hydrolyze cellulose to sugars and convert sugars to ethanol at high yields. Recently, great advances have been made by genetically engineering microorganisms to address this problem (Lynd et al., 2005). One strategy is to engineer the native cellulolytic microorganisms to improve the product yield and productivity. The main objective of this strategy is to improve the product yield and end-product tolerance to satisfy the industrial requirements. Metabolic engineering has been successfully applied to enhance ethanol production and eliminate by-product formation in cellulolytic clostridia. Higher ethanol yields and productivities were obtained in T. saccharolyticum by knockout of pta/ack and ldh genes responsible for acetate and lactate production, respectively (Shaw et al., 2008). Higher ethanol titer and less lactate production were found by heterologous expression of the Z. mobilis ethanol synthesis pathway (pyruvate decarboxylase and alcohol dehydrogenase) in C. cellulolyticum (Guedon et al., 2002). Recent development of gene-transfer systems for cellulolytic clostridia, such as C. cellulolyticum (Jennert et al., 2000; Tardif et al., 2001) and C. thermocellum (Tyurin et al., 2004), has greatly enhanced the ability to metabolically engineer clostridia to improve their ethanol production from cellulose. A high ethanol-tolerance (60 g/L) strain of C. thermocellum was reported by Strobel and Lynn (2004), although the ethanol titer produced by the parental strain, as well as other thermophiles, is limited to 26 g/L (Lynd et al., 2002). The increased production of ethanol and decreased production of lactate was achieved by expressing pyruvate decarboxylase and alcohol dehydrogenase (Guedon et al., 2002). More recently, the isobutanol-producing pathway was successfully cloned into cellulose-utilizing C. cellulolyticum (Higashide et al., 2011). Five genes responsible for converting pyruvate to isobutanol were cloned and heterologously expressed in C. cellulolyticum. However, the mutant grew slowly on cellulose and gave a low isobutanol titer (0.6 g/L) and productivity because of the metabolic burden and imbalance of heterologous enzymes in the host.

The second strategy is to engineer noncellulolytic microorganisms capable of producing ethanol or a desirable chemical product from sugar at a high yield and productivity to express cellulas and hemicellulas to directly utilize cellulose and hemicellulose (Lynd et al., 2005). The primary objective of such development is to engineer a heterologous cellulase system that enables growth and fermentation on pretreated lignocellulose. To date, the heterologous production of cellulases has been pursued primarily with bacterial hosts producing ethanol at high yields (engineered strains of E. coli, K. oxytoca, and Z. mobilis) and the yeast S. cerevisiae (Olson et al., 2011). For example, Ryu and Karim (2011) codisplayed endoglucanase, exoglucanase, and β-glucosidase from C. cellulolyticum on the surface of E. coli, which produced 3.59 g/L of ethanol from 10 g/L of phosphoric acid swollen cellulose (PASC), a 95.4% of the theoretical yield. Cellulase expression in strains of K. oxytoca resulted in increased hydrolysis yields (but no growth without added cellulase) for microcrystalline cellulose (Avicel) and anaerobic growth on amorphous cellulose (Zhou et al., 2001). To date, dozens of saccharolytic enzymes have been functionally expressed in S. cerevisiae. However, anaerobic growth on cellulose as the result of such expression has not been fully demonstrated (Den Haan et al., 2007; Sun and Cheng, 2002). Production of heterologous cellulolytic enzymes (Bayer et al., 2008), which is the extracellular hydrolyzing enzyme complex of some cellulolytic bacteria, is another possible way to engineer noncellulolytic microorganisms. It has been reported that cellulolysome could be constructed by expressing cohesin of S. cerevisiae and hydrolyzing subunit of cellulases from C. thermocellum, C. cellulolyticum, and Ruminococcus flavefaciens to form cellulolysome on the surface of S. cerevisiae (Tsai et al., 2010; Wen et al., 2009). These heterogeneous cellulolysomes could catalyze the hydrolysis of PASC into glucose and improve the ethanol fermentation by yeasts. However, no growth of yeasts on PASC was observed. Nevertheless, some industrial strains of S. cerevisiae with the ability to ferment all lignocellulose-derived sugars (pentoses and glucose) with ethanol yield of 0.4 g/g sugar have been developed (Becker and Boles, 2003; Hahn-Hagerdal et al., 2007; Kuyper et al., 2005). Kondo and coworkers expressed cellulases (Fujita et al., 2004), xylanases (Katahira et al., 2004), and amylases (Shigechi et al., 2004) on the cell surface of different S. cerevisiae strains. High cell density suspensions of the recombinant strains fermented amorphous cellulose, raw starch, and birchwood xylan to ethanol with ethanol yields of 0.45, 0.44, and 0.3 g/g substrate, respectively. To date, no work has been reported on the cloning of cellulase or hemicellulase in butanol-producing strains such as Clostridium acetobutylicum and Clostridium beijerinckii for direct fermentation of cellulose or lignocellulosics to butanol.

1.3.4 Plant Genetic Engineering to Improve Biomass Feedstock

Genetic engineering has been applied to develop transgenic plant with improved biomass characteristics for
hydrolysis and biofuel production (Lange, 2007; Simmons et al., 2008). Plant genetic engineering can increase the whole crop biomass yield and decrease the costs of pretreatment and enzymatic hydrolysis processes. Several strategies have been used (Ragauskas, 2006; Sticklen, 2006). The first strategy is to overexpress and engineer cellulase enzymes for cellulose hydrolysis in plants. The catalytic domain of 1,4-β-endoglucanase E1 from Acidothermus cellulolyticus has been successfully expressed in rice and maize (Sticklen, 2006). When the crude extract of rice total soluble proteins was added to AFEX pretreated rice straw or maize stover, 30% and 22% of the cellulose of these plants was converted into glucose, respectively. The second strategy is to engineer the lignin synthesis pathway to decrease lignin content or change the composition of lignin, thereby reducing the cost of pretreatment (Weng et al., 2008). For example, the downregulation of 4-coumarate:coenzyme A ligase (Pt4CL1), one of the major enzymes involved in lignin biosynthesis, resulted in a 45% decrease in lignin and a 15% increase in cellulose, doubling the plant cellulose:lignin ratio without any change in lignin composition (Li et al., 2003). Downregulating the gene encoding 4-coumarate 3-hydroxylase increased the proportion of p-hydroxyphenyl units relative to the normally dominant guaiacyl and syringyl units. This led to an increase in crop digestibility, which might increase biofuel production (Ralph et al., 2006).

Another strategy is to engineer the plant growth regulator or photosynthetic pathway to increase biomass yield. Two rate-limiting enzymes in the chloroplast carbon-fixing “dark reaction” from cyanobacteria were overexpressed in tobacco, resulting in an elevated rate of photosynthesis and increased plant dry weight (Van Camp, 2005). The manipulation of nitrogen metabolism genes has also been a successful approach to increasing biomass production (Good et al., 2004; Jing et al., 2004). Genetic engineering has also been applied to corn to modify some properties to improve biofuel production (Torney et al., 2007).

1.3.5 Thermochemical Platform for Lignocellulosic Biorefinery

Another route or platform for biomass conversion is the syngas or thermochemical platform involving the gasification of biomass at 650–900°C by reacting with air, oxygen, and steam to gaseous products (CO, CO₂, H₂, CH₄), which can then be converted by classical chemical reactions with metallic catalysts to various chemicals such as ethanol, methanol, and butanol (Basu, 2010; Cherubini, 2010; Demirbas, 2009; Wright and Brown, 2007). The syngas generated from biomass can also be converted biologically using chemoautotrophic bacteria such as homoacetogens that use the Wood–Ljungdahl pathway to fix CO₂ and H₂ to acetic acid and ethanol (see Chapter 19). In addition, liquefaction or pyrolysis of biomass at 450–500°C in the absence of any reactive compounds or oxidants can be used to produce pyrolysis oils as fuels. The thermochemical platform has the advantage that all components of the lignocellulosic biomass, including lignin, which is resistant to biological conversion, can be converted to chemicals. However, there are several challenges to overcome in order for the thermochemical platform to compete economically with fossil fuels, especially coal. These challenges arise from (1) the high oxygen (and phosphorous and nitrogen) contents in biomass; (2) large variations in biomass composition, which differs greatly among different plant species and even within the same species harvested in different seasons or regions; and (3) the high moisture content and low solid density in most of the plant biomass, which increase the difficulty and cost in biomass storage and transportation. In general, it is difficult to use existing catalysts for the thermochemical reactions and build a biomass gasification or liquefaction plant at an economically feasible scale, except perhaps for the wood-based gasification process in the pulp and paper industry (Consonni et al., 2010; Peterson and Haase, 2009).

1.4 AQUACULTURES AND ALGAE BIOREFINERY

Microalgae offer another promising resource for biofuels, especially biodiesel, production. Many microalgae have a high lipid content (as high as 70% of dry weight) that can be extracted and used to produce biodiesel. It has been estimated that microalgae have the highest biodiesel production efficiency based on the land use (12,000–98,500 L/ha/year) that is up to 220-fold of oil crops (soybean: 446; sunflower: 952; rapeseed: 1190; jatropha: 1892; oil palm: 5950) (Schenk et al., 2008). The current oil-producing crops would not be able to supply more than 50% of our current energy demand even if they were cultivated on all the arable land on the Earth.

In contrast, the area required for microalgae cultivation for supplying global oil demand would be 0.3–2.7% of global land mass or 2.5–20.5% of global arable land based on the biomass yield of 10–50 g/m² day with a 30–50% content of triacylglycerides (Schenk et al., 2008). In fact, aquacultures of microalgae, either in outdoor ponds or closed bioreactors, can be situated on nonarable land and thus would not have any negative effect on the arable land for crops for food and feed. Therefore, algal oil production can supply the so-called second- or third-generation biofuels in the future.
However, current microalgal cultivation technologies are still years away from economical for biofuel production. In general, outdoor cultivation has a lower initial capital cost but is prone to contamination and low light and CO₂ efficiencies (Lee, 2011). Closed bioreactor systems, including plate, tubular, and airlift bioreactors is expensive to operate and difficult to scale up (Costa and de Morais, 2011). The slow cell growth, low cell density (usually less than 1–10 g/L), and large water content (>90% cell weight) of cell biomass make microalgal cultures expensive to justify the relatively high production costs for the relatively low-priced biofuels. Therefore, the microalgal biorefinery must also consider the utilization of all the cell components and extract the high-value products such as carotenoids (e.g., lutein and astaxanthin) and polyunsaturated fatty acids (PUFAs) (e.g., eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]). Figure 1.5 shows a microalgal biorefinery for the production of algal oil and other value-added products. Some species of microalgae have a high content (50–60%) of carbohydrate, mainly starch or glycogen in the cytoplasm and cellulose in the inner cell wall, but little or no hemicellulose and lignin. Microalgal biomass is thus a good source of glucose for fermentative production of biofuels and chemicals. No harsh pretreatment or fermentation inhibitors are expected since there is no lignin or hemicellulose present in microalgal biomass. Microalgal biomass is a promising feedstock especially for the production of ethanol and methane with high energy yields under anaerobic conditions (Lakaniemi et al., 2012). More detailed discussion about microalgae as a valuable bioresource is given in Chapter 5.

In addition to microalgae, macroalgae from marine cultures have also been proposed as a renewable resource for the “third-generation” ethanol (and other biofuels) production (Goh and Lee, 2010). Many marine macroalgae are rich in carbohydrates (polysaccharides of galactan, mannan, etc.) with little or no lignin, and can be mass cultivated in oceans and harvested, hydrolyzed to monosaccharides, and then used as feedstock for fermentation to produce fuels (e.g., ethanol and butanol) and chemicals (Jeong and Park, 2010). The macroalgae thus can provide another alternative and promising biorefining platform for fuel and chemical production.

1.5 CHEMICAL AND BIOLOGICAL CONVERSIONS FOR FUEL AND CHEMICAL PRODUCTION

1.5.1 Biofuels

As discussed in the previous sections, many different biofuels, bio-based chemicals, biopolymers, and high-value food and nutritional products can be produced from traditional crops and lignocellulosic biomass in a biorefinery. In addition to ethanol and biodiesel, advanced biofuels such as higher alcohols (e.g., butanol), fatty acid ethyl ester (FAEE), alkanes, alkenes (olefins), and terpenes can also be produced from biomass using

![Figure 1.5](image-url)
either native or engineered microorganisms (Jang et al., 2012; Peralta-Yahya et al., 2012). Currently, bioethanol is the major biofuel on the market. Bioethanol production reached ~13.95 billion gallons in 2011 in the United States and ~23.4 billion gallons worldwide (http://ethanolrfa.org/pages/World-Fuel-Ethanol-Production). However, lately biobutanol has attracted a lot of attention as an advanced transportation fuel for its many desirable characteristics including higher energy density, lower vapor pressure (and thus low explosive risk), and lower water solubility (thus lower corrosiveness) compared with ethanol. These properties make butanol a better and more desirable biofuel than ethanol. In addition, butanol can be dispersed through existing pipelines and filling stations. Butanol can also be readily upgraded to jet fuels. Commercial production of n-butanol using solventogenic clostridia has been in operation for several years in China and isobutanol using engineered yeast has also begun in the United States in 2012 (Gevo). Other higher alcohols such as propanol and pentanol have also been investigated. These are discussed in Chapter 13.

As an alternative to biodiesel production, which is currently limited by the supply of vegetable oil or triacylglycerides, scientists have developed engineered E. coli expressing Z. mobilis pyruvate decarboxylase and alcohol dehydrogenase, which convert pyruvate to ethanol, and an acyltransferase from Acinetobacter baylyi that can directly synthesize FAEE from glucose and oleic acid (Elbahloul and Steinbüchel, 2010; Kalscheuer et al., 2006). Steen et al. (2010) engineered E. coli to overexpress a wax-ester synthase (atfA), a native thioesterase without the leader sequence ('tesA) and the gene encoding for the enzyme in the first step of β-oxidation pathway (fadD). The mutant had increased fatty acid biosynthesis and was able to produce up to 0.4 g/L of FAEEs from glucose with the addition of ethanol in the culture medium. Duan et al. (2011) further developed an engineered E. coli that produced 0.92 g/L of FAEEs from glucose in fed-batch fermentation. Similarly, Yu et al. (2011) expressed a bacterial acyltransferase in S. cerevisiae for FAEE production from glycerol; however, only a trace amount (<10 mg/L) of FAEEs was produced. Although these studies demonstrated the feasibility of direct synthesis of biodiesels, the technology is still far from commercial application.

Microbial biosynthesis of C15 to C17 alkanes and alkenes, which can be used in diesel fuel, in E. coli expressing an acyl–acyl carrier protein reductase and an aldehyde decarboxylase in the alkane biosynthesis pathway natively present in some cyanobacteria has also been demonstrated (Schirmer et al., 2010). Rude et al. (2011) showed that terminal alkenes could be produced from fatty acids by E. coli expressing a P450 fatty acid decarboxylase from Jeotgalicoccus. A polyketide synthase present in Synechococcus sp. can also be used to produce terminal alkenes through decarboxylation (Mendez-Perez et al., 2011). In addition, the biosynthesis of long-chain (C25–C31) alkenes by a head-to-head condensation of two fatty acids in Shewanella oneidensis has also been reported (Sukovich et al., 2010). LS9 has been exploiting engineered E. coli for the production of FAEE, fatty alcohols, alkanes, and olefins because of its ability in fatty acid biosynthesis with a high rate of 0.3 g/L h per gram of dry cell weight (Peralta-Yahya et al., 2012).

Isoprenoid pathways have also been exploited for the production of terpene-based biofuels. Wang et al. (2011) engineered E. coli to produce α-farnesene (a triterpene, C30H42) using a codon-optimized α-farnesene synthase and an exogenous mevalonate (MVA) pathway. The engineered E. coli strain produced 0.38 g/L of α-farnesene, which can be chemically hydrogenated to farnesene and used in jet fuel. Amyris has developed an engineered S. cerevisiae for farnesene production. In addition, Peralta-Yahya et al. (2011) used engineered E. coli and S. cerevisiae to produce bisabolene (C15H26), a sesquiterpene present in the essential oils of plants, reaching a titer of greater than 0.9 g/L for both engineered organisms. Because these terpenes are insoluble in water, their recovery and purification from fermentation broth are simpler and could be less expensive compared with other biofuels.

1.5.2 Bio-Based Chemicals

Globally, over 80 million tons of industrial chemicals valued at over $2 trillion is manufactured each year from petroleum-based feedstocks. As chemicals usually have a higher value than fuels and yet have a relatively larger market than nutritional products, the biorefinery industry is focusing more on the production of chemicals that can serve as platform chemicals, including several carboxylic acids and alcohols. Table 1.8 lists some of the building block chemicals that can be produced from biomass via microbial fermentation. Chemicals with bi- or multifunctional groups such as diols, diamines, and dicarboxylic acids can be used as monomers to produce polymers and plastics currently manufactured from petroleum-based feedstocks (e.g., ethylene, propylene, and butadienes) (Ji et al., 2012; Lee et al., 2011; Zeng and Sabra, 2011). Some of them have been or will soon be in commercial production, including 1,3-PDO (DuPont and Tate & Lyle), succinic acid (Myriant, DSM, BASF), 3-hydroxypropionic acid (for acrylic acid production) (OPX Biotechnologies and Dow Chemical), 1,4-butanediol (Genomatica), and isoprene (Genencor and Goodyear). Biodegradable
<table>
<thead>
<tr>
<th>Chemical (Annual Production)</th>
<th>Structure</th>
<th>Microorganism/Process</th>
<th>Titer (g/L)</th>
<th>Productivity (g/L h)</th>
<th>Yield (g/g)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol (67,200,000 MT; 85.2 billion gallons)</td>
<td>S. cerevisiae</td>
<td>131</td>
<td>1.71</td>
<td>0.467</td>
<td>Liu et al. (2012)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Z. mobilis</td>
<td>~120</td>
<td>~3.5</td>
<td>~0.5</td>
<td>Lin and Tanaka (2006)</td>
<td></td>
</tr>
<tr>
<td>n-Propanol</td>
<td>E. coli (glucose/xylose)</td>
<td>54/42</td>
<td>1.1/0.87</td>
<td>&gt;0.5</td>
<td>Ohta et al. (1991a)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>E. coli</td>
<td>10.8</td>
<td>0.144</td>
<td>0.107</td>
<td>Jun et al. (2012)</td>
<td></td>
</tr>
<tr>
<td>Isopropanol</td>
<td>E. coli</td>
<td>40.1</td>
<td>0.67</td>
<td>0.24</td>
<td>Inokuma et al. (2010)</td>
<td></td>
</tr>
<tr>
<td>n-Butanol (2,800,000 MT)</td>
<td>C. acetobutylicum</td>
<td>20.3</td>
<td>0.37</td>
<td>0.23</td>
<td>Lu et al. (2012)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>E. coli</td>
<td>15</td>
<td>0.2</td>
<td>0.35</td>
<td>Shen et al. (2011)</td>
<td></td>
</tr>
<tr>
<td>Isobutanol</td>
<td>E. coli</td>
<td>22</td>
<td>0.19</td>
<td>0.34</td>
<td>Atsumi et al. (2008)</td>
<td></td>
</tr>
<tr>
<td>Acetone (5,700,000 MT)</td>
<td>C. acetobutylicum</td>
<td>10.2</td>
<td>0.19</td>
<td>0.12</td>
<td>Lu et al. (2012)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>E. coli</td>
<td>7.1</td>
<td>0.15</td>
<td>0.17</td>
<td>May et al. (2012)</td>
<td></td>
</tr>
<tr>
<td>Acetic acid (~10,000,000 MT)</td>
<td>Clostridium thermoaceticum</td>
<td>100</td>
<td>0.8</td>
<td>0.8</td>
<td>Parekh and Cheryan (1994)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clostridium formicoaceticum</td>
<td>78</td>
<td>0.95</td>
<td>0.95</td>
<td>Huang et al. (1998)</td>
<td></td>
</tr>
<tr>
<td>Propionic acid (180,000 MT)</td>
<td>Propionibacterium acidipropionici</td>
<td>68.9</td>
<td>1.55</td>
<td>0.48</td>
<td>Liang et al. (2012)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clostridium tyrobutyricum</td>
<td>86.9</td>
<td>1.10</td>
<td>0.46</td>
<td>Jiang et al. (2011)</td>
<td></td>
</tr>
<tr>
<td>Butyric acid (80,000 MT)</td>
<td>E. coli</td>
<td>0.26</td>
<td>–</td>
<td>–</td>
<td>McKenna and Nielsen (2011)</td>
<td></td>
</tr>
<tr>
<td>Styrene (6,800,000 MT)</td>
<td>E. coli</td>
<td>135</td>
<td>3.5</td>
<td>0.51</td>
<td>Kaur et al. (2012)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C. butyricum (glycerol)</td>
<td>93.7</td>
<td>3.3</td>
<td>0.51</td>
<td>Wilkens et al. (2012)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>E. coli (glycerol)</td>
<td>5.6</td>
<td>0.078</td>
<td>0.21</td>
<td>Clomburg and Gonzalez (2011)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T. thermosaccharolyticum</td>
<td>9</td>
<td>0.36</td>
<td>0.24</td>
<td>Sanchez-Riera et al. (1987)</td>
<td></td>
</tr>
<tr>
<td>1,4-Butanediol (1,360,000 MT)</td>
<td>E. coli</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Burk (2010)</td>
<td></td>
</tr>
<tr>
<td>2,3-Butanediol (1,250,000 MT)</td>
<td>K. pneumoniae</td>
<td>150</td>
<td>4.21</td>
<td>0.43</td>
<td>Ma et al. (2009)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>K. oxytoca</td>
<td>130</td>
<td>1.64</td>
<td>0.48</td>
<td>Ji et al. (2010)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Serratia marcescens</td>
<td>152</td>
<td>2.67</td>
<td>0.41</td>
<td>Zhang et al. (2010)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lactobacillus delbrueckii</td>
<td>135</td>
<td>3.4</td>
<td>0.9</td>
<td>Kadam et al. (2006)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>E. coli</td>
<td>138</td>
<td>3.54</td>
<td>0.99</td>
<td>Zhu et al. (2007)</td>
<td></td>
</tr>
<tr>
<td>Lactic acid (450,000 MT)</td>
<td>E. coli</td>
<td>38.7</td>
<td>0.54</td>
<td>0.35</td>
<td>Rathnasingh et al. (2009)</td>
<td></td>
</tr>
<tr>
<td>3-Hydroxypropionic acid</td>
<td>E. coli (glycerol)</td>
<td>24.4</td>
<td>0.18</td>
<td>1.02</td>
<td>Huang et al. (2012)</td>
<td></td>
</tr>
<tr>
<td>Acrylic acid (4,200,000 MT)</td>
<td>K. pneumoniae</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Straathof et al. (2005)</td>
<td></td>
</tr>
<tr>
<td>Succinic acid (30,000 MT)</td>
<td>Anaerobiosprillum succiniciproducens</td>
<td>83</td>
<td>10.4</td>
<td>0.88</td>
<td>Meynial-Salles et al. (2008)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Corynebacterium glutamicum</td>
<td>146</td>
<td>3.2</td>
<td>0.9</td>
<td>Okino et al. (2008)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>E. coli</td>
<td>86.6</td>
<td>0.9</td>
<td>0.92</td>
<td>Jantama et al. (2008)</td>
<td></td>
</tr>
</tbody>
</table>

(Continued)
polymers such as polylactic acid derived from lactic acid (Cargill) and polyhydroxybutyrate (Metabolix and ADM) have also been in commercial production. The commercial success of these bio-based chemicals will be determined by their production costs or process economics, which depends largely on the product titer, yield, and productivity in the fermentation process. As can be seen in Table 1.8, not all chemicals can be produced by microorganisms at a sufficiently high titer, productivity, or yield for commercial application, largely because of the toxicity of the chemical to cells.

In addition to the biological conversion routes discussed before, many chemicals and fuels can also be produced from biomass via chemical conversion routes. For example, biomass-derived sugars can be dehydrated with acid catalysts to form furan derivatives, such as hydroxymethylfurfural (HMF), followed by aldol condensation with ketones (e.g., acetone) and then hydrodeoxygenation to form liquid alkanes of C7–C15 that can be used in diesel and jet fuels (Huber et al., 2005). Xing et al. (2010) used a similar process to produce primarily C13 and C12 alkanes from xylose in hemicellulose extracts, achieving 76% of the theoretical yield or 0.46 g alkane per gram xylose for the process. Another process using a platinum–rhenium (Pt-Re) catalyst converts sugars and polyols to primarily hydrophobic alcohols, ketones, carboxylic acids, and heterocyclic compounds that can provide reactive intermediates for the production of fine chemicals and polymers (Kunkes et al., 2008). This process can also provide a route for the synthesis of branched alkanes and olefins, and alkylated aromatics as high-octane components of gasoline. Recently, Anbarasan et al. (2012) reported a hybrid process integrating chemical catalysis with extractive fermentation to produce fuels. In their process, acetone, butanol, and ethanol (ABE) produced in a solventogenic fermentation by clostridia are extracted with an organic solvent, and ABE are then converted to C5−C11 ketones by a palladium-catalyzed alkylation, with the overall carbon yield of up to ~58%. The C5−C11 ketones can be deoxygenated to paraffins and used in petro, diesel, and jet fuels. These chemical catalysis processes offer promising alternatives for biofuel production; however, their

### Table 1.8 (Continued)

<table>
<thead>
<tr>
<th>Chemical (Annual Production)</th>
<th>Structure</th>
<th>Microorganism/Process</th>
<th>Titer (g/L)</th>
<th>Productivity (g/L h)</th>
<th>Yield (g/g)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fumaric acid (90,000 MT)</td>
<td><img src="image" alt="Fumaric acid" /></td>
<td><em>Rhizopus oryzae</em>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>92</td>
<td>4.25</td>
<td>0.85</td>
<td>Cao et al. (1996)</td>
</tr>
<tr>
<td>Malic acid (200,000 MT)</td>
<td><img src="image" alt="Malic acid" /></td>
<td><em>Aspergillus flavus</em>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>113</td>
<td>0.59</td>
<td>0.94</td>
<td>Battat et al. (1991)</td>
</tr>
<tr>
<td>Itaconic acid (80,000 MT)</td>
<td><img src="image" alt="Itaconic acid" /></td>
<td><em>E. coli</em>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>69</td>
<td>0.69</td>
<td>1.04</td>
<td>Jantama et al. (2008)</td>
</tr>
<tr>
<td>Adipic acid (2,600,000 MT)</td>
<td><img src="image" alt="Adipic acid" /></td>
<td><em>E. coli</em> fermentation of glucose to cis, cis-muconic acid, followed by hydrogenation</td>
<td>36.8</td>
<td>0.77</td>
<td>0.18</td>
<td>Niu et al. (2002); Polen et al. (2012)</td>
</tr>
<tr>
<td>Glutaric acid (42,000 MT)</td>
<td><img src="image" alt="Glutaric acid" /></td>
<td><em>E. coli</em></td>
<td>1.13</td>
<td>0.016</td>
<td>0.153</td>
<td>Moon et al. (2009)</td>
</tr>
<tr>
<td>Isoprene (80,000 MT)</td>
<td><img src="image" alt="Isoprene" /></td>
<td><em>E. coli</em></td>
<td>60</td>
<td>2</td>
<td>0.11</td>
<td>Cervin et al. (2009)</td>
</tr>
<tr>
<td>Putrescine (10,000 MT)</td>
<td><img src="image" alt="Putrescine" /></td>
<td><em>E. coli</em></td>
<td>24.2</td>
<td>0.75</td>
<td>0.168</td>
<td>Qian et al. (2009)</td>
</tr>
<tr>
<td>Cadaverine (1,600,000 MT)</td>
<td><img src="image" alt="Cadaverine" /></td>
<td><em>E. coli</em></td>
<td>9.61</td>
<td>0.32</td>
<td>0.131</td>
<td>Qian et al. (2011)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Sources: Almeida et al. (2012); Lee et al. (2011); Zeng and Sabra (2011).

<sup>b</sup>Fermentation with glucose as substrate, unless otherwise indicated.

MT, metric tons.
industrial applications may be limited by the high costs of the catalysts used in the processes.

1.5.3 Hybrid Chemical and Biological Conversion Processes

Although many of the building block chemicals can be produced via biological routes, some of them could be more efficiently produced in a hybrid biological/chemical process. For example, direct microbial production of bio-based acrylic acid from carbohydrate is very difficult because of the high cytotoxicity of acrylic acid. A better process is to produce 3-hydroxypropionic acid by fermentation first and then followed by catalytic dehydration to acrylic acid. Likewise, adipic acid can be better produced from glucose via E. coli fermentation to cis, cis-muconic acid, followed by catalytic hydrogenation. In addition, more ethanol, propanol, or butanol could be produced from glucose in a hybrid process as illustrated in Figure 1.6. For example, a two-step process with homoacetogenic fermentation followed by catalytic hydrogenation can give an overall ethanol yield of 0.72 g/g, which is 50% higher than that from yeast fermentation (~0.46 g/g). Similarly, propionic acid fermentation followed by hydrogenation can give an overall propanol yield of ~0.5 g/g sugar, whereas propanol fermentation with engineered microorganisms usually give a low yield of <0.3 g/g glucose. The butanol yield is usually only 0.2–0.25 g/g sugar in ABE fermentation and ~0.3 g/g sugar in fermentation with engineered E. coli, whereas a higher butanol yield of 0.4 g/g could be obtained via butyric acid fermentation followed by hydrogenation (Yang, 2008). Compared with direct fermentation of sugar, much more alcohols could be produced in these two-step hybrid processes, significantly lowering the feedstock cost that often accounts for more than 50% of the final product cost. In addition, alcohols such as ethanol, propanol, and butanol can be catalytically dehydrated to corresponding alkenes, which are major feedstock chemicals in current petroleum refineries. In Brazil, Braskem, a Brazilian petrochemical company headquartered in São Paulo, currently produces “green” polyethylene and ethylene from sugarcane, illustrating a trend or desire of moving from petroleum-based feedstock toward bio-based feedstock for sustainability and carbon credit in the traditional petrochemical industry.

1.5.4 Biorefinery Feedstock Economics

As illustrated in Figure 1.7, biorefinery and petroleum refinery are generally moving in opposite directions; the former converts more complex and oxidized molecules such as carbohydrates to organic acids and alcohols, whereas the latter converts simpler and more reduced small molecules such as alkenes to the desirable chemical products. Therefore, biorefinery will have to compete with petroleum refinery for the same or similar chemical market, and the ultimate deciding factors would be the cost of the raw materials (biomass vs. crude oil) and the efficiencies of the process technologies. Over the last two decades, the crude oil prices increased fivefold from ~US$20 to surpass US$100 per barrel, whereas corn prices increased threefold from ~US$100 per metric ton to ~US$300 per ton. Although the prices of corn and other agricultural products would continue to increase with increasing oil prices, the market prices of corn and other major agricultural commodities were relatively stable and did not change significantly until in 2005.
when increasingly more corn was used for ethanol production. Furthermore, lignocellulosic biomass is the fourth largest energy source. The United States alone is capable of producing 1.3 billion dry tons of biomass from both agricultural and forestry resources at $60 or less per ton annually (Munasinghe and Khanal, 2010; Perlack et al., 2005). These abundant inexpensive renewable bioresources will not cause food/feed versus fuel controversy and thus can be used to produce fuels and chemicals, adding value to biorefinery and agricultural industries while simultaneously solving waste disposal problems and reducing greenhouse gas (CO₂) emission. In addition, aquacultures could supply plenty of algal biomass for biofuel production. Therefore, it can be expected that as the oil prices continue to rise, petroleum feedstock would become more expensive than biomass feedstock, and biorefinery could overtake petroleum refinery in the foreseeable future.

1.6 CONCLUSIONS AND FUTURE PROSPECTS

The biorefinery industry has developed rapidly in the last few years. In addition to bioethanol and biodiesel, several bio-based products are already or will soon be in commercial production replacing petroleum-based products in the market. With continuing developments and advances in new energy crops, aquacultures, synthetic biology for cell engineering, and conversion technologies, biorefining will increasingly play a more important role in the supply of energy, fuels, and chemicals for sustainable economic growth with minimal or no negative impact on the environment.

However, there are many challenges facing the biorefinery industry. First, the current infrastructures built on the petroleum-based manufacturing and products may not be relevant to bio-based manufacturing and products. For example, the supply of the biomass feedstock may be seasonal and limited by the geographical area. Also, the relatively low density of biomass would hinder its storage and transportation, thus severely limiting its ability to support a mega-scale biorefinery that could benefit from the economy of scale. Second, as the bioproducts are usually produced at a relatively low concentration, a large amount of water would be required in a biorefinery such as in the production of bioethanol. For example, a plant producing 100 million gallons of ethanol per year would use the equivalent of the water supply for about 5000 people (The National Academy of Sciences, 2007). This could cause a serious problem on the supply of fresh water for drinking and other uses. Water recycling and using sea or salt water in biomass production and conversion (fermentation) are thus important to the biorefinery industry. Finally, not all of the current petroleum-based chemicals can be economically produced from biomass or via bioconversion. Continuing research and development in both process engineering and cell engineering technologies are needed to improve the conversion efficiency and reduce the product costs. In this regard, modern technologies in metabolic pathway engineering, synthetic biology, and systems biology offer immense opportunities for the further development of the biorefinery industry (Curran and Alper, 2012; Dhamankar and Prather, 2011; Jang et al., 2012).

REFERENCES


