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

Bioprocessing Technologies in Biorefinery for Sustainable Production of Fuels, Chemicals, and Polymers, First Edition. Edited by Shang-Tian Yang, Hesham A. El-Enshasy, and Nuttha Thongchul.

biorefinery was developed to produce high-value wax products using a supercritical CO_2 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 CO_2 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 CO_2 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 polyhydroxyalkanoates (PHAs) are currently produced by microbial fermentation in corn refinery (Beval and Fransse, 2006). In addition, new processes to produce butanol, 1,3-PDO (Bio-PDO), and other platform chemicals such as succinic acid, 3-hydroxyl propionic acid, adipic acid, and acrylic acid that can be converted to various polymers (plastics) have also been developed

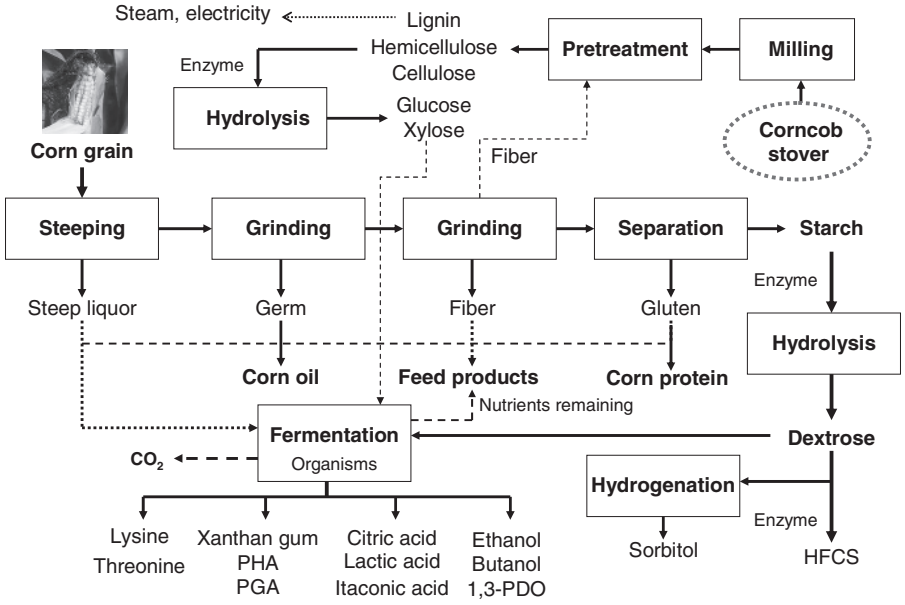


Figure 1.1 Integrated corn biorefinery (wet milling). In addition to corn grain, corncob, stover, and fiber are also used as feedstock to fermentation for fuel and chemical production. HFCS, high-fructose corn syrup; PHA, polyhydroxyalkanoate; PGA, poly-γ-glutamate; 1,3-PDO, 1,3-propanediol.

(Lee et al., 2011). Figure 1.1 illustrates the concept of a corn biorefinery based on the wet milling process.

In the corn wet milling process, the corn grain is first steeped in warm and acidic water to loosen the gluten bond within the corn. After steeping, the germ is separated from the corn to extract corn oil. The corn and water slurry from germ separation is grinded again to release starch and gluten from the fiber in the corn kernel. The fiber can be used as animal feed or be converted into fermentable sugars. Gluten obtained from the starch–gluten suspension by centrifugation can be used as animal feed. Some of the starch is dried and marketed as unmodified cornstarch, some is modified into specialty starch, and most of starch is converted into syrups and dextrose. Dextrose can be converted to chemicals, fuels, or enzymes by microbial fermentation (see Fig. 1.1). Various products from corn are shown in Table 1.1.

In corn wet milling, many by-products including corn fiber, corn gluten meal, and corn steep liquor (CSL) can be used to produce ethanol and other value-added products. Corn fiber contains starch (~24%), cellulose (~15%), hemicellulose (~35%), protein, and oil (Gaspar et al., 2007). Corn fiber can be treated by hot water, dilute acid hydrolysis, and enzymatic hydrolysis to convert starch, cellulose, and hemicellulose into glucose, xylose, and arabinose, which can be used for ethanol production (Dien, 2005). In addition, xylose can be converted to xylitol via a biological reaction (Moon et al., 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

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

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

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 Ozbek, 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 polylactic 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

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

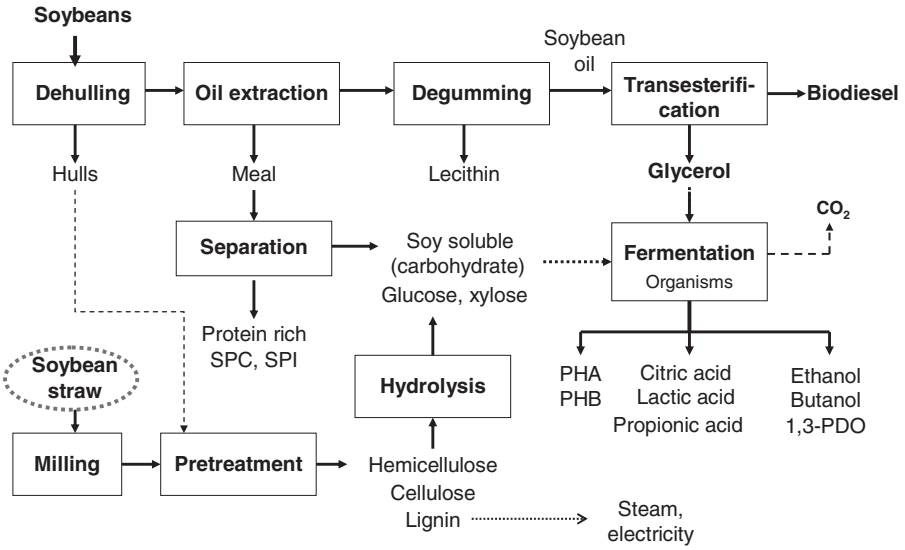


Figure 1.2 Integrated soybean biorefinery for biodiesel production from soybean oil and chemical production from glycerol and other soybean by-products. SPC, soy protein concentrate; SPI, soy protein isolate; PHA, polyhydroxyalkanoate; PHB, poly(3-hydroxybutyric acid); 1,3-PDO, 1,3-propanediol.

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

Components	wt % (Dry Basis)	Products
Oil	~21	Biodiesel and glycerol, which can be converted to various chemicals
Protein	~40	Food or feed products, pharmaceuticals, adhesives, plastics and coating
Carbohydrate	~34	Chemicals and biofuels
Fiber	~5	Feed products

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), glyceric acid, 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).

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

	Soybean Meal (%)	Soybean Protein Concentrate (%)	Soybean Protein Isolate (%)
Protein	48	64	92
Fat	0.3	0.3	0.5
Fiber	3.0	4.5	<1
Carbohydrate	30	15	–
Ash	7	7	4
Moisture	10	10	<5

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 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).

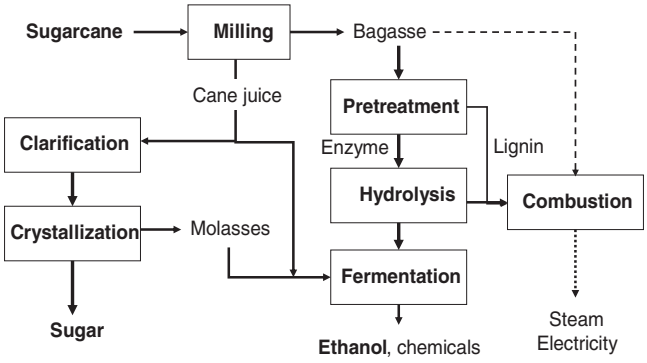


Figure 1.3 Integrated sugarcane biorefinery for fuel and chemical production from cane juice, molasses, and bagasse.

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 directed 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 cellodextrins 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 (SSCF) (Lynd et al., 2002, 2005; Ohgren et al.,

TABLE 1.5. Organic Components of Some Lignocellulosic Biomass (Dry Basis, wt %)

Feedstock	Cellulose	Hemicellulose	Lignin	Other
Sugarcane bagasse	40	24	25	11
Corn stover	40	25	17	18
Corncob	39	35	15	11
Corn fiber	15	35	8	42[a]
Cotton stalk	31	11	30	–
Rice straw	35	25	12	28
Soybean straw	25	12	18	45
Wheat bran	10–15	35–39	8.3–12.5	–
Wheat straw	38.2	21.2	23.4	17.2
Wheat chaff	38	36	16	11
Grasses	25–40	35–50	10–30	–
Switch grass	45	31	12	12
Hard wood (hybrid poplar)	44.7	18.6	26.4	10.3
Soft wood (pine)	44.6	21.9	27.7	5.8
Waste paper	76	13	11	0

[a]Including 23.7% starch.
Source: Yang (2007).

2006). Nevertheless, extensive energy-intensive thermochemical 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 pretreated and hydrolyzed to simple sugars in order to be converted to chemicals and biofuels by microorganisms. 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, and (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-

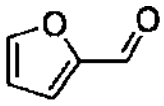
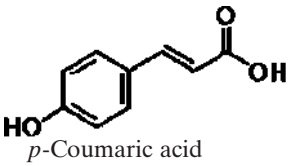
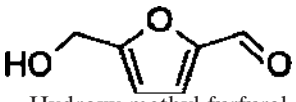
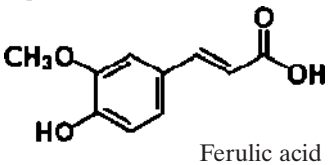
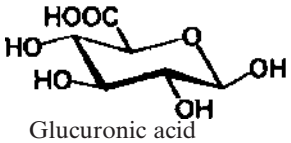
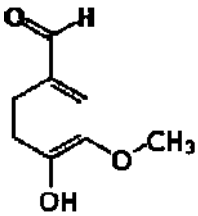
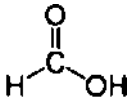
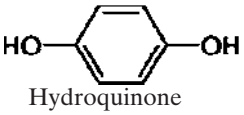
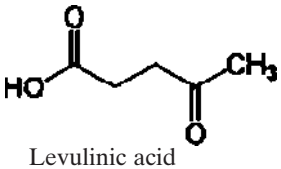
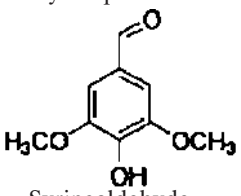
TABLE 1.6. Some Pretreatment Methods for Lignocellulosic Biomass

Pretreatment Methods	Operating Conditions	Advantages and Disadvantages
Steam explosion	Uses steam at 210–290°C, 20–50 bar for 2 minutes, followed by sudden pressure release	Simple and high downstream enzymatic efficiency High energy requirement, low xylose yield of 45–65%, and less efficient for soft wood
Liquid hot water	Uses compressed hot water 200–230°C for up to 15 minutes	High xylose yield of 88–98% and high downstream enzymatic efficiency High energy requirement
Ammonia fiber explosion (AFEX)	Uses liquid ammonia (5–15%) and steam explosion (160–180°C) for 10–30 minutes	Simple and high downstream enzymatic efficiency High cost of ammonia and less efficient for high-content lignin biomass
Acid hydrolysis	Uses 0.5–1.5% H_2SO_4 or HCl at 160–220°C for several minutes	High xylose yield of 75–90%, current industrial method Requires neutralization before hydrolysis and generates some toxic by-products
Alkali hydrolysis	Uses lime or NaOH at lower temperatures and pressures for a longer time (hours)	High downstream enzymatic efficiency Long time
Biological pretreatment	Uses fungi for several days	Simple and low energy requirement Low yield and long time

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 CO_2 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 H_2SO_4 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 1.7. Some Potential Inhibitors from Lignocellulose after Thermochemical Pretreatment

Sugar Degradation Products	Lignin Degradation Products
 Furfural	 *p*-Coumaric acid
 Hydroxy methyl furfural (HMF)	 Ferulic acid
 Glucuronic acid	 Vanillin
 Formic acid	 Hydroquinone
 Levulinic acid	 Syringaldehyde

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 bioconversion 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

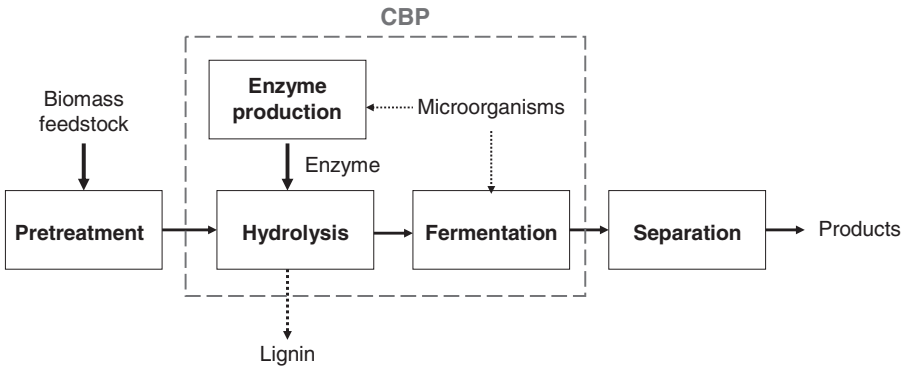


Figure 1.4 General bioprocess flow sheet in lignocellulosic biorefinery for fuel and chemical production. The consolidated bioprocess (CBP) combines enzyme production, hydrolysis, and fermentation in one operation step as indicated by the dashed box.

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 cellulases and hemicellulases 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 cellulosome (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 cellulosome could be constructed by expressing cohesin of *S. cerevisiae* and hydrolyzing subunit of cellulases from *C. thermocellum*, *C. cellulolyticum*, and *Ruminococcus flavifaciens* to form cellulosome on the surface of *S. cerevisiae* (Tsai et al., 2010; Wen et al., 2009). These heterogeneous cellulosomes 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, CO_2, H_2, CH_4), 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 CO_2 and H_2 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/m^2 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

(Demirbas and Demirbas, 2011; Gao et al., 2012; Weyer et al., 2010).

However, current microalgae 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 CO_2 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 microalgae cultures expensive to justify the relatively high production costs for the relatively low-priced biofuels. Therefore, the microalgae 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 microalgae 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 microalgae 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

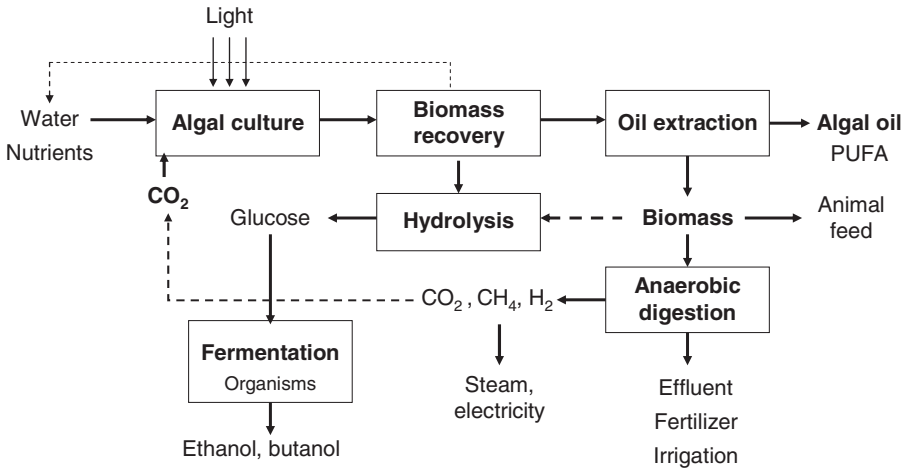


Figure 1.5 Integrated microalgae biorefinery for production of algal oil (biodiesel) and other value-added products. Some microalgae are rich in carbohydrate and can be harvested and used as feedstock for fermentation to produce ethanol and other chemicals. PUFA, polyunsaturated fatty acid.

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.

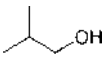
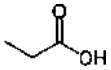
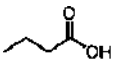
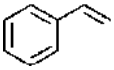
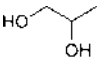
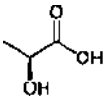
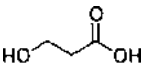
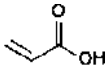
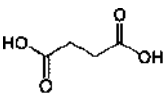
Microbial biosynthesis of C_{13} to C_{17} alkanes and alkenes, which can be used in diesel fuel, in *E. coli* expressing an acyl–acyl carrier protein reductase and an aldehyde decarbonylase in the alkane biosynthesis pathway natively present in some cyanobacteria has also been demonstrated (Schirmer 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 (C_{24}–C_{31}) 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, $C_{15}H_{24}$) 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 ($C_{15}H_{24}$), 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

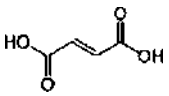
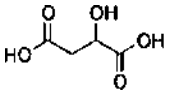
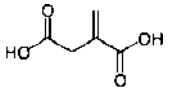
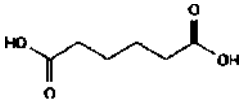
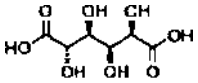
Globally, over 80 million tons of industrial chemicals valued at over $2 trillion is manufactured each year from petroleum-based feedstocks. As chemicals usually have a higher value than fuels and yet have a relatively larger market than nutritional products, the biorefinery industry is focusing more 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 1.8. Some Building Block Chemicals from Biorefineries

Chemical (Annual Production)[a]	Structure	Microorganism/Process[b]	Titer (g/L)	Productivity (g/L h)	Yield (g/g)	Reference
Monofunctional						
Ethanol		*S. cerevisiae*	131	1.71	0.467	Liu et al. (2012)
(67,200,000 MT; 85.2 billion gallons)		*Z. mobilis*	~120	~3.5	~0.5	Lin and Tanaka (2006)
		E. coli (glucose/xylose)	54/42	1.1/0.87	>0.5	Ohta et al. (1991a)
n-Propanol		*E. coli*	10.8	0.144	0.107	Jun et al. (2012)
Isopropanol		*E. coli*	40.1	0.67	0.24	Inokuma et al. (2010)
n-Butanol		*C. acetobutylicum*	20.3	0.37	0.23	Lu et al. (2012)
(2,800,000 MT)		*E. coli*	15	0.2	0.35	Shen et al. (2011)
Isobutanol		*E. coli*	22	0.19	0.34	Atsumi et al. (2008)
Acetone		*C. acetobutylicum*	10.2	0.19	0.12	Lu et al. (2012)
(5,700,000 MT)		*E. coli*	7.1	0.15	0.17	May et al. (2012)
Acetic acid		*Clostridium thermoaceticum*	100	0.8	0.8	Parekh and Cheryan (1994)
(~10,000,000 MT)		*Clostridium formicoaceticum*	78	0.95	0.95	Huang et al. (1998)
Propionic acid		*Propionibacterium acidipropionici*	68.9	1.55	0.48	Liang et al. (2012)
(180,000 MT)			97	0.05	0.54	Zhang and Yang (2009)
Butyric acid		*Clostridium tyrobutyricum*	86.9	1.10	0.46	Jiang et al. (2011)
(80,000 MT)						
Styrene		*E. coli*	0.26	–	–	McKenna and Nielsen (2011)
(6,800,000 MT)						
Multifunctional						
1,3-Propanediol		*E. coli*	135	3.5	0.51	Kaur et al. (2012)
(120,000 MT)		*C. butyricum* (glycerol)	93.7	3.3	0.51	Wilkens et al. (2012)
1,2-Propanediol		*E. coli* (glycerol)	5.6	0.078	0.21	Clomburg and Gonzalez (2011)
(1,500,000 MT)		*T. thermosaccharolyticum*	9	0.36	0.24	Sanchez-Riera et al. (1987)
1,4-Butanediol		*E. coli*	–	–	–	Burk (2010)
(1,360,000 MT)						
2,3-Butanediol		*K. pneumoniae*	150	4.21	0.43	Ma et al. (2009)
(1,250,000 MT)		*K. oxytoca*	130	1.64	0.48	Ji et al. (2010)
		Serratia marcescens	152	2.67	0.41	Zhang et al. (2010)
Lactic acid		*Lactobacillus delbrueckii*	135	3.4	0.9	Kadam et al. (2006)
(450,000 MT)		*E. coli*	138	3.54	0.99	Zhu et al. (2007)
3-Hydroxypropionic acid		*E. coli* (glycerol)	38.7	0.54	0.35	Rathnasingh et al. (2009)
		K. pneumoniae	24.4	0.18	1.02	Huang et al. (2012)
Acrylic acid		Dehydration of lactic acid or 3-hydroxypropionic acid	–	–	–	Straathof et al. (2005)
(4,200,000 MT)						
Succinic acid		*Anaerobiosprillum succiniciproducens*	83	10.4	0.88	Meynial-Salles et al. (2008)
(30,000 MT)		*Corynebacterium glutamicum*	146	3.2	0.9	Okino et al. (2008)
		E. coli	86.6	0.9	0.92	Jantama et al. (2008)

(*Continued*)

TABLE 1.8. (*Continued*)

Chemical (Annual Production)[a]	Structure	Microorganism/Process[b]	Titer (g/L)	Productivity (g/L h)	Yield (g/g)	Reference
Fumaric acid (90,000 MT)		*Rhizopus oryzae*	92	4.25	0.85	Cao et al. (1996)
		Rhizopus arrhizus	130	0.92	1.0	Ling and Ng (1989)
Malic acid (200,000 MT)		*Aspergillus flavus*	113	0.59	0.94	Battat et al. (1991)
		Zygosaccharomyces rouxii	75	0.52	0.40	Taing and Taing (2007)
		E. coli	69	0.69	1.04	Jantama et al. (2008)
Itaconic acid (80,000 MT)		*Aspergillus terreus*	82.3	0.57	0.54	Yahiro et al. (1995)
Adipic acid (2,600,000 MT)		*E. coli* fermentation of glucose to *cis*, *cis*-muconic acid, followed by hydrogenation	36.8	0.77	0.18	Niu et al. (2002); Polen et al. (2012)
Glucaric acid (42,000 MT)		*E. coli*	1.13	0.016	0.153	Moon et al. (2009)
			2.37	0.049	–	Moon et al. (2010)
Isoprene (80,000 MT)		*E. coli*	60	2	0.11	Cervin et al. (2009)
Putrescine (10,000 MT)		*E. coli*	24.2	0.75	0.168	Qian et al. (2009)
Cadaverine (1,600,000 MT)		*E. coli*	9.61	0.32	0.131	Qian et al. (2011)

[a]*Sources*: Almeida et al. (2012); Lee et al. (2011); Zeng and Sabra (2011).
[b]Fermentation with glucose as substrate, unless otherwise indicated.
MT, metric tons.

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 C_7–C_{15} that can be used in diesel and jet fuels (Huber et al., 2005). Xing et al. (2010) used a similar process to produce primarily C_{13} and C_{12} 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 C_5–C_{11} ketones by a palladium-catalyzed alkylation, with the overall carbon yield of up to ~58%. The C_5–C_{11} 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

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 bioethanol obtained 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.

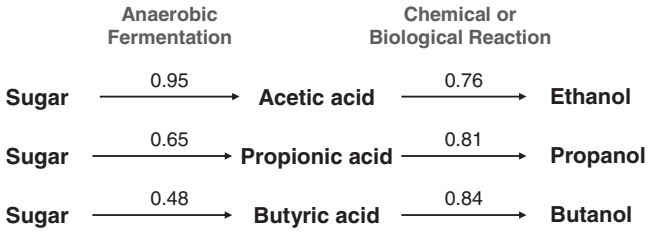


Figure 1.6 Two-step processes for the production of ethanol, propanol, and butanol from sugar. Numbers above arrows indicate the product yields (wt/wt) from each step.

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

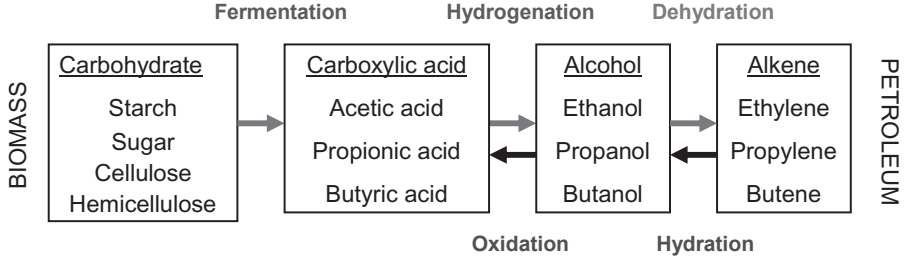


Figure 1.7 Production of some carboxylic acids and alcohols from biomass in biorefinery or from alkene in petroleum refinery.

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 (CO_2) 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

Aden A, Ruth M, Ibsen K, Jechura J, Neeves K, Sheehan J, Wallace K, Montague L, Slayton A, Lukas J (2002). Lignocellulosic biomass to ethanol process design and economics utilizing co-current dilute acid prehydrolysis and enzymatic hydrolysis for corn stover. National Renewable Energy Laboratory, Golden, CO, NREL/TP-510-32438.

Agarwal L, Dutt K, Meghwanshi GK (2008). Anaerobic fermentative production of lactic acid using cheese whey and corn steep liquor. Biotechnol. Lett. 30:631–635.

Agrawal M, Mao Z, Chen RR (2011). Adaptation yields a highly efficient xylose fermenting *Zymomonas mobilis* strain. Biotechnol. Bioeng. 108:777–785.

Aithani D, Mohanty AK (2006). Value-added new materials from byproduct of corn based ethanol industries: Blends of plasticized corn gluten meal and poly(ε-caprolactone). Ind. Eng. Chem. Res. 45:6147–6152.

Almeida JRM, Fávaro LCL, Quirino BF (2012). Biodiesel biorefinery: Opportunities and challenges for microbial production of fuels and chemicals from glycerol waste. Biotechnol. Biofuels 5:48.

Alper H, Moxley J, Nevoigt E, Fink GR, Stephanopoulos G (2006). Engineering yeast transcription machinery for improved ethanol tolerance and production. Science 314:1565–1568.

Al-Zuhair S (2007). Production of biodiesel: Possibilities and challenges. Biofuels Bioprod. Bioref. 1:57–66.

Amartey S, Jeffries TW (1994). Comparison of corn steep liquor with other nutrients in the fermentation of D-xylose by *Pichia stipitis* CBS 6054. Biotechnol. Lett. 16:211–214.

Anand P, Saxena RK (2012). A comparative study of solvent-assisted pretreatment of biodiesel derived crude glycerol on growth and 1,3-propanediol production from *Citrobacter freundi*. New Biotechnol. 29:199–205.

Anbarasan P, Baer ZC, Sreekumar S, Gross E, Binder JB, Blanch HW, Clark DC, Toste FD (2012). Integration of chemical catalysis with extractive fermentation to produce fuels. Nature 491:235–239.

Apar DK, Ozbek B (2007). Hydrolysis and solubilization of corn gluten by Neutrase. J. Chem. Technol. Biotechnol. 82:1107–1114.

Ashiuchi M, Misono H (2002). Biochemistry and molecular genetics of poly-gamma-glutamate synthesis. Appl. Microbiol. Biotechnol. 59:9–14.

Atsumi S, Hanai T, Liao JC (2008). Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels. Nature 451:86–89.

Balat M, Balat H, Oz C (2008). Progress in bioethanol processing. Prog. Energy Combust. Sci. 34:551–573.

Baral A, Bakshi BR (2010). Thermodynamic metrics for aggregation of natural resources in life cycle analysis: Insight via application to some transportation fuels. Environ. Sci. Technol. 44:800–807.

Barbirato F, Chedaille D, Bories A (1997). Propionic acid fermentation from glycerol: Comparison with conventional substrates. Appl. Microbiol. Biotechnol. 47:441–446.

Basu P (2010). Biomass gasification and pyrolysis practical design. Elsevier, Burlington, MA.

Battat E, Peleg Y, Bercovitz A, Rokem JS, Goldberg I (1991). Optimization of L-malic acid production by *Aspergillus flavus* in a stirred fermentor. Biotechnol. Bioeng. 27: 1108–1116.

Bayer EA, Lamed R, White BA, Flint HJ (2008). From cellulosomes to cellulosomics. Chem. Rec. 8:364–377.

Becker J, Boles E (2003). A modified *Saccharomyces cerevisiae* strain that consumes L-arabinose and produces ethanol. Appl. Environ. Microbiol. 69:4144–4150.

Behzadi S, Farid M (2007). Review: Examining the use of different feedstock for the production of biodiesel. Asia Pac. J. Chem. Eng. 2:480–486.

Berk Z (1992). Technology of production of edible flours and protein products from soybeans. FAO Agric. Serv. Bull. pp. 1–10.

Beval MW, Fransse MCR (2006). Investing in green and white biotech. Nat. Biotechnol. 24:765–767.

Bormann EJ, Roth M (1999). The production of polyhydroxybutyrate by *Methylobacterium rhodesianum* and *Ralstonia eutropha* in media containing glycerol and casein hydrolysates. Biotechnol. Lett. 21:1059–1063.

Bozell JJ, Petersen GR (2010). Technology development for the production of biobased products from biorefinery carbohydrates—The US Department of Energy's "Top 10" revisited. Green Chem. 12:539–554.

Brehmer B, Sanders J (2009). Assessing the current Brazilian sugarcane industry and directing developments for maximum fossil fuel mitigation for the international petrochemical market. Biofuels Bioprod. Bioref. 3:347–360.

Brennan TCR, Datta S, Blanch HW, Simmons BA, Holmes BM (2010). Recovery of sugars from ionic liquid biomass liquor by solvent extraction. Bioenerg. Res. 3:123–133.

Brumbley SM, Purnell MP, Petrasovits LA, Nielsen LK, Twine PH (2007). Developing the sugarcane biofactory for high-value biomaterials. Int. Sugar J. 109:5–15.

Burk MJ (2010). Sustainable production of industrial chemicals from sugars. Int. Sugar J. 112:30–35.

Cao NJ, Du JX, Gong CS, Tsao GT (1996). Simultaneous production and recovery of fumaric acid from immobilized *Rhizopus oryzae* with a rotary biofilm contactor and an adsorption column. Appl. Environ. Microbiol. 62:2926–2931.

Castoldi MC, Camaraa LDT, Monteiro RS (2007). Experimental and theoretical studies on glucose hydrogenation to produce sorbitol. React. Kinet. Catal. Lett. 91:341–352.

Cervin MA, Whited GM, Chotani GK, Valle F, Fioresi C, Sanford KJ, et al. (2009). Compositions and methods for producing isoprene. US patent application, US 2009/0203102 A1.

Cherubini F (2010). The biorefinery concept: Using biomass instead of oil for producing energy and chemicals. Energy Convers. Manag. 51:1412–1421.

Choi YB, Rhee JS, Lee YB (2004). Extraction of isoflavones from soybean hypocotyl using aqueous ethanol. Food Sci. Biotechnol. 13:719–723.

Clomburg JM, Gonzalez R (2011). Metabolic engineering of *Escherichia coli* for the production of 1,2-propanediol from glycerol. Biotechnol. Bioeng. 108:867–879.

Consonni S, Katofsky RE, Larson ED (2010). A gasification-based biorefinery for the pulp and paper industry. Chem. Eng. Res. Des. 87:1293–1317.

Costa JAV, de Morais MG (2011). The role of biochemical engineering in the production of biofuels from microalgae. Bioresour. Technol. 102:2–9.

Curran KA, Alper HS (2012). Expanding the chemical palate of cells by combining systems biology and metabolic engineering. Metab. Eng. 14:289–297.

De Azeredo LAI, De Lima MB, Coelho RRR (2006). A low-cost fermentation medium for thermophilic protease production by *Streptomyces* sp. 594 using feather meal and corn steep liquor. Curr. Microbiol. 53:335–339.

Demain AL, Newcomb M, Wu JHD (2005). Cellulase, clostridia, and ethanol. Microbiol. Mol. Biol. Rev. 69:124–154.

Demirbas A (2005). Biodiesel production from vegetable oils via catalytic and non-catalytic supercritical methanol transesterification methods. Prog. Energy Combust. Sci. 31:466–487.

Demirbas A, Demirbas MF (2011). Importance of algae oil as a source of biodiesel. Energy Convers. Manag. 52:163–170.

Demirbas MF (2009). Biorefineries for biofuel upgrading: A critical review. Appl. Energy 86:S151–S161.

Den Haan R, Rose SH, Lynd LR, van Zyl WH (2007). Hydrolysis and fermentation of amorphous cellulose by recombinant *Saccharomyces cerevisiae*. Metab. Eng. 9:87–94.

Dhamankar H, Prather KLJ (2011). Microbial chemical factories: Recent advances in pathway engineering for synthesis of value added chemicals. Curr. Opin. Struct. Biol. 21:488–494.

Dharmadi Y, Murarka A, Gonzalez R (2006). Anaerobic fermentation of glycerol by *Escherichia coli*: A new platform for metabolic engineering. Biotechnol. Bioeng. 94:821–829.

Dias MO, Junqueira TL, Cavalett O, Cunha MP, Jesus CD, Rossell CE, Maciel Filho R, Bonomi A (2012). Integrated

versus stand-alone second generation ethanol production from sugarcane bagasse and trash. Bioresour. Technol. 103:152–161.

Dien B (2005). Enzymes generate sugars from corn fiber. Ind. Bioproc. 27:3–4.

Ding SY, Himmel ME (2006). The maize primary cell wall microfibril: A new model derived from direct visualization. J. Agric. Food Chem. 54:597.

Dishisha T, Ståhl A, Lundmark S, Hatti-Kaul R (2012). An economical biorefinery process for propionic acid production from glycerol and potato juice using high cell density fermentation. Bioresour. Technol. doi:dx.doi.org/10.1016/j.biortech.2012.08.098.

Doleyres Y, Beck P, Vollenweider S, Lacroix C (2005). Production of 3-hydroxypropionaldehyde using a two-step process with *Lactobacillus reuteri*. Appl. Microbiol. Biotechnol. 68:467–474.

Du C, Lin SKC, Koutinas A (2007). Succinic acid production from wheat using a biorefining strategy. Appl. Microbiol. Biotechnol. 76:1263–1270.

Duan YK, Zhu Z, Cai K, Tan XM, Lu XF (2011). De novo biosynthesis of biodiesel by *Escherichia coli* in optimized fed-batch cultivation. PLoS One 6:e20265.

Eggink G, Eenink AH, Huizing HJ (1994). PHB-producing microorganism and process for removing glycerol from a culture medium. WO9409146.

Elbahloul Y, Steinbüchel A (2010). Pilot-scale production of fatty acid ethyl esters by an engineered *Escherichia coli* strain harboring the p(Microdiesel) plasmid. Appl. Environ. Microbiol. 76:4560–4565.

Emptage M, Haynie SL, Laffend LA, Pucci JP, Whited G (2003). Process for the biological production of 1,3-propanediol with high titer. US Patent 6514733.

Erickson DB (1995). Practical handbook of soybean processing and utilization. AOCS Press, Champaign, IL.

Ezeji H, Blaschek HP (2008). Fermentation of dried distillers' grains and solubles (DDGS) hydrolysates to solvents and value-added products by solventogenic *Clostridia*. Bioresour. Technol. 99:5232–5242.

Fabien E, Deswarte I, Clark JH, Wilson AJ, Hardy JE, Marriott R, Chahal SP, Jackson C, Heslop G, Birkett M, Bruce TJ, Whiteley G (2007). Toward an integrated straw-based biorefinery. Biofuels Bioprod. Bioref. 1:245–254.

Friedl A (2012). Lignocellulosic biorefinery. Environ. Eng. Manag. J. 11:75–79.

Fujita Y, Ito J, Ueda M, Fukuda H, Kondo A (2004). Synergistic saccharification, and direct fermentation to ethanol, of amorphous cellulose by use of an engineered yeast strain codisplaying three types of cellulolytic enzyme. Appl. Environ. Microbiol. 70:1207–1212.

Ganjyal GM, Reddy N, Yang YQ, Hanna MA (2004). Biodegradable packaging foams of starch acetate blended with corn stalk fibers. J. Appl. Polym. Sci. 93:2627–2633.

Gao Y, Gregor C, Liang Y, Tang D, Tweed C (2012). Algae biodiesel—A feasibility report. Chem. Cent. J. 6:S1.

Gaspar M, Kalman G, Reczey K (2007). Corn fiber as a raw material for hemicellulose and ethanol production. Process Biochem. 42:1135–1139.

Gennadios A (2002). Protein-based films and coatings. CRC Press, Boca Raton, FL.

Goh CS, Lee KT (2010). A visionary and conceptual macroalgae based third-generation bioethanol (TGB) biorefinery in Sabah, Malaysia as an underlay for renewable and sustainable development. Renew. Sust. Energ. Rev. 14:842–848.

Good AG, Shrawat AK, Meunch DG (2004). Can less yield more? Is reducing nutrient input into the environment compatible with maintaining crop production? Trends Plant Sci. 12:597.

Greer D (2005). Creating cellulosic ethanol. Spinning straw into fuel. Biocycle 46:61–65.

Gregg DJ, Boussaid A, Saddler JN (1998). Techno-economic evaluations of a generic wood-to-ethanol process: Effect of increased cellulose yields and enzyme recycle. Bioresour. Technol. 63:7–12.

Guedon E, Desvaux M, Petitdemange H (2002). Improvement of cellulolytic properties of *Clostridium cellulolyticum* by metabolic engineering. Appl. Environ. Microbiol. 68:53–58.

Gupta B, Revagade N, Hilborn J (2007). Poly(lactic acid) fiber: An overview. Prog. Polym. Sci. 32:455–482.

Haas MJ, McAloon AJ, Yee WC, Foglia TA (2006). A process model to estimate biodiesel production costs. Bioresour. Technol. 97:671–678.

Hahn-Hagerdal B, Karhumaa K, Fonseca C, Spencer-Martins I, Gorwa-Grauslund MF (2007). Towards industrial pentose-fermenting yeast strains. Appl. Microbiol. Biotechnol. 74:937–953.

Hamelinck CN, van Hooijdonk G, Faaij APC (2005). Ethanol from lignocellulosic biomass: Techno-economic performance in short-, middle- and long-term. Biomass Bioenergy 28:384–410.

He YQ, Tan TW (2006). Use of response surface methodology to optimize culture medium for production of lipase with *Candida* sp 99-125. J. Mol. Catal. B Enzym. 43:9–14.

Higashide W, Li H, Yang Y, Liao J (2011). Metabolic engineering of *Clostridium cellulolyticum* for production of isobutanol from cellulose. Appl. Environ. Microbiol. 77:2727–2733.

Himmel ME, Ding SY, Johnson DK, Adney WS, Nimlos MR, Brady JW, Foust TD (2007). Biomass recalcitrance: Engineering plants and enzymes for biofuels production. Science 315:804.

Huang Y, Li Z, Shimizu K, Ye Q (2012). Simultaneous production of 3-hydroxypropionic acid and 1,3-propanediol from glycerol by a recombinant strain of *Klebsiella pneumoniae*. Bioresour. Technol. 103:351–359.

Huang YL, Mann K, Novak JM, Yang ST (1998). Acetic acid production from fructose by *Clostridium formicoaceticum* in a fibrous-bed bioreactor. Biotechnol. Prog. 14:800–806.

Huber GW, Chheda JN, Barrett CJ, Dumesic JA (2005). Production of liquid alkanes by aqueous-phase processing of biomass-derived carbohydrates. Science 308:1446–1450.

Inokuma K, Liao JC, Okamoto M, Hanai T (2010). Improvement of isopropanol production by metabolically engineered *Escherichia coli* using gas stripping. J. Biosci. Bioeng. 110:696–701.

Jang Y-S, Park JM, Choi S, Choi YJ, Seung DY, Cho JH, Lee SY (2012). Engineering of microorganisms for the production of biofuels and perspectives based on systems metabolic engineering approaches. Biotechnol. Adv. 30:989–1000.

Jantama K, Haupt MJ, Svoronos SA, Zhang XL, Moore JC, Shanmugam KT, Ingram LO (2008). Combining metabolic engineering and metabolic evolution to develop nonrecombinant strains of *Escherichia coli* C that produce succinate and malate. Biotechnol. Bioeng. 99:1140–1153.

Jennert KCB, Tardiff C, Young DI, Young M (2000). Gene transfer to *Clostridium cellulolyticum* ATCC 35319. Microbiology 146:3071–3080.

Jeong G-T, Park D-H (2010). Production of sugars and levulinic acid from marine biomass *Gelidium amansii*. Appl. Biochem. Biotechnol. 161:41–52.

Jerez A, Partal P, Martinez I, Gallegos C, Guerrero A (2005). Rheology and processing of gluten based bioplastics. Biochem. Eng. J. 26:131–138.

Ji XJ, Huang H, Zhu JG, Ren LJ, Nie ZK, Du J, et al. (2010). Engineering *Klebsiella oxytoca* for efficient 2,3-butanediol production through insertional inactivation of acetaldehyde dehydrogenase gene. Appl. Microbiol. Biotechnol. 85:1751–1758.

Ji XJ, Huang H, Ouyang P-K (2012). Microbial 2,3-butanediol production: A state-of-the-art review. Biotechnol. Adv. 29:351–364.

Jiang L, Wang J, Liang S, Cai J, Xu Z, Cen P, Yang S, Li S (2011). Enhanced butyric acid tolerance and bioproduction by *Clostridium tyrobutyricum* immobilized in a fibrous bed bioreactor. Biotechnol. Bioeng. 108:31–40.

Jing ZP, Gallardo F, Pascual MB, Sampalo R, Romero J, de Navarra AT, Canovas FM (2004). Improved growth in a field trial of transgenic hybrid poplar overexpressing glutamine synthetase. New Phytol. 164:137.

Jørgensen H, Kristensen JB, Felby C (2007). Enzymatic conversion of lignocellulose into fermentable sugars: Challenges and opportunities. Biofuels Bioprod. Bioref. 1:119–134.

Jun CY, Park JH, Kim TY, Lee SY (2012). Metabolic engineering of *Escherichia coli* for the production of 1-propanol. Metab. Eng. 14:477–486.

Kadam SR, Patil SS, Bastawde KB, Khire JA, Gokhale DV (2006). Strain improvement of *Lactobacillus delbrueckii* NCIM 2365 for lactic acid production. Process Biochem. 41:120–126.

Kalman G, Recseg K, Gaspar M, Reczey K (2006). Novel approach of corn fiber utilization. Appl. Biochem. Biotechnol. 129–132:738–750.

Kalscheuer R, Stolting T, Steinbuchel A (2006). Microdiesel: *Escherichia coli* engineered for fuel production. Microbiology 152:2529–2536.

Kamm B, Kamm M (2004a). Biorefinery systems. Chem. Biochem. Eng. Q. 18:1–6.

Kamm B, Kamm M (2004b). Principles of biorefineries. Appl. Microbiol. Biotechnol. 64:137–145.

Kamm B, Hille C, Schonicke P, Dautzenberg G (2010). Green biorefinery demonstration plant in Havelland (Germany). Biofuels Bioprod. Bioref. 4:253–262.

Katahira S, Fujita Y, Mizuike A, Fukuda H, Kondo A (2004). Construction of a xylan-fermenting yeast strain through codisplay of xylanolytic enzymes on the surface of xylose-utilizing *Saccharomyces cerevisiae* cells. Appl. Environ. Microbiol. 70:5407–5414.

Kaur G, Srivastava AK, Chand S (2012). Advances in biotechnological production of 1,3-propanediol. Biochem. Eng. J. 64:106–118.

Kim JM, Whang JH, Kim KM, Koh JH, Suh HJ (2004). Preparation of corn gluten hydrolysate with angiotensin I converting enzyme inhibitory activity and its solubility and moisture sorption. Process Biochem. 39:989–994.

Klapatch TR, Guerinot ML, Lynd LR (1996). Electrotransformation of *Clostridium thermosaccharolyticum*. J. Ind. Microbiol. 16:342–347.

Koutinas A, Wang R-H, Webb C (2007). The biochemurgist—Bioconversion of agricultural raw materials for chemical production. Biofuels Bioprod. Bioref. 1:24–38.

Kumar P, Barrett DM, Delwiche MJ, Stroeve P (2009). Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. Ind. Eng. Chem. Res. 48:3713–3729.

Kumar R, Choudhary V, Mishra S, Varma IK, Mattiason B (2002). Adhesives and plastics based on soy protein products. Ind. Crops Prod. 16:155–172.

Kunkes EL, Simonetti DA, West RM, Serrano-Ruiz JC, Gärtner CA, Dumesic JA (2008). Catalytic conversion of biomass to monofunctional hydrocarbons and targeted liquid-fuel classes. Science 322:417–421.

Kurian JV (2005). A new polymer platform for the future—Sorona® from corn derived 1,3-propanediol. J. Polym. Environ. 13:159–167.

Kuyper M, Hartog MMP, Toirkens MJ, Almering MJH, Winkler AA, Van Dijken JP, Pronk JT (2005). Metabolic engineering of a xyloseisomerase-expressing *Saccharomyces cerevisiae* strain for rapid anaerobic xylose fermentation. FEMS Yeast Res. 5:399–409.

Lakaniemi A-M, Tuovinen OH, Puhakka JA (2012). Anaerobic conversion of microalgal biomass to sustainable energy carriers—A review. Bioresour. Technol. doi:10.1016/j.biortech.2012.08.096.

Lange JP (2007). Lignocellulose conversion: An introduction to chemistry, process and economics. In: Centi G, van Santen R, eds. Catalysis for renewables. Wiley-VCH, Weinheim.

Lau MW, Gunawan C, Balan V, Dale BE (2010). Comparing the fermentation performance of *Escherichia coli* KO11, *Saccharomyces cerevisiae* 424A(LNH-ST) and *Zymomonas mobilis* AX101 for cellulosic ethanol production. Biotechnol. Biofuels 3:11.

Lee D, Yu AHC, Saddler JN (1995). Evaluation of cellulase recycling strategies for the hydrolysis of lignocellulosic substrates. Biotechnol. Bioeng. 45:328–336.

Lee DH (2011). Algal biodiesel economy and competition among biofuels. Bioresour. Technol. 102:43–49.

Lee JO, Park MH, Choi YH (2007). New fermentation technique for complete digestion of soybean protein. J. Microbiol. Biotechnol. 17:1904–1907.

Lee JW, Kim HU, Choi S, Yi J, Lee SY (2011). Microbial production of building block chemicals and polymers. Curr. Opin. Biotechnol. 22:758–767.

Lee PC, Lee WG, Lee SY, Chang HN (2001). Succinic acid production with reduced by-product formation in the fermentation of *Anaerobiospirillum succiniciproducens* using glycerol as a carbon source. Biotechnol. Bioeng. 72:41–48.

Li L, Zhou Y, Cheng X, Sun J, Marita JM, Ralph J, Chiang VL (2003). Combinatorial modification of multiple lignin traits in trees through multigene cotransformation. Proc. Natl. Acad. Sci. U.S.A. 100:4939.

Liang Z, Li L, Li S, Cai Y, Yang S-T, Wang J (2012). Enhanced propionic acid production from Jerusalem artichoke hydrolysate by immobilized *Propionibacterium acidipropionici* in a fibrous-bed bioreactor. Bioprocess Biosyst. Eng. 35:915–921.

Lin Y, Tanaka S (2006). Ethanol fermentation from biomass resources: Current state and prospects. Appl. Microbiol. Biotechnol. 69:627–642.

Ling LB, Ng TK (1989). Fermentation process for carboxylic acids. US Patent 4877731.

Liu C-G, Wang N, Lin Y-H, Bai F-W (2012). Very high gravity ethanol fermentation by flocculating yeast under redox potential-controlled conditions. Biotechnol. Biofuels 5:61.

Liu HJ, Du W, Liu DH (2007). Progress of the biodiesel and 1,3-propanediol integrated production. Progr. Chem. 19:1185–1189.

Lu C, Zhao J, Yang ST, Wei D (2012). Fed-batch fermentation for butanol production from cassava bagasse hydrolysate in a fibrous bed bioreactor with continuous gas stripping. Bioresour. Technol. 104:380–387.

Lynd LR, Weimer PJ, van Zyl WH, Pretorius IS (2002). Microbial cellulose utilization: Fundamentals and biotechnology. Microbiol. Mol. Biol. Rev. 66:506–577.

Lynd LR, van Zy WH, McBride JE, Laser M (2005). Consolidated bioprocessing of cellulosic biomass: An update. Curr. Opin. Biotechnol. 16:577–583.

Ma C, Wang A, Qin J, Li L, Ai X, Jiang T, Tang H, Xu P (2009). Enhanced 2,3-butanediol production by *Klebsiella pneumoniae* SDM. Appl. Microbiol. Biotechnol. 82:49–57.

Mai V, Lorenz WW, Wiegel J (1997). Transformation of *Thermoanaerobacterium* sp. strain JW/SL-YS485 with plasmid pIKM1 conferring kanamycin resistance. FEMS Microbiol. Lett. 148:163–167.

Mandl MG (2010). Status of green biorefining in Europe. Biofuels Bioprod. Bioref. 4:268–274.

Marchetti JM, Miguel VU, Errazu AF (2007). Possible methods for biodiesel production. Renew. Sustain. Energy Rev. 11:1300–1311.

May A, Fischer R-J, Thumb SM, Schaffer S, Verseck S, Durre P, Bahl H (2012). A modified pathway for the production of acetone in *Escherichia coli*. Metab. Eng. doi:10.1016/j.ymben.2012.08.001.

McKenna R, Nielsen DR (2011). Styrene biosynthesis from glucose by engineered *E. coli*. Metab. Eng. 13:544–554.

Mendez-Perez D, Begemann MB, Pfleger BF (2011). Modular synthase-encoding gene involved in alpha-olefin biosynthesis in *Synechococcus* sp. strain PCC 7002. Appl. Environ. Microbiol. 77:4264–4267.

Meynial-Salles I, Dorotyn S, Soucaille P (2008). A new process for the continuous production of succinic acid from glucose at high yield, titer, and productivity. Biotechnol. Bioeng. 99:129–135.

Moon KH, Lee WJ, Kim JH (2002). Biological production of xylitol by *Candida tropicalis* and recombinant *Saccharomyces cerevisiae* containing xylose reductase gene. In: Marten MR, Park TH, Nagamune T, eds. Biological systems engineering. American Chemical Society, Washington, DC, pp. 53–68.

Moon TS, Yoon SH, Lanza AM, Roy-Mayhew JD, Prather KLJ (2009). Production of glucaric acid from a synthetic pathway in recombinant *Escherichia coli*. Appl. Environ. Microbiol. 75:4660–14660.

Moon TS, Dueber JE, Shiue E, Prather KLJ (2010). Use of modular, synthetic scaffolds for improved production of glucaric acid in engineered *E. coli.* Metab. Eng. 12:298–305.

Moore GRP, do Canto LR, Amante ER, Soldi V (2005). Cassava and corn starch in maltodextrin production. Quim. Nova 28:596–600.

Mosier N, Wyman C, Dale B, Elander R, Lee YY, Holtzapple M, Ladisch M (2005). Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour. Technol. 96:673–686.

Munasinghe PC, Khanal SK (2010). Biomass-derived syngas fermentation into biofuels: Opportunities and challenges. Bioresour. Technol. 101:5013–5022.

Nakamura CE, Whited GM (2003). Metabolic engineering for the microbial production of 1,3-propanediol. Curr. Opin. Biotechnol. 14:454–459.

Nass LL, Pereira PAA, Ellis D (2007). Biofuels in Brazil: An overview. Crop Sci. 47:2228–2237.

Nel S (2010). The potential of biotechnology in the sugarcane industry: Are you ready for the next evolution? Int. Sugar J. 112:11–16.

Nishiyama Y, Langan P, Chanzy H (2002). Crystal structure and hydrogen-bonding system in cellulose I from synchrotron

X-ray and neutron fiber diffraction. J. Am. Chem. Soc. 124:9074.

Niu W, Draths KM, Frost JW (2002). Benzene-free synthesis of adipic acid. Biotechnol Prog. 18:201–211.

Nonato RV, Mantelatto PE, Rossell CEV (2001). Integrated production of biodegradable plastic, sugar and ethanol. Appl. Microbiol. Biotechnol. 57:1–5.

Offermann R, Seidenberger T, Thrän D, Kaltschmitt M, Zinoviev S, Miertus S (2011). Assessment of global bioenergy potentials. Mitig. Adapt. Strateg. Glob. Change 16:103–115.

Ohgren K, Bengtsson O, Gorwa-Grauslund MF, Galbe M, Hahn-Hagerdal B, Zacchi G (2006). Simultaneous saccharification and co-fermentation of glucose and xylose in steam-pretreated corn stover at high fiber content with *Saccharomyces cerevisiae* TMB3400. J. Biotechnol. 126:488–498.

Ohta K, Beall DS, Mejia JP, Shanmugam KT, Ingram LO (1991a). Genetic improvement of *Escherichia coli* for ethanol production—Chromosomal integration of *Zymomonas mobilis* genes encoding pyruvate decarboxylase and alcohol dehydrogenase II. Appl. Environ. Microbiol. 57:893–900.

Ohta K, Beall DS, Mejia JP, Shanmugam KT, Ingram LO (1991b). Metabolic engineering of *Klebsiella oxytoca* M5A1 for ethanol production from xylose and glucose. Appl. Environ. Microbiol. 57:2810–2815.

Okino S, Noburyu R, Suda M, Jojima T, Inui M, Yukawa H (2008). An efficient succinic acid production process in a metabolically engineered *Corynebacterium glutamicum* strain. Appl. Microbiol. Biotechnol. 81:459–464.

Olson DG, McBride JE, Shaw AJ, Lynd LR (2011). Recent progress in consolidated bioprocessing. Curr. Opin. Biotechnol. 23:396–405.

Omoni AO, Aluko RE (2005). Soybean foods and their benefits: Potential mechanisms of action. Nutr. Rev. 63:272–283.

Orecchioni A-M, Duclairoir C, Renard D, Nakache E (2006). Gliadin characterization by sans and gliadin nanoparticle growth modelization. J. Nanosci. Nanotechnol. 6:3171–3178.

Papanikolaou S, Ruiz-Sanchez P, Pariset B, Blanchard F, Fick M (2000). High production of 1,3-propanediol from industrial glycerol by a newly isolated *Clostridium butyricum* strain. J. Biotechnol. 77:191–208.

Parekh SR, Cheryan M (1994). High concentrations of acetate with a mutant strain of *C. thermoaceticum*. Biotechnol. Lett. 16:139–142.

Park SJ, Choi J, Lee SY (2005). Engineering of *Escherichia coli* fatty acid metabolism for the production of polyhydroxyalkanoates. Enzyme Microb. Technol. 36:579–588.

Peralta-Yahya PP, Ouellet M, Chan R, Mukhopadhyay A, Keasling JD, Lee TS (2011). Identification and microbial production of a terpene-based advanced biofuel. Nat. Commun. 2:483.

Peralta-Yahya PP, Zhang F, del Cardayre SB, Keasling JD (2012). Microbial engineering for the production of advanced biofuels. Nature 488:320–328.

Perlack RD, Wright LL, Turhollow AF, Graham RL, Stokes BJ, Erbach DC (2005). Biomass as feedstock for a bioenergy and bioproducts industry: The technical feasibility of a billion-ton annual supply. U.S. Department of Energy & U.S. Department of Agriculture, Oak Ridge, TN.

Perrard A, Gallezot P, Joly JP (2007). Highly efficient metal catalysts supported on activated carbon cloths: A catalytic application for the hydrogenation of D-glucose to D-sorbitol. Appl. Catal. A Gen. 331:100–104.

Peterson D, Haase S (2009). Market assessment of biomass gasification and combustion technology for small- and medium-scale applications. U.S. Department of Energy Technical Report NREL/TP-7A2-46190.

Polen T, Spelberg M, Bott M (2012). Toward biotechnological production of adipic acid and precursors from biorenewables. J. Biotechnol. doi:10.1016/j.jbiotec.2012.07.008.

Pu Y, Zhang D, Singh PM, Ragauskas AJ (2008). The new forestry biofuels sector. Biofuels Bioprod. Bioref. 2:58–73.

Qian ZG, Xia XX, Lee SY (2009). Metabolic engineering of *Escherichia coli* for the production of putrescine: A four carbon diamine. Biotechnol. Bioeng. 104:651–662.

Qian ZG, Xia XX, Lee SY (2011). Metabolic engineering of *Escherichia coli* for the production of cadaverine: A five carbon diamine. Biotechnol. Bioeng. 108:93–103.

Qureshi N, Saha BC, Hector RE, Cotta MA, Hughes SR (2008). Butanol production from wheat straw by simultaneous saccharification and fermentation using *Clostridium beijerinckii*: Part I—Batch fermentation. Biomass Bioenergy 32:168–175.

Ragauskas AJ (2006). The path forward for biofuels and biomaterials. Science 311:484.

Ragauskas AJ, Nagy M, Kim DH, Eckert CA, Hallett JP, Liotta CL (2006). From wood to fuels: Integrating biofuels and pulp production. Ind. Biotechnol. 2:55–65.

Ralph J, Akiyama T, Kim H, Lu F, Schatz PF, Marita JM, Ralph SA, Reddy MSS, Chen F, Dixon RA (2006). Effects of coumarate 3-hydroxylase down-regulation on lignin structure. J. Biol. Chem. 281:8843–8853.

Ramos LP, Breuil C, Saddler JN (1993). The use of enzyme recycling and the influence of sugar accumulation on the cellulose hydrolysis by *Trichoderma* cellulases. Enzyme Microb. Technol. 15:19–25.

Rathnasingh C, Raj SM, Jo JE, Park S (2009). Development and evaluation of efficient recombinant *Escherichia coli* strains for the production of 3-hydroxypropionic acid from glycerol. Biotechnol. Bioeng. 104:729–739.

Reddy CSK, Ghai R, Rashmi, Kalia VC (2003). Polyhydroxyalkanoates: An overview. Bioresour. Technol. 87:137–146.

Reddy N, Yang Y (2005). Biofibers from agricultural byproducts for industrial applications. Trends Biotechnol. 23:22–27.

Rostagno MA, Araujo JMA, Sandi D (2002). Supercritical fluid extraction of isoflavones from soybean flour. Food Chem. 78:111–117.

Rude MA, Baron TS, Brubaker S, Alibhai M, Del Cardayre SB, Schirmer A (2011). Terminal olefin (1-alkene) biosynthesis by a novel P450 fatty acid decarboxylase from *Jeotgalicoccus* species. Appl. Environ. Microbiol. 77:1718–1727.

Ryu S, Karim MN (2011). A whole cell biocatalyst for cellulosic ethanol production from dilute acid-pretreated corn stover hydrolysates. Appl. Microbiol. Biotechnol. 91:529–542.

Samarasinghe S, Easteal AJ, Edmonds NR (2008). Biodegradable plastic composites from corn gluten meal. Polym. Int. 57:359–364.

Sanchez-Riera F, Cameron DC, Cooney CL (1987). Influence of environmental factors in the production of R(S)-1,2-propanediol by *Clostridium thermosaccharolyticum*. Biotechnol. Lett. 9:449–454.

Schenk PM, Thomas-Hall SR, Stephens E, Marx UC, Mussgnug JH, Posten C, Kruse O, Hankamer B (2008). Second generation biofuels: High-efficiency microalgae for biodiesel production. Bioenerg. Res. 1:20–43.

Schirmer A, Rude MA, Li XZ, Popova E, del Cardayre SB (2010). Microbial biosynthesis of alkanes. Science 329:559–562.

Schlosser S, Blahušiak M (2011). Biorefinery for production of chemicals, energy and fuels. Elektroenergetika 4:8–16.

Shaw AJ, Podkaminer KK, Desai SG, Bardsley JS, Rogers SR, Thorne PG, Hogsett DA, Lynd LR (2008). Metabolic engineering of a thermophilic bacterium to produce ethanol at high yield. Proc. Natl. Acad. Sci. U.S.A. 105:13769–13774.

Shen CR, Lan EI, Dekishima Y, Baez A, Cho KM, Liao JC (2011). Driving forces enable high-titer anaerobic 1-butanol synthesis in *Escherichia coli*. Appl. Environ. Microbiol. 77:2905–2915.

Shigechi H, Koh J, Fujita Y, Matsumoto T, Bito Y, Ueda M, Satoh E, Fukuda H, Kondo A (2004). Direct production of ethanol from raw corn starch via fermentation by use of a novel surface-engineered yeast strain codisplaying glucoamylase and α-amylase. Appl. Environ. Microbiol. 70:5037–5040.

Shih I-L, Van Y-T (2000). The production of poly-(γ-glutamic acid) from microorganisms and its various applications. Bioresour. Technol. 79:207–225.

Shukla R, Cheryan M (2001). Zein: The industrial protein from corn. Ind. Crops Prod. 13:171–192.

Simmons BA, Loque D, Blanch HW (2008). Next-generation biomass feedstocks for biofuel production. Genome Biol. 9:242.

Singh A, Kumar PKR, Schugerl K (1991). Adsorption and reuse of cellulases during saccharification of cellulosic materials. J. Biotechnol. 19:205–212.

Solange IM, Dragone G, Guimaraes PMR, Silva JPA, Carneiro LM, Roberto IC, Vicente A, Domingues L, Teixeira JA (2010). Technological trends, global market, and challenges of bio-ethanol production. Biotechnol. Adv. 28:817–830.

Steen EJ, Kang YS, Bokinsky G, Hu ZH, Schirmer A, McClure A, del Cardayre SB, Keasling JD (2010). Microbial production of fatty acid-derived fuels and chemicals from plant biomass. Nature 463:182–559.

Stephanopoulos G (2007). Challenges in engineering microbes for biofuels production. Science 315:801–804.

Sticklen M (2006). Plant genetic engineering to improve biomass characteristics for biofuels. Curr. Opin. Biotechnol. 17:315–319.

Straathof AJJ, Sie S, Franco TT, van der Wielen LAM (2005). Feasibility of acrylic acid production by fermentation. Appl. Microbiol. Biotechnol. 67:727–734.

Strobel HP, Lynn B (2004). Proteomic analysis of ethanol sensitivity in *Clostridium thermocellum*. In Proceeding of the National Meeting of the American Society of Microbiology, May 23–27. New Orleans, LA. Abstract number 0-094.

Sukovich DJ, Seffernick JL, Richman JE, Hunt KA, Gralnick JA, Wackett LP (2010). Structure, function, and insights into the biosynthesis of a head-to-head hydrocarbon in *Shewanella oneidensis* strain MR-1. Appl. Environ. Microbiol. 76:3842–3849.

Sun N, Rahman M, Qin Y, Maxim ML, Rodríguez H, Rogers RD (2009). Complete dissolution and partial delignification of wood in the ionic liquid 1-ethyl-3-methylimidazolium acetate. Green Chem. 11:646–655.

Sun Y, Cheng J (2002). Hydrolysis of lignocellulosic materials for ethanol production: A review. Bioresour. Technol. 83:1–11.

Sung M-H, Park C, Kim C-J, Poo H, Soda K, Ashiuchi M (2005). Natural and edible biopolymer poly-γ-glutamic acid: Synthesis, production, and applications. Chem. Rec. 5:352–366.

Szuhaj BF (1989). Lecithins: Sources, manufacture, and uses. American Oil Chemists' Society, Champaign, IL.

Taing O, Taing K (2007). Production of malic and succinic acids by sugar-tolerant yeast *Zygosaccharomyces rouxii*. Eur. Food Res. Technol. 224:343–347.

Tanyildizi MS, Ozer D, Elibol M (2007). Production of bacterial α-amylase by *B. amyloliquefaciens* under solid substrate fermentation. Biochem. Eng. J. 37:294–297.

Tardif C, Maamar H, Balfin M, Belaich JP (2001). Electrotransformation studies in *Clostridium cellulolyticum*. J. Ind. Microbiol. Biotechnol. 16:1–4.

The National Academy of Sciences (2007). Water implications of biofuels production in the United States. Report in brief.

Thomsen MH (2005). Complex media from processing of agricultural crops for microbial fermentation. Appl. Microbiol. Biotechnol. 68:598–606.

Tilman D, Hill J, Lehman C (2006). Carbon-negative biofuels from low-input high-diversity grassland biomass. Science 314:1598–1600.

Torney F, Moeller L, Scarpa A, Wang K (2007). Genetic engineering approaches to improve bioethanol production from maize. Curr. Opin. Biotechnol. 18:193–199.

Tsai SL, Goyal G, Chen W (2010). Surface display of a functional minicellulosome by intracellular complementation using a synthetic yeast consortium and its application to cellulose hydrolysis and ethanol production. Appl. Environ. Microbiol. 76:7514–7520.

Tyurin M, Desai SG, Lynd LR (2004). Electrotransformation of *Clostridium thermocellum*. Appl. Environ. Microbiol. 70:883–890.

Van Camp W (2005). Yield enhancement genes: Seeds for growth. Curr. Opin. Biotechnol. 16:147.

Vancauwenberge JE, Slininger PJ, Bothast RJ (1990). Bacterial conversion of glycerol to 3-hydroxypropionaldehyde. Appl. Environ. Microbiol. 56:329–332.

Van Walsum GP, Allen SG, Spencer MJ, Laser MS, Antal MJ, Lynd LR (1996). Conversion of lignocellulosics pretreated with liquid hot water to ethanol. Appl. Biochem. Biotechnol. 57(58):157–170.

Wang C, Yoon S-H, Jang H-J, Chung Y-R, Kim J-Y, Choi E-S, Kim S-W (2011). Metabolic engineering of *Escherichia coli* for a farnesene production. Metab. Eng. 13:648–655.

Wang QJ, Chen SW, Zhang JB (2008). Co-producing lipopeptides and poly-gamma-glutamic acid by solid-state fermentation of *Bacillus subtilis* using soybean and sweet potato residues and its biocontrol and fertilizer synergistic effects. Bioresour. Technol. 99:3318–3323.

Wen F, Sun J, Zhao H (2009). Yeast surface display of trifunctional minicellulosomes for simultaneous saccharification and fermentation of cellulose to ethanol. Appl. Environ. Microbiol. 76:1251–1260.

Weng J-K, Li X, Bonawitz ND, Chapple C (2008). Emerging strategies of lignin engineering and degradation for cellulosic biofuel production. Curr. Opin. Biotechnol. 19:166–172.

Westervelt R (2004). DuPont and Tate & Lyle form joint venture for propanediol production. Chem. Week 166:10.

Weyer KM, Bush DR, Darzins A, Willson BD (2010). Theoretical maximum algal oil production. Bioenerg. Res. 3:204–213.

Wilkens E, Ringel AK, Hortig D, Willke T, Vorlop K-D (2012). High-level production of 1,3-propanediol from crude glycerol by *Clostridium butyricum* AKR102a. Appl. Microbiol. Biotechnol. 93:1057–1063.

Winkelhausen E, Kuzmanova S (1998). Microbial conversion of D-xylose to xylitol. J. Ferment. Bioeng. 86:1–14.

Wright MM, Brown RC (2007). Comparative economics of biorefineries based on the biochemical and thermochemical platforms. Biofuels Bioprod. Bioref. 1:49–56.

Wyman CE, Dale BE, Elander RT, Holtzapple M, Ladisch MR, Lee YY (2005a). Coordinated development of leading biomass pretreatment technologies. Bioresour. Technol. 96:1959–1966.

Wyman CE, Dale BE, Elander RT, Holtzapple M, Ladisch MR, Lee YY (2005b). Comparative sugar recovery data from laboratory scale application of leading pretreatment technologies to corn stover. Bioresour. Technol. 96:2026–2032.

Xing R, Subrahmanyam AV, Olcay H, Qi W, van Walsum GP, Pendse H, Huber GW (2010). Production of jet and diesel fuel range alkanes from waste hemicellulose-derived aqueous solutions. Green Chem. 12:1933–1946.

Yahiro K, Takahama T, Park YS, Okabe M (1995). Breeding of *Aspergillus terreus* mutant Tn-484 for itaconic acid production with high-yield. J. Ferment. Bioeng. 79:506–508.

Yang B, Wyman CE (2008). Pretreatment: The key to unlocking low-cost cellulosic ethanol. Biofuels Bioprod. Bioref. 2:26–40.

Yang F, Hanna MA, Sun R (2012). Value-added uses for crude glycerol—A byproduct of biodiesel production. Biotechnol. Biofuels 5:13.

Yang ST (2007). Bioprocessing—From biotechnology to biorefinery. Chapter1. In: Yang ST, ed. Bioprocessing for value-added products from renewable resources. Elsevier, New York, pp. 1–24.

Yang ST (2008). Methods of producing butanol. US Patent 20080248540.

Yazdani SS, Gonzalez R (2007). Anaerobic fermentation of glycerol: A path to economic viability for the biofuels industry. Curr. Opin. Biotechnol. 18:213–219.

Ye XP, Julson J, Kuo M, Womac A, Myers D (2007). Properties of medium density fiberboards made from renewable biomass. Bioresour. Technol. 98:1077–1084.

Yu KO, Jung J, Kim SW, Park CH, Han SO (2011). Synthesis of FAEEs from glycerol in engineered *Saccharomyces cerevisiae* using endogenously produced ethanol by heterologous expression of an unspecific bacterial acyltransferase. Biotechnol. Bioeng. 109:110–115.

Yu L, Dean K, Li L (2006). Polymer blends and composites from renewable resources. Prog. Polym. Sci. 31:576–602.

Yuan WJ, Chang BL, Ren JG, Liu JP, Bai FW, Li YY (2012). Consolidated bioprocessing strategy for ethanol production from Jerusalem artichoke tubers by *Kluyveromyces marxianus* under high gravity conditions. J. Appl. Microbiol. 112:38–44.

Zaldivar J, Nielsen J, Olsson L (2001). Fuel ethanol production from lignocellulose: A challenge for metabolic engineering and process integration. Appl. Microbiol. Biotechnol. 56:17–34.

Zavrel M, Bross D, Funke M, Büchs J, Spiess AC (2009). High throughput screening for ionic liquids dissolving (ligno-) cellulose. Bioresour. Technol. 100:2580–2587.

Zeng A-P, Sabra W (2011). Microbial production of diols as platform chemicals: Recent progresses. Curr. Opin. Biotechnol. 22:749–757.

Zhang A, Yang S-T (2009). Engineering *Propionibacterium acidipropionici* for enhanced propionic acid tolerance and fermentation. Biotechnol. Bioeng. 45:79–386.

Zhang LY, Sun JA, Hao YL, Zhu JW, Chu J, Wei DZ, et al. (2010). Microbial production of 2,3-butanediol by a surfactant (serrawettin)-deficient mutant of *Serratia marcescens* H30. J. Ind. Microbiol. Biotechnol. 37:857–862.

Zhang YHP, Himmel ME, Mielenz JR (2006). Outlook for cellulase improvement: Screening and selection strategies. Biotechnol. Adv. 24:452–481.

Zhao H, Baker GA, Song Z, Olubajo O, Crittle T, Peters D (2008). Designing enzyme-compatible ionic liquids that can dissolve carbohydrates. Green Chem. 10:696–705.

Zhou S, Davis FC, Ingram LO (2001). Gene integration and expression and extracellular secretion of *Erwinia chrysanthemi* endoglucanase CelY (celY) and CelZ (celZ) in ethanologenic *Klebsiella oxytoca* P2. Appl. Environ. Microbiol. 67:6–14.

Zhu Y, Eiteman MA, DeWitt K, Altman E (2007). Homolactate fermentation by metabolically engineered *Escherichia coli* strains. Appl. Environ. Microbiol. 73:456–464.

Zverlov VV, Berezina O, Velikodvorskaya GA, Schwarz WH (2006). Bacterial acetone and butanol production by industrial fermentation in the Soviet Union: Use of hydrolyzed agricultural waste for biorefinery. Appl. Microbiol. Biotechnol. 71:587–597.