BIOPROCESSING TECHNOLOGIES
IN BIOREFINERY FOR
SUSTAINABLE PRODUCTION
OF FUELS, CHEMICALS,
AND POLYMERS
BIOPROCESSING TECHNOLOGIES IN BIOREFINERY FOR SUSTAINABLE PRODUCTION OF FUELS, CHEMICALS, AND POLYMERS

Edited by

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In June 2008, crude oil prices had rapidly increased to a high of over $130 per barrel from ~$20 per barrel in 2002. Since then we have seen a resurgent interest in biofuels and rapid development of the biorefinery industry worldwide. While the oil prices have fluctuated wildly between $80 and $100 in the last 2 years, public interest in biofuels and bio-based chemicals remains high because of their green nature and sustainability. Biomass represents an abundant carbon-neutral renewable feedstock for production of fuels and chemicals, replacing fossil fuels and petrochemicals. Current biorefineries use corn, soybeans, and sugarcane for bioethanol and biodiesel production, which can benefit from integrated biorefining that extracts high-value nutritional products while using the main feedstock component for biofuels and chemicals production and further converting low-value by-products to additional marketable products such as energy (heat and electricity) and animal feed. Lignocellulosic biomass, including forestry and agricultural residues, is the second-generation feedstock in biorefineries and offers the opportunity to meet 30% of U.S. fuel and chemical needs by 2030. In addition, aquacultures of micro- and macro-algae, which use CO₂ and sunlight for their growth, could provide all of the future fuel needs without affecting the current agriculture land use. With continuing developments and advances in new energy crops, aquaculture, synthetic biology for cell engineering, and conversion technologies, biorefining will play an increasingly 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 also many challenges facing the biorefinery industry. Today, lignocellulosic refining is not yet economical because of the recalcitrant nature of the feedstock and high costs in pretreatment and enzymatic hydrolysis of cellulose. Processes using microalgae with photosynthesis for cell growth and oil production are difficult to scale up and are far from economical. To achieve sustainable and economical production of biofuels and biobased chemicals, new advances in process engineering and metabolic engineering for biomass conversion will be required. Furthermore, a biorefinery should utilize all components of biomass feedstock to produce energy, fuels, and chemicals to maximize product values, minimize waste generation, and improve process economics, which requires the integration of technologies from various areas, including new energy crops with higher biomass yields and better processability, better and cheaper enzymes for hydrolysis, novel and improved cells and catalysts for biomass conversion to fuels, chemicals, and other marketable products, and more efficient processes for the production of these bio-based products at a commercial scale.

This book provides a comprehensive review of bioprocessing technologies important to corn, soybean, and lignocellulosic biomass-based biorefineries for production of ethanol, biodiesel, chemicals, and other value-added products. The first chapter gives an overview of the concept of and current trends in integrated biorefineries. The book then provides an overview of various biomass feedstocks, including current sugar- and starch-rich crops, new energy crops rich in oils, sugars, and/or polysaccharides, and microalgae (Chapters 2–5), followed by a chapter focusing on pretreatment technologies for lignocellulosic biomass in a biorefinery. Chapters 7–10 cover important hydrolytic enzymes used in biorefineries for the hydrolysis of starch and lignocelluloses and provides a detailed review of enzyme source, type and characteristics, protein and genetic engineering of
the enzymes, and their industrial production and applications. Chapters 11–15 cover bioconversion technologies for current and future biofuels, including ethanol, biodiesel, butanol, hydrogen, biogas, and other advanced biofuels. Chapters 16–23 cover some of the most important specialty chemicals, building block chemicals, and biopolymers that can be produced via fermentation. Metabolic engineering and novel fermentation process technologies for economical production of these chemicals are reviewed and discussed in sufficient detail to allow both experts and nonexperts to comprehend recent progress in this field. Finally, Chapter 24 discusses phytochemicals and functional food ingredients that can be extracted from plant materials.

We started working on this book project in 2008 when the oil price had just peaked. From the inception to the completion of this book, we have experienced many ups and downs just like those in the global economics and oil prices. We are very grateful to all the contributing authors, who are leading experts in their respective research fields from the United States, Europe, and Asia. Without their contributions, this book would not have been finished for publication. We also deeply appreciate the efforts, patience, and understanding of our editor at Wiley.

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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 Blahušiač, 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. Kouitinas 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 high-value fuels and chemicals. For example, a straw-based...
biorefinery was developed to produce high-value wax products using a supercritical CO₂ extraction technology along with a number of chemicals and energy (Fabien et al., 2007). Therefore, the traditional agricultural processing industry should incorporate the integrated biorefinery concept to minimize the negative impact of biofuel production on food supply while maximizing its revenues.

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

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

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

1.2 BIOREFINERIES USING CORN, SOYBEANS, AND SUGARCANE

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

1.2.1 Corn Refinery

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

The lipo-protein 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 (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 ground 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).

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**Figure 1.1** Integrated corn biorefinery (wet milling). In addition to corn grain, corn cob, 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.

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

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

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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).
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 (Amartey and Jeffries, 1994). Value-added biodegradable high-performance engineering plastics and composites can be produced using corn gluten by plasticizing with glycerol/ethanol and blending with commercial polymers (Aithani and Mohanty, 2006; Jerez et al., 2005; Samarasinghe et al., 2008). Gliadins extracted from gluten have been investigated to produce nanosized colloidal carriers that can ensure a controlled and targeted drug delivery (Orecchioni et al., 2006). Corn gluten was also used as substrate in solid-state fermentation to produce enzymes (Tanyildizi et al., 2007). Corn proteins extracted from gluten can be used to produce protein-based films and coatings in the food industry (Gennadios, 2002). A tasteless and odorless corn protein isolate with high nutritional values can be extracted as a high-value product from corn germ and used in food and beverage industries.

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

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

### 1.2.2 Soybean Biorefinery

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

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

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

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

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

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

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

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

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.
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 (Doleynes et al., 2005; Vancuwenberge 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, dihydroxyaceton (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).

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 Aluks, 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

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