Chapter 13
Integrated Consolidated Bioprocessing for Conversion of Lignocellulosic Feedstock to Biofuels and Value-Added Bioproducts

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What Will You Learn from This Chapter?
This chapter will provide basic information about consolidated bioprocessing (CBP), including native and recombinant strategies and their application in biofuel production. It will address the integrated CBP process to produce biopolymers (e.g., polyhydroxyalkanoates, extracellular polysaccharides), organic compounds (e.g., 1,3-propanediol), and biogas (methane). The chapter will also discuss the production of biofuels by integrating the CBP process with fuel cells and other bio-electrochemical systems. A detailed discussion will be provided on the thermophilic anaerobic digestion (TAD) process to produce methane from agricultural biomass using thermophilic microorganisms as well as biological oxidation of methane to methanol using methanotrophic bacteria. The chapter will conclude with presenting different approaches in modeling CBP processes for existing applications.

13.1 Introduction

Recent decades witnessed an exponentially increasing trend in industrialization and motorization throughout the world. This has led to rapid depletion of fossil fuels and increase in emission of greenhouse gases (GHGs) and other toxic pollutants in the environment. The use of fuel alternatives to replace the depleting fossil fuels will help to meet the growing energy demand as well as mitigating environmental issues. Biofuels are one of the most promising sources of energy for replacing fossil fuels, and they can supplement solar, wind, and other clean energy resources in the renewable energy industry in the foreseeable future (Krishnaraj and Yu 2014).

Biofuels are the renewable fuel products that are produced from biomass using biological processes. They include biohydrogen, bioethanol, biobutanol, biodiesel, biogas (biomethane), and biomethanol (Barnard et al. 2010). Biofuels are economical and eco-friendly sources of energy for sustainable development, since they provide renewability, biodegradability, and generate cleaner exhaust gases, greatly contributing to lessening the emission of GHGs and improving air quality (Sani and Krishnaraj 2017). The use of lignocellulosic biomass as a feedstock for biofuel production would be an added advantage.

Lignocellulosic biomasses are derived from trees, grass, aquatic plants, agricultural wastes, municipal wastes, and various industrial by-products, which are locally available (Chong et al. 2009; van Maris et al. 2006). They are the structural components of plant cell wall and mainly composed of cellulose, hemicelluloses, and lignin, in the range of 20–50%, 12–50%, and 10–40% (w/w), respectively. The composition of the different lignocellulosic biomass varies with different species. For instance, the composition of cellulose and hemicellulose in hardwood ranges from 40–55% to 24–40%, respectively. In wheat straw, the composition of cellulose and hemicellulose constitutes around 30% and 50% of the biomass, respectively (Bajpai 2016). These lignocellulosic substrates can be utilized as promising feedstocks for liquid and gaseous biofuel production through different bioprocess operations.

Lignocellulosic woody biomasses possess strong physical structure and have high lignin content which make these considerably recalcitrant to degradation by microorganisms (Zhu et al. 2010). The cellulose and hemicellulose in lignocellulosic materials are tightly bound to lignin; therefore, pretreatment processes for delignification and depolymerization are often necessary to obtain fermentable sugar substrates such as pentose and hexose. The lignin constitutes around 27–32% in woody plants and ranges around 14–25% in herbaceous plants (Chen et al. 1996). The lignin content of alfalfa fibers, pine straw, wheat straw, and flax fibers constitutes about 34%, 23%, 20%, and 15%, respectively (Watkins et al. 2015).

Different strategies for the pretreatment of lignocellulosic biomass such as mechanical comminution, pyrolysis, steam explosion, ammonia fiber explosion, carbon dioxide explosion, ozonolysis, acid hydrolysis, and the organosolv process have been reported in the literature (Kumar et al. 2009). However, these pretreatment processes for degradation of lignocellulosic biomass raise serious concerns in upstream processing, including the increased energy requirement leading to decreased overall efficiency of the process and high cost of the product.

The microbial digestibility of the resulting hydrolysates, downstream processing, industrial scalability, and biorefinery protocols can also be affected by the pretreatment processes of lignocellulosic feedstock. Different pretreatment methods also demand sophisticated techniques for chemical recovery and wastewater treatment to comply with certain environmental regulations (Lin and Tanaka 2006; Chong et al. 2009; Zhu et al. 2010). Meanwhile, it is challenging to develop an economical and efficient pretreatment process to degrade lignocellulosic materials. In many cases, the pretreatment processes also generate toxic compounds inhibiting the microbial growth and lessening the rates of bioprocessing and product yield (Talluri et al. 2013). Minimizing the total energy consumption during biofuel production has been considered quite critical to achieve the maximum yield and efficiency.

Some of the above-mentioned limitations can be overcome by the use of extremophiles, which can improve the rates of utilization of lignocellulose as well as improving the efficiency of the bioprocess. The potential of these organisms to thrive over a wide range of operating conditions and to metabolize a broad range of substrate including recalcitrant materials will be an added advantage. Extremophiles are microorganisms that can thrive and mediate catalysis in extreme conditions of temperature, pressure, acidity, alkalinity, or salinity, in which most of the other organisms cannot survive (Rampelotto 2010). They can synthesize stable enzymes and other macromolecules even at harsh environmental conditions. The extremophiles and their enzymes have a broad range of applications. It is now widely accepted that extremophilic microorganisms will provide valuable resources for exploitation in novel biotechnological processes including integrated bioprocesses for biofuel production (Bhalla et al. 2013). There has been a lot of interest worldwide to develop bioprocesses using different kinds of extremophiles to produce different biofuels which include first-, second-, third-, and fourth-generation biofuels. The second-generation biofuels which includes biofuels from nonedible lignocellulosic materials have several advantages over the first-generation biofuels that are produced from food crops. The use of second-generation biofuels helps to

overcome the ethical issues raised by the first-generation biofuels related to conflicts with food supply and agricultural land usage (Barnard et al. 2010). The extremophilic bioprocesses for production of second-generation biofuels from energy crops or lignocellulosic biomass will help to overcome the limitations of the mesophilic bioprocesses, such as poor rates or efficiency.

The extremophilic organisms are promising candidates for integrated bioprocessing because they can mediate a wide range of catalytic reactions. As in the case of any other bioprocess, choosing ideal microorganisms is one of the key requirements for CBP. In an integrated extremophilic bioprocess, different extremophilic bioprocesses are developed and integrated with each other to complete full transformation of feedstocks to the desired products (biofuels), and unutilized fractions can be further bioprocessed to generate other value-added products. Thus, an integrated continuous and sequential process can be developed to produce biofuels and other value-added bioproducts from lignocellulosic wastes in a sustainable and economical manner.

Integrated extremophilic bioprocessing is therefore a higher value approach to microbial biofuel production from lignocellulosic materials and wastes, compared to conventional bioprocesses using lignocellulosic biomass in which the unutilized fractions and by-products are discarded, reducing economic viability and ecological advantage. Different microorganisms are known to produce biogas through a thermophilic aerobic digestion (TAD) process or to produce value-added products such as exopolysaccharides (EPSs), polyhydroxyalkanoates (PHAs), and 1,3-propanediol (1,3-PDO) (Gebreeyessus and Jenicek 2016; Nicolaus et al. 2010; Chen 2009; Saxena et al. 2009). These value-added products can be refined through downstream processing, and they have different applications in different sectors including plastics, biomedical products, food packaging, and pharmaceutical delivery systems.

This chapter will focus on integrated extremophilic bioprocessing to produce biofuels which involves several steps starting from lignocellulosic materials as a primary feedstock. In this processing scheme, CBP will be used as the first step to produce biofuels including biohydrogen, bioethanol, and biobutanol from lignocellulosic feedstocks. These liquid and gaseous biofuels will be transformed to electrical energy using fuel cells/bioelectrochemical systems. The organic by-products and unhydrolyzed fractions from CBP will be further utilized as substrates for production of methane (through TAD process), biopolymers, and 1,3-PDO. The methane obtained can be utilized to generate methanol and lipid with methanotrophs. The latter can be transesterified to produce biodiesel. The glycerol generated from the biodiesel production process as by-product can be fed back as substrate for biopolymer and 1,3-PDO production. The unutilized liquid fraction from TAD and aerobic fermentation of value-added products can also be recycled as nitrogen and water sources. Finally, the heat required by CBP can be generated by combustion of methane and bioH_2 generated through TAD and CBP. This integrated bioprocessing will be an environmentally friendly biofuel producing process with high production efficiency and minimal waste generation, thus increasing the scope of commercialization. A scheme showing an Integrated CBP for production of biofuels and other value-added products is shown in Fig. 13.1.

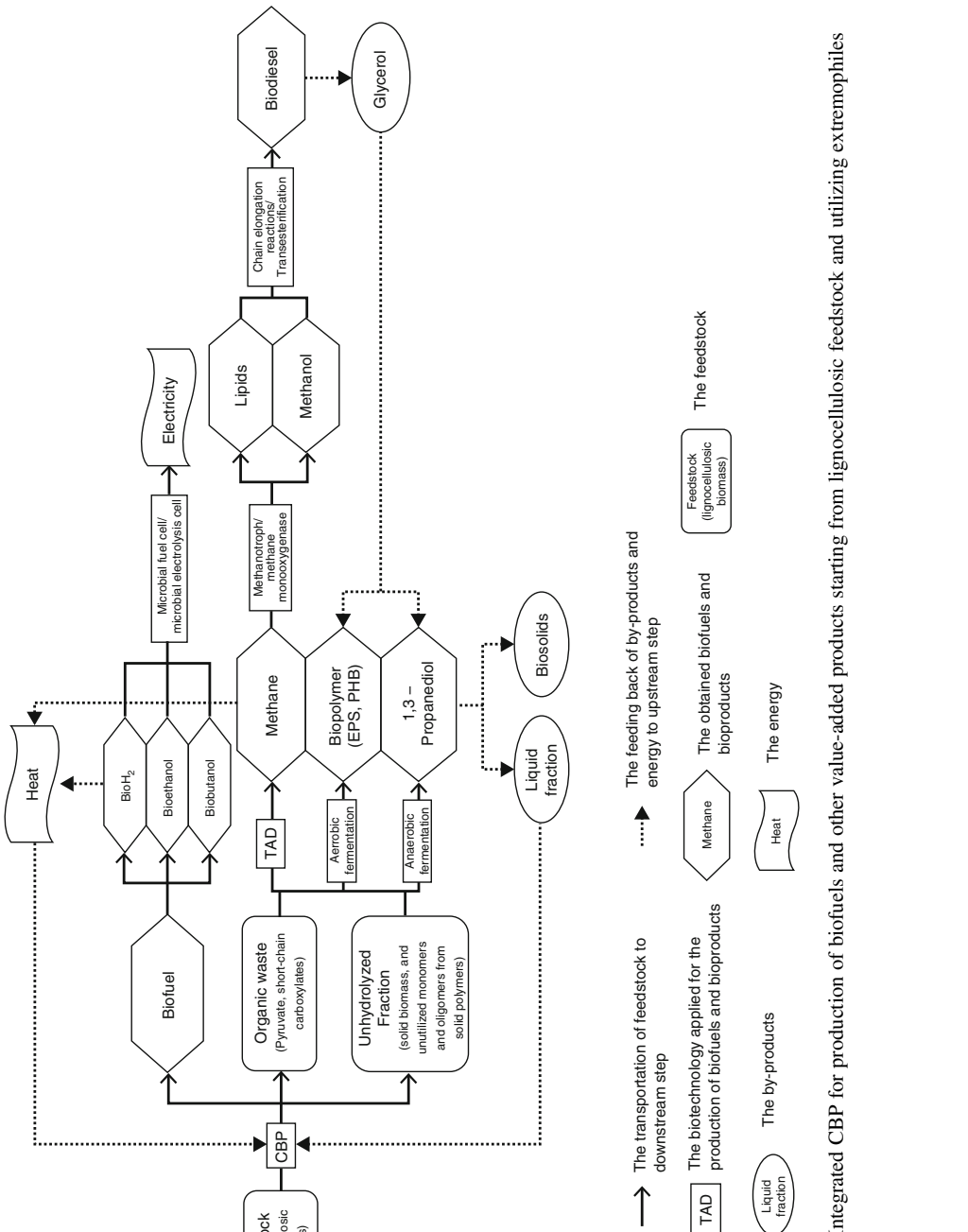

Fig. 13.1 Integrated CBP for production of biofuels and other value-added products starting from lignocellulosic feedstock and utilizing extremophiles

13.2 Consolidated Bioprocessing (CBP) by Extremophilic Microorganisms

CBP is a one pot bioprocessing strategy for the conversion of lignocellulosic substrates into desired products such as biofuels using a single bioreactor. CBP combines enzyme production, cellulose hydrolysis, and fermentation for final products in only one step without adding hydrolytic enzymes. CBP is widely considered as a promising procedure for hydrolysis and fermentation of lignocellulosic woody biomass and wastes with appealingly low cost (Olson et al. 2011; Lynd et al. 2005). Since there is no need to externally supplement enzymes to the system for the hydrolysis of lignocellulosic woody biomass, the processing cost of CBP can be considerably lower than that of separated hydrolyzation and fermentation steps, especially when the enzyme loading for hydrolysis is high. CBP can also attain extensive saving due to high yield of the substrate and feedstock utilization efficiency. CBP using extremophiles, especially thermophiles, is promising because of the high efficacy of lignocellulosic hydrolases, higher operating temperature, and enzyme–microorganism synergy, or combination of these. The cost of CBP can be much lower than that of traditional process for biofuel production from woody biomass. In a traditional process, the hydrolysates of lignocellulose inhibit the lignocellulolytic enzymes, thereby lowering the rates of saccharification. On the other hand, in CBP, the hydrolysates will be fermented immediately and thus the product inhibition for saccharification will be no longer effective. Although CBP has several advantages over traditional processes, especially the use of a single reactor, it has certain challenges. Some operating conditions may not be optimal for both saccharification and fermentation. The organic acids which are mostly generated as by-products in CBP lead to a lower biofuel yield compared with the theoretical maximum value. The heat energy that is required to maintain thermophilic CBP is also relatively intensive. Finally, the leftover lignocellulosic substrates are considered hardly utilizable for further bioprocessing to produce other bioproducts (Mazzoli 2012).

The CBP process has been developed using different thermophilic strains such as *Caldicellulosiruptor saccharolyticus*, *Caldicellulosiruptor bescii*, and *Thermoanaerobacterium thermosaccharolyticum* for biohydrogen production (Talluri et al. 2013; Cao et al. 2014; Cha et al. 2013); *Clostridium thermocellum* and *Thermoanaerobacterium saccharolyticum* for bioethanol production (Schuster and Chinn 2013); and mesophilic strains such as *Caldicellulosiruptor cellulolyticum* and *Clostridium* spp. for biobutanol production (Higashide et al. 2011; Mazzoli 2012). The ideal microorganisms for CBP can express and synthesize different kinds of glycoside hydrolases such as cellulase and xylanase. They help in the enzymatic hydrolysis of lignocellulosic feedstocks to soluble monosaccharides and disaccharides which can be used for biofuel production. All the enzymes involved in CBP should demonstrate proper synergy and can be well coordinated, which means the products from one enzymatic step will not inhibit the activities of any other enzymes for the next several steps. The biosynthesis of hydrogen, ethanol, and butanol takes place through several series of redox biochemical reactions involving the Embden–Meyerhof route and NAD/NADP, ATP, and ferredoxin, which also generates several kinds of organic acids as by-products including lactate, acetate, and butyrate. Higher biofuel yields can

be attained by the repression of competing metabolic pathways coupled with certain biofuel producing pathways. Metabolic engineering strategies are aimed at developing microorganisms with resistance over adverse operating conditions and which can produce biofuels such as hydrogen, ethanol, or butanol at high rates.

13.2.1 The Native Strategy of CBP

The native strategy of CBP makes use of the microorganisms with inherent capability to decompose insoluble lignocellulosic biomass to fermentable monosaccharides (Olson et al. 2011). Several thermophilic strains, such as *Thermoanaerobacter* sp., *Caldicellulosiruptor* sp., *Thermoanaerobacterium saccharolyticum*, and *Geobacillus thermoglucosidasius* have been shown to utilize a broad range of lignocellulosic substrates to produce biofuel with high yield through an anaerobic process which is favorable for CBP (Cao et al. 2014; Talluri et al. 2013; Cha et al. 2013; Bhalla et al. 2013; Argyros et al. 2011; Shaw et al. 2008).

Thermophiles are capable of producing a range of extracellular enzymes and can hydrolyze a broad range of different organic substrates. They can yield high rates of production in a relatively short period when compared with traditional mesophilic fermentation process. Thermophilic microbial strains also possess versatile metabolic pathways which are advantageous for utilizing a wide range of lignocellulosic biomass and waste to produce biohydrogen and other value-added products by anaerobic CBP (Bhalla et al. 2013). Table 13.1 summarizes the different native consolidated extremophilic bioprocesses that are documented in the literature.

Thermophilic anaerobic bacterium *Clostridium thermocellum* can naturally hydrolyze lignocellulosic substrates and utilize the hydrolysates primarily to produce bioethanol and biohydrogen in one single-step conversion. *C. thermocellum* is suitable for CBP since it performs fermentation using lignocellulosic substrate to produce bioethanol and acetic acid as major final products. Although *C. thermocellum* can hardly utilize pentose sugar including xylose from degradation of hemicellulose, CBP can still be carried out with co-cultures of *C. thermocellum*, *C. thermosaccharolyticum*, and *Thermoanaerobacter ethanolicus* (Harish et al. 2010), which makes the whole process promising for continuous and efficient lignocellulose hydrolysis, monosaccharides utilization, and bioethanol production . Ethanol production through co-cultures coupling *C. thermocellum* with *C. themosacharolyticum* using banana waste could attain up to 0.41 g ethanol/g substrate, which is relatively higher compared with that of the single wild type of *C. thermocellum*, which reached 0.08–0.37 g ethanol/g substrate (Harish et al. 2010). The use of thermophilic co-cultures for simultaneous lignocellulosic material hydrolyzation and simple sugar utilization can be a preferred strategy, especially when the whole CBP for biofuel production cannot be accomplished by a single strain.

Caldicellulosiruptor saccharolyticus, an anaerobic and thermophilic strain, can be considered as an ideal candidate for biohydrogen production using CBP as they can efficiently hydrolyze cellulose. This strain could degrade untreated switchgrass

Table 13.1 Consolidated bioprocessing by native extremophiles

Organism	Substrates	Biofuel products	Yield	Operating conditions	Reference
Clostridium thermocellum	Banana agro-waste	Ethanol	0.41 g/g substrate	60 °C	Harish et al. (2010)
Caldicellulosiruptor saccharolyticus	Switchgrass	Hydrogen	11.2 mmol/g substrate	65 °C	Talluri et al. (2013)
Thermoanaerobacterium thermosaccharolyticum	Microcrystalline cellulose, corn cob, corn stalk, and wheat straws	Hydrogen	10.86, 3.27, 3.47, and 3.53 mmol/g substrate	60 °C	Cao et al. (2014)
Clostridium thermocellum	Dried distillers grain, barley hulls, and fusarium head blight contaminated barley hulls	Hydrogen	1.27, 1.24, and 1.18 mmol/g glucose equivalent utilized	60 °C	Magnusson et al. (2008)
Caldicellulosiruptor saccharolyticus	Wheat straw	Hydrogen	3.8 mol/mol glucose equivalent consumed	70 °C	Ivanova et al. (2009)

and micro-crystalline cellulose and produce biohydrogen (23.2 mmol/L) using hydrolysates in a single step (Talluri et al. 2013). Combined saccharification and fermentation for biohydrogen production utilizing lignocellulosic substrate may be developed to provide a breakthrough that would provide a more effective process at lower cost.

Some other thermophilic microorganisms have also been reported as promising strains for CBP of biohydrogen production, due to their ability to degrade untreated woody biomass. *Caldicellulosiruptor saccharolyticus* is a gram-positive thermophilic anaerobic strain, which can utilize various substrates including lignocellulose, starch, pectin, pentose, and hexose for growth. The ability of this organism for simultaneous fermentation utilizing untreated lignocellulosic woody biomass represents an excellent feature of this bacterium for biohydrogen production through CBP (Ivanova et al. 2009).

Clostridium thermocellum, a gram-positive, acetogenic, cellulolytic, thermophilic bacterium, was shown to synthesize a series of cellulolytic enzymes and form cellulosome, which is critical for the bacteria to attach to lignocellulosic feedstock and hydrolyze the polysaccharides to simple sugars such as glucose, which can be utilized for biohydrogen production (Magnusson et al. 2008). Therefore *C. thermocellum*, which represents a group of thermophilic strains producing cellulosome, provides the potential for directly producing biohydrogen through CBP from lignocellulosic materials.

Clostridium sp., a gram-positive anaerobic bacterium, is widely used for biobutanol production, and this process is usually considered as acetone–butanol–ethanol fermentation (ABE) since the strains can produce acetone, butanol, and ethanol simultaneously in the ratio of 3:6:1 using a series of carbohydrate feedstock including polysaccharides and lignocellulosic substrates. Several reports are available on the production of biobutanol from hydrolysates of lignocellulosic materials as carbon source; however, the concentration of hydrolysates is limited and can inhibit cell growth and butanol yield. Meanwhile, inhibition from the final product (butanol) is another issue in the ABE process. CBP can overcome this drawback by removing the accumulated butanol immediately in the continuous process. As the by-products during butanol fermentation—acetone and ethanol—account for 40% of the final products, attempts to decrease the by-products during butanol production through metabolic engineering techniques can benefit from the CBP process.

13.2.2 The Recombinant Strategy of CBP

In a recombinant CBP strategy, a recombinant strain is developed with capacities including efficient depolymerization of lignocellulosic materials, a wide range of utilization of different kinds of monosaccharides, and tolerance for toxic compounds from hydrolysates and metabolites during CBP. This strategy attempts to develop a recombinant microorganism for degrading lignocellulosic substrates using a heterogeneous genome to overexpress lignocellulolytic enzymes towards production of biofuels and value-added products (Olson et al. 2011). Therefore, one of the most

challenging steps for recombinant strains is the expression of heterogeneous enzymes with sufficient amount and activity. The cellulases for hydrolysis of lignocellulosic substrates can be either extracellular enzymes or surface binding enzymes as cellulosomes. However, developing the strains for the synthesis of extracellular enzymes will be an advantage. The extracellular release of enzymes will help to overcome the mass transfer limitations and will help in increasing the rates of reaction/product.

Until now more than 400 different genes related to the hydrolysis of lignocellulosic woody biomass have already been reported in the literature (Parisutham et al. 2014). Multiple genes may be required in order to express and synthesize an enzyme system for efficient degradation of lignocellulosic biomass. The gene of β-glucosidase can also be transferred into recombinant strains to lessen the effect of feedback inhibition on cellulase by cellobiose during hydrolysis of lignocellulosic substrate. The activities of different enzymes need to be controlled to avoid feedback inhibition. For example, the activity of β-glucosidase for cellobiose degradation in the recombinant strain should be higher than those of the enzymes for glucose metabolism in order to decrease the cellobiose accumulated during CBP (Martinez et al. 2008). The composition of cellulose, hemicellulose, and lignin in lignocellulosic materials varies with different sources. The ideal recombinant strains for CBP should be able to hydrolyze them irrespective of their varying ratios, and it requires the strains to express different kinds of enzymes for degrading variable lignocellulosic substrates.

Besides the transfer and expression of heterogeneous genes for lignocellulolytic enzymes, the recombinant strains should possess an efficient protein secreting system in order to produce sufficient amounts of enzymes with higher activities for degradation of lignocellulosic substrates. On the other hand, attempts can also be made to engineer the enzymes with higher catalytic rates in order to improve the overall efficiency of the process. One native thermophilic and cellulolytic strain *Clostridium thermocellum* can be considered as a promising candidate for CBP of biobutanol production, due to the high-level conversion of substrate, low risk of contamination, and improved product recovery (Higashide et al. 2011).

Metabolic engineering techniques can be used in certain microorganisms which have already been shown to be promising as native biofuel producing strains in CBP for improving efficient process and decreased by-products. A pyrF-based genetic system in *Clostridium thermocellum* has been developed by deleting pyrF gene through metabolic engineering for making targeted gene knockouts which could not produce acetate, and can rapidly solubilize cellulose during biofuel fermentation (Tripathi et al. 2010). Some other genes of crucial enzymes that are responsible for the production of undesired by-products during CBP have been deleted, such as the genes encoded lactate dehydrogenase (ldh) and phosphotransacetylase (pta). The genetic system modification provides a method to build engineered CBP strains with high yield of bioethanol compared with the wild-type strain. The developed Δldh and Δpta mutant of *Clostridium thermocellum* exhibited a high ethanol selectivity of 40:1 ethanol and has 4.2 times higher ethanol yield when compared with the wild-type strain. (Argyros et al. 2011).

Metabolic engineering can be carried out for minimizing certain by-products. Gene knock out is an ideal way for developing higher yield of cellulosic biofuels by deleting the genes responsible for unrelated products. *Clostridium cellulolyticum*, a mesophilic strain, was modified as metabolically engineered strain for CBP to produce isobutanol from cellulosic substrates through directing the conversion pathway of pyruvate to isobutanol (Higashide et al. 2011). The thermophilic strain *Anaerocellum thermophilum* is a strictly anaerobic bacterium which grows optimally at 75 °C, and this strain is able to utilize different substrates such as crystalline cellulose and untreated plant biomass to produce hydrogen and organic acids as final products (Kataeva et al. 2009). This strain is a good source for different thermostable lignocellulolytic enzymes. The genes for these enzymes can be isolated, cloned, and expressed in other genetically engineered strains for CBP.

Thermostable endo-xylanase from a thermophilic strain was cloned and expressed heterologously in *E. coli*, and the recombinant enzymes exhibited high specific activity of 461 U/mg of protein at 70 °C on xylan. (Bhalla et al. 2014a). The thermostable hemicellulases can be promising for CBP processes in the biofuel industry. In addition, the use of high temperature in thermophilic CBP helps in increasing the mass transfer and decreasing the viscosity, leading to enhanced solubility of substrates and products, thereby contributing to better performance of the bioprocess and biofuel production. Depending on the improved thermostability at relatively high temperature, the hydrolysis performance of recombinant endo-xylanase and β-xylosidase can be improved for thermophilic CBP. The recombinant lignocellulolytic enzymes have a longer active life under higher temperature, broader active pH range, broad substrate specificity, high specific activity, and thermostability, which significantly favor commercial applications in CBP.

Xylanolytic enzymes are required for complete degradation of hemicellulose in lignocellulosic biomass. Among the different xylanolytic enzymes, β-xylosidase is responsible for hydrolyzation of xylo-oligosaccharides to xylose as monosaccharide. An active β-xylosidase can also lessen the end-product inhibition issues for xylanases and cellulases raised by xylose oligomers. Endo-xylanases and β-xylosidases are both hemicellulases which aid in the conversion of the xylan fraction in lignocellulosic biomass and enhance the performance of cellulases. The enzyme cocktail of endo-xylanase, β-xylosidase, and cellulase has been shown to have a synergistic effect on lignocellulose hydrolyzation, generating fermentable monosaccharides (Bhalla et al. 2014b) (Table 13.2).

13.2.3 Integrated Biofuel Production and Fuel Cell/Microbial Fuel Cell

Fuel cells are electrochemical energy devices that convert chemical energy into electrical energy. Unlike batteries, fuel cells require continuous sources of fuels (e.g., electron donor and electron acceptor) to carry out the reaction in order to generate electricity (Edwards et al. 2008; Winter and Brodd 2004). Fuel cells are

Table 13.2 Consolidated bioprocessing by recombinant extremophiles

Organism	Substrates	Biofuel products	Yield	Operating conditions	Recombinant strategy	Reference
Clostridium thermocellum and *Thermoanaerobacterium saccharolyticum*	Avicel	Ethanol	38.1 g/L	55 °C	Deletion of lactate dehydrogenase and phosphotransacetylase	Argyros et al. (2011)
Thermoanaerobacterium saccharolyticum	Xylan	Ethanol	37 g/L	50 °C	Knockout of genes involved in organic acid formation (acetate kinase, phosphate acetyltransferase, and L-lactate dehydrogenase)	Shaw et al. (2011)
Caldicellulosiruptor bescii	Switchgrass	Hydrogen	Around 23 mmol/L	75 °C	Deletion of the L-lactate dehydrogenase gene	Cha et al. (2013)
Clostridium cellulolyticum	Crystalline cellulose	Isobutanol	660 mg/L	34 °C	Expressing enzymes that convert pyruvate to isobutanol by using an engineered valine biosynthesis pathway	Higashide et al. (2011)

promising strategies for direct transformation of the biofuels produced from CBP to applicable energy.

Fuel cells consist of anode, cathode, and electrolyte in which, for instance, hydrogen, methane, ethanol, sugars, and acetate produced from CBP can be used as electron donors, and carbon dioxide produced in CBP can be used as electron acceptors. The oxidation of the hydrogen produces electrons which travel across the external circuit to reach the cathode, where they reduce the electron acceptor, thereby producing electricity. The electrons flow through the external circuit and produce DC current. The CBP process can be integrated to improve the overall performance of the system, which includes higher yield of products, minimal wastes, and lower cost of the products. The use of crude wastes produced from CBP in an electro-chemical system will decrease the power output and may have greater chance of fouling/damaging the membrane electrode assemblies. Under those conditions, the wastes that are obtained through CBP should be processed before feeding them to fuel cells as sources of electron donors/acceptors.

Microbial fuel cells (MFCs) are similar to chemical fuel cells, but differ in the way they make use of the metabolic machinery of microorganisms for bioelectricity generation. The fuels and the electrode surfaces in a MFC must be nontoxic to microorganisms. The microorganisms act as a whole cell bioelectrocatalyst which can be used to oxidize the electron donor or reduce the electron acceptor at the anode or cathode, respectively. Methane and hydrogen can be oxidized in the anode whereas carbon dioxide can be reduced at the cathodic side of the MFC using suitable microorganisms (Pham et al. 2006; Rabaey and Verstraete 2005; Du et al. 2007). In most cases, the microorganisms that are used in MFCs as electrocatalysts are consortia from waste water (Logan et al. 2006). However, the use of extremophiles will be advantageous for developing MFCs which utilize the waste from CBP. MFCs have already been used for hydrogen production using a microbial electrolysis cell (Wang et al. 2011). For integrated bioprocessing, MFCs can be combined with a CBP reactor wherein the wastes from CBP can be used as fuels for MFC and the electrical energy generated from the MFC can be utilized for operating/supplementing the CBP process. Reports are also available on the computational molecular approaches for screening the electroactive microorganisms for electrocatalytic activity and cellulolytic activity (Krishnaraj et al. 2014a, 2017; Dodda et al. 2016; Krishnaraj and Pal 2017). These techniques will be useful for identifying suitable microorganisms for MFCs in the developed CBP process.

Different configurations of MFC have been reported in the literature for the production of bioelectricity with different substrates such as lignocellulosic biomass, glucose, ethanol, acetate, winery effluent, food industry effluent, and tannery effluent (Bhuvaneswari et al. 2013; Krishnaraj et al. 2014b, 2015; Rajeswari et al. 2016). Reports have been made on the different electrode functionalization strategies that allow the growth and proliferation of the microorganisms on the electrode surface and contribute to better substrate utilization and higher power output (Krishnaraj et al. 2013; Karthikeyan et al. 2016). Attempts have also been made to develop economical proton exchange membranes using polyvinyl alcohol, polyvinyldine

chloride, and polystyrene sulfonic acid for fuel cell applications, which would help to cut down the costs of installation by replacing the Nafion (Bella et al. 2016).

13.3 Integrated CBP Utilizing Organic Waste and Unhydrolyzed Fraction

The organic wastes from CBP mainly contain organic acids acetate, propionate, lactate, and butyrate (Lin and Tanaka 2006), which are valuable products and can be further processed through secondary bioprocesses. Due to the unutilized lignocellulosic fractions and the organic acids generated from upstream CBP, opportunities exist to transform these waste streams to biogas and other value-added products in order to maximize the value of these streams. Meanwhile, the remaining nutrients and water can also be recycled. The organic wastes obtained from CBP can be used as organic feedstocks to produce biopolymers, short-chain carbohydrates, and methane. The microbial strains that are used for integrated CBP should be able to breakdown the complex and variable substrates and must overcome substrate/product inhibitions as well. Several biological processes can be integrated with the production of organic acids from lignocellulosic substrates through CBP, such as reduction of carboxylates to certain alcohols, and biological elongation of short-chain carboxylates to longer chain biopolymers (Agler et al. 2011). In this section, the other bioprocesses in the integrated CBP system will be reviewed, including methane generation through the TAD process, biopolymers and 1,3-PDO production through aerobic or anaerobic fermentation, and biodiesel production.

13.3.1 Biopolymers

13.3.1.1 Exopolysaccharides

Exopolysaccharides (EPSs) are high molecular weight carbohydrate biopolymers that are composed of sugar residues and are secreted by microorganisms into the surrounding environment (Nicolaus et al. 2010; Poli et al. 2010). Microorganisms can synthesize a wide variety of EPSs that serve a broad and diverse range of functions, such as intercellular signal transmission, molecular recognition, protection against predation, construction of a comfortable extracellular environment, pathogenic process, and so on (Nicolaus et al. 1999; Moriello et al. 2003). A great number of microorganisms are able to produce EPSs and excrete them out of the cell either as soluble or insoluble polymers. The EPSs can attach to the cellular surface or exist in extracellular medium as glue with indefinite form. The industrial microbial EPSs such as xanthan, dextran, curdlan, gellan, and pullulan are usually generated by pathogenic mesophilic strains, and their production processes are costly. Lignocellulosic biomass resources are considered inexpensive feedstocks for EPS production. Extremophilic microorganisms can be advantageously harnessed for the production of EPSs as they can thrive in a wide range of conditions, possess robust

Table 13.3 EPS produced by extremophiles

Organism	Substrates	Yield	Operating conditions	Monosaccharide composition	Reference
Bacillus Licheniformis	Sucrose	366 mg/L	50 °C	Fructose, fucose, glucose, galactosamine, mannose	Spanò et al. (2013)
Halomonas sp.	Beet molasses	12.4 g/L	137.2 g/L NaCl	Fructose	Küçükaşik et al. (2011)
Pseudoalteromonas sp.	Lactose	5.25 g/L	15 °C	Glucose, arabinose, galactose, xylose,	Qin et al. (2007)
Cronobacter sakazakii	Sucrose	3.15 g/L	pH 10	Glucose, mannose, galactose, xylose, arabinose	Jain et al. (2012)

hydrolytic machinery for lignocellulose degradation, and can produce EPSs with unique characteristics, such as high thermostability (Nicolaus et al. 2010).

In order to survive in extreme conditions, extremophiles have to adapt to the hostile environments through unique mechanisms, and the biosynthesis of EPSs is one of the vital mechanisms in extremophiles which allow them to survive under harsh environments. As a kind of response and adaptation under extreme conditions, extremophiles produce EPSs to protect themselves to endure the extremes of temperature, pressure, acidity, alkalinity, or salinity. The aerobic extremophilic strains can synthesize lignocellulolytic enzymes which enhance the lignocellulose utilization and thereby enhance EPS production using the organic wastes and unhydrolyzed fractions from upstream CBP in biofuel production. Thus, the lignocellulolytic extremophiles can degrade the unutilized fractions from CBP and secrete EPSs. The cellulase activities of extremophiles need to be optimized during the EPS production, since the EPS production can influence bacterial cellulase activity (Öner 2013). To provide a value-added polymer bioproduct, EPS biosynthesis combined with integrated bioprocessing can be feasible through extremophilic process with significant lignocellulolytic activity (Table 13.3).

13.3.1.2 Polyhydroxyalkanoates (PHAs)

Polyhydroxyalkanoates (PHAs) are polyesters composed of hydroxyl fatty acids. They are synthesized by microorganisms and can be stored as lipid inclusions. Some extremophiles are able to produce and accumulate lipid inclusions containing PHAs which are utilized as intercellular carbon and energy sources (Poli et al. 2011). Being derived from renewable feedstock and having biodegradable and biocompatible properties, PHAs are attractive alternatives to petroleum-based plastics which have many deleterious effects on the environment. Halophilic strains including *Haloferax*, *Haloarcula*, *Natrialba*, *Haloterrigena*, *Halococcus*, *Haloquadratum, Halorubrum*, *Natronobacterium*, *Natronococcus, Halobacterium*, and *Halomonas* have been

shown as promising extremophilic sources for production of PHAs (Du et al. 2012; Poli et al. 2011).

The traditional PHA fermentation is costly due to the use of relatively expensive carbon source as feedstocks, which represents approximately 50% of the overall cost (Dietrich et al. 2013). Therefore, it is necessary to use inexpensive lignocellulosic substrates as carbon source for PHA production process. The unhydrolyzed fractions from upstream CBP of biofuels can be used for PHA synthesis. This will help to cut down the operation costs of the processes. To improve the physicochemical properties and meet a broad range of industrial needs, PHAs can be engineered with different monomer composition and molecular structures. However, the organic acids such as acetic acid are prone to inhibit the PHA synthesis, and the tolerance of PHA-producing strains for these kinds of inhibitors need to be considered before developing a process (Dietrich et al. 2013). The study of inhibitor tolerance and PHA production may identify ways to screen and improve PHA production using unhydrolyzed lignocellulosic material from CBP.

13.3.2 1,3-Propanediol (1,3-PDO)

1,3-Propanediol (1,3-PDO) is a valuable bifunctional molecule, which can be produced from renewable resources using microorganisms. Due to the presence of two hydroxyl groups, 1,3-PDO finds applications in the synthesis of polymers, such as polyesters and polyurethanes. The natural producers of 1,3-PDO are mainly bacteria such as *Klebsiella*, *Clostridia*, *Citrobacter*, *Enterobacter*, and *Lactobacilli* (Saxena et al. 2009; Nakamura and Whited 2003).

The by-product stream generated during CBP biofuel production can be utilized as feedstock for 1,3-PDO production, and the crude glycerol generated from biodiesel production can be utilized with the stream (Saxena et al. 2009). As a major by-product from the biodiesel industry, glycerol is an economical substrate for 1,3-PDO production. The surplus glycerol waste from biodiesel production can be used to produce 1,3-PDO (Rastogi et al. 2013).

To maximize the production of 1,3-propanediol, genetic engineering strategies such as fermentation through immobilized cells and two-stage fermentation can be used since these are applicable in achieving high conversion rate. Lama et al. (2017) reported a metabolic engineering approach for producing 1,3-propanediol (1,3-PDO) from glucose using *Klebsiella pneumoniae* J2B. Homologous overexpression of glycerol dehydratase and 1,3-PDO oxidoreductase from *Saccharomyces cerevisiae* were overexpressed, and disruption of glycerol oxidation pathways in *Klebsiella pneumoniae* J2B increased the production of 1,3-PDO with 0.27 and 0.52 mol/mol with glucose and glycerol, respectively. These strategies will aid in 1,3-PDO production from unutilized CBP hydrolysates. The coculture fermentation is also able to utilize mixtures of glycerol and other monosaccharides such as glucose as substrate for 1,3-PDO production. Co-fermentation improves the growth rate and yield of 1,3-PDO by suppressing the formation of other by-products.

13.3.3 Methane

13.3.3.1 Thermophilic Anaerobic Digestion (TAD)

Thermophilic anaerobic digestion (TAD) is a process engineered to decompose organic substrates using different thermophilic, anaerobic microorganisms to produce biogas having methane as a major component. The digestate from the TAD process is rich in nitrogen (Li et al. 2011; Weiland 2010), which can be fed back to CBP of biofuel as a nitrogen source. Agricultural wastes, food wastes, and wastewater sludge can be used as the feedstock for the TAD process. The chemical oxygen demand (COD) and biological oxygen demand (BOD) of these kinds of wastes can be reduced through TAD (Li et al. 2011). TAD has been considered as a developed technology for waste-to-energy conversion, and during the TAD process the methane is released directly without any downstream separation steps.

TAD can be separated into two types: solid-state TAD and liquid-state TAD. In solid-state TAD, the solid content of the feedstocks is usually no less than 15% (Li et al. 2011; Weiland 2010). One of the advantages of solid-state TAD is that the residues of the process can be further fed back as feedstocks for CBP of biofuels. During TAD, the unhydrolyzed fractions from CBP can be continuously degraded into simple soluble molecules with the aid of microorganisms or extracellular enzymes. Consortia of microorganisms are broadly used for the TAD process which makes it a synergistic process, and the members of the consortia for TAD usually include lignocellulolytic bacteria, acetogenic bacteria, and methanogenic bacteria. For the TAD process, a wide range of organic solid wastes with varying compositions and characteristics can be used as feedstocks, and the thermophilic conditions accelerate the anaerobic digestion process and increase the yield of biogas.

The methanogenic consortia for TAD utilize the acetate produced by acetogenic bacteria to generate methane as the final product. Therefore, the organic wastes from CBP can be further utilized as feedstock for the TAD process for methane production, such that TAD will not be limited by the hydrolysis of lignocellulosic substrates. The growth rate of thermophilic consortia is significantly higher than those in mesophilic processes, which makes TAD more efficient and economical. Thermophilic processes for methane production also require a smaller reactor volume and shorter hydraulic retention time. Meanwhile, an optimum carbon/nitrogen ratio in the feedstock can avoid the accumulation of acetate and improve the methane yield and stability of the TAD process. The optimum pH value for TAD usually ranges from 7.0 to 8.0, and it needs to be prudently controlled in order to avoid inhibition for methanogens. The proper amount of trace elements is also necessary for the growth of methanogenic consortia (Weiland 2010). The energy required for maintaining thermophilic temperature during TAD is not extremely energy intensive, since the heat can be provided by methane itself and the biofuels generated through upstream CBP. The water can be recycled after TAD and fed back for CBP. For a highly efficient TAD process, the mixing and process control also need to be optimized. Additionally, a well-developed

and stable microbial consortia is crucial for methane production through the TAD process since they can influence the final yield of methane.

13.3.3.2 Biological Oxidation of Methane to Methanol Using Methanotrophs

Methane is a gas at normal atmospheric temperature and pressure which makes it expensive for storage and transportation. Hence, production of liquid fuels from renewable substrates is advantageous over gaseous fuels. In addition, methane is a strong GHG, having an effect that is 23 times stronger than carbon dioxide (Weiland 2010). Given that current industrial production of methanol is energy intensive and is not environmentally benign, the development of CBP to convert methane produced from the TAD process offers important advantages.

Methane can be oxidized to methanol through a biological process using methanotrophic bacteria or methane monooxygenase with high selectivity under mild conditions (Park and Lee 2013). However, based on the current literature, the conversion rate of methane to methanol is not high enough for industrial application. Methanotrophs are capable of utilizing methane as carbon and energy source through methane monooxygenase under aerobic conditions (Khoshtinat et al. 2010). Some of the methanotrophic strains thrive in extreme environmental conditions (Dedysh et al. 2000; Vorobev et al. 2011). Methanol is not the final product of the metabolism pathway of methanotrophic bacteria, and it can be further used as a precursor for further biosynthetic process of other metabolites. Methanol can be further oxidized to formaldehyde using methanol dehydrogenase, and formaldehyde can be subsequently oxidized to formate (Khoshtinat et al. 2010). Development of genetically or metabolically engineered methanotrophic strains is a way forward to attain high selectivity for the production of methanol.

Methane monooxygenase can be used for the conversion of methane to methanol directly. The engineered methane monooxygenase is required for enhanced and stable catalytic activity for a technically and economically applicable oxidation process. The methanol produced in this process can be further utilized as raw material for the production of biodiesel through transesterification in the CBP (Khoshtinat et al. 2010). It would be of significant value to combine the conversion of methane to methanol in the integrated CBP, producing methanol continuously using the methane obtained from the upstream TAD process.

The methanotrophic strains can also generate lipids through oxidation of methane (Blumenberg et al. 2004). There are a few reports on harnessing the methanotrophic communities for the production of lipids, but so far the lipids from methanotrophic strains have not been explored for industrial application. The specific lipid composition associated with certain consortia can be analyzed and recognized, so that these consortia-specific lipids can provide important information on the structure of microbial communities and the identification of methanotrophic bacteria (Jahnke et al. 1995). Both nonpolar lipids and polar lipids can be produced using methanotrophic strains. The methanotrophs also produce unique monounsaturated fatty acid that are suitable for biodiesel production. The lipid production through methanotrophic strains

can be combined with integrated CBP for biodiesel production along with the generation of methanol using methanotrophs.

13.3.4 Applications of the Bioproducts Obtained Through Integrated CBP

In order to commercialize bioproducts, e.g., EPSs, the cost of their production and downstream processing need to be optimized. Until now, microbial extremophilic EPSs have not been well explored for use as industrial biopolymers, although several reports have been made. Due to some superior properties, certain microbial EPSs are promising alternatives to traditional polysaccharides and have several advantages over a number of synthetic polymers. In the light of recent reports on bacterial EPSs with unique physiochemical properties, new commercial applications of the EPSs will be explored. Current high-value markets for EPSs with great potential include cosmetics, pharmaceuticals, and biomedical industries (Kumar et al. 2007; Freitas et al. 2011; Nicolaus et al. 2010).

PHAs can be degraded to carbon dioxide and water by several microorganisms. The materials made with PHAs are promising alternatives to replace traditional petroleum-based plastics (Du et al. 2012). PHAs can also be blended with other polymers or copolymerized, increasing their property range and providing a range of potential applications in packaging, drug delivery, and medical bio-implants. PHA-based copolymers also find application in the production of bulk chemicals such as heat-sensitive adhesives, latex, and smart gels and can be used as processing aids in plastics and textile manufacturing. PHAs are processed into fibers for various biomedical substrates and are used for developing controlled drug release matrices (Chen 2009).

1,3-PDO is also a value-added product which has great industrial demand. For example, it finds several applications in the polymer and cosmetic industries. It can be used as a monomer for synthesis of polyester and biodegradable plastics, and its properties can be modified in order to satisfy different requirements. Additionally, 1,3-PDO has been studied as an industrial biocide and preservative.

Biodiesel is biodegradable, nontoxic, sulfur-free, renewable, and can be produced from agricultural and woody wastes. Biodiesel can be produced from edible, nonedible, and waste vegetable oils. Transesterification is an important method to produce biodiesel from lipids. During the transesterification process, the biodiesel and triglycerides are used to produce glycerol and other alkyl esters (Agarwal 2007). The methanol, ethanol, and butanol obtained from integrated CBP can be used for transesterification to biodiesel. Besides the application as biofuels, the alcohols and methane produced during integrated CBP can be used to generate heat directly to maintain the temperature for thermophilic processes in CBP. The liquid fraction obtained from integrated CBP can be fed as a nitrogen source for various processes in integrated CBP in order to provide a no-waste bioprocess.

13.4 Modeling of CBP

To develop integrated CBP with higher reliability and efficiency, simulation software such as ASPEN Plus can be used for evaluating the economic performance of CBP through integrated flowsheet configuration. A combined framework can be generated through ASPEN Plus to simulate the integrated bioprocesses for biofuel production. The simulation strategy estimates several different parameters for biofuel production processes, and the parameters can be optimized through the model. In this way, ASPEN Plus can be used to design advanced reactors for the whole CBP process.

Traditional ethanol production through bioprocess needs an enzymatic pretreatment step of the lignocellulosic materials, which leads to increased cost for the commercialization of biofuel production process. The total cost can be estimated through ASPEN Plus software which includes the cost for commercial enzymes. In the case of bioethanol, companies such as Poet and DuPont have already reported that the cost of enzymes is approximately $0.50 per gallon of ethanol (Geddes et al. 2011). Therefore, utilization of CBP without the pretreatment step using supplementary lignocellulolytic enzymes provides a way to decrease the total cost of biofuel production significantly. The ASPEN modeling framework, developed by the National Renewable Energy Laboratory (NREL), has been used to simulate the cost of ethanol production through CBP and compare it with simultaneous saccharification and co-fermentation (SSCF) processes using dedicated lignocellulolytic enzymes (Lynd et al. 2005; Cardona and Sánchez 2007).

The cost of dedicated cellulase production and SSCF is ¢9.85/gallon ethanol and ¢8.98/gallon ethanol, respectively, for a total of ¢18.9/gallon ethanol. However, the total cost of CBP for bioethanol production is just ¢4.23/gallon ethanol, which means the cost of supplementary enzymes can be more than half the total cost of ethanol production through SSCF in order to maintain appropriate hydrolysis rate. Meanwhile, the reacting time required for SSCF is 7 days which is much longer than that of CBP (1.5–3 days). Without supplementary enzymes, the total cost of ethanol production through CBP is around one-fourth of the total cost of SSCF (Lynd et al. 2005). The ASPEN simulation can also be used to evaluate the processing cost of the biofuel production using switchgrass by CBP and electricity generation from the biofuel obtained (Laser et al. 2009). CBP has great potential to cut down costs as well as improve ethanol yield; additionally, the power required for CBP is considerably reduced compared with traditional bioconversion technology. For integrated CBP, the thermochemical process of biodiesel production and related heat and power plant required can be simulated by ASPEN Plus software (Wu et al. 2006). The choice of process technology, configuration, feedstock, and size of the reactors can be postulated using simulation software to provide detailed information for industrial application.

As a carbon-free energy carrier, biohydrogen is a fuel resource with zero emission. The ASPEN Plus simulation program can also be used to solve mass and energy balances for biohydrogen produced through CBP. The pH value of process

streams can be calculated using the data generated for ionic species through the ASPEN model, in order to manage the effects of recirculation on the osmolality of the fermentation broth. The parameters obtained through the model can be compared with the experimental results generated from large-scale fermentation of biohydrogen via CBP. The thermophilic fermentation of biohydrogen should be deployed in anaerobic conditions and at high temperature. In the simulation model, the integrated pathway of hydrogen biosynthesis can be simplified and described in the following reactions:

$$\text{For hexose } C_6H_{12}O_6 + 2H_2O \rightarrow 4H_2 + 2CH_3COOH + 2CO_2$$
$$\text{For pentose } 3C_5H_{10}O_5 + 5H_2O \rightarrow 10H_2 + 5CH_3COOH + 5CO_2$$

The inhibition of hydrogen production from increasing hydrogen partial pressure also needs to be considered during the design of the configuration for simulation modeling. The effluent streams can be recycled to reduce the water and heat consumption of CBP. Cost analysis has already been carried out which demonstrates that, currently, the cost of biohydrogen production is still not competitive with traditional fossil fuels. Therefore, significant system improvements are necessary for biohydrogen production through CBP (Foglia et al. 2009, 2011a, b; Choi and Anh 2015; Yasin et al. 2013; Wukovits et al. 2007; Hay et al. 2013).

13.5 Conclusions and Future Perspectives

In order to remove commercial barriers and attain competitive yield and cost compared with traditional fossil fuels, a number of efforts have been made to improve CBP over the past several years. The establishment of economically feasible fermentation processes for biofuels requires the microorganisms to be in an integrated CBP, to attain high yield and a relatively low product inhibition effect. The strains should be able to utilize lignocellulosic substrates with considerable differences in compositions. Engineered cellulolytic-recombinant strains help to improve the performance of CBP. For other kinds of value-added bioproducts, it is important to identify suitable strains, especially extremophiles, to utilize the organic wastes and residues from the upstream steps with high biosynthetic rate and final yield of the products. Since most of the residues and unutilized fractions will be fed to the next steps or back into the CBP, the whole integrated CBP leads to minimal waste generation. For further improvement of the integrated CBP, novel extremophilic strains with desired properties need to be developed. In addition, various biotechnology strategies such as genetic engineering, metabolic engineering, mutagenesis, and protein engineering can be used for developing an integrated CBP in order to produce value-added bioproducts with improved yields and properties. In summary, due to the application of extremophiles and lignocellulosic substrates, integrated CBP can be economical and eco-friendly. In the near future, biofuels and other value-added bioproducts obtained from integrated CBP promise to be competitive alternatives to traditional fossil fuels and petroleum-based synthetic materials.

Take Home Message

- Biofuels are renewable, eco-friendly, and economical energy resources and are promising alternatives to traditional fossil fuels.
- The application of CBP with extremophiles using lignocellulosic substrates as feedstock can provide cost-effective production of biofuels, and pretreatment processing of lignocellulosic materials can be eliminated by using CBP with lignocellulolytic extremophiles.
- Integrated CBP is a sequential or continuous process to produce several kinds of gaseous and liquid biofuels and other value-added bioproducts using lignocellulosic substrates as primary feedstock. An integrated CBP can be developed by the combination of CBP for biofuels with several other bioprocesses for the production of biopolymers, 1,3-PDO, methane, and biodiesel.
- In integrated CBP, the liquid residues can be fed back to the upstream bioprocesses as nutrient, while the heat required for CBP can be generated directly from the biofuels obtained. The development of integrated CBP will be a feasible method for sustainable and efficient production of different kinds of biofuels and value-added bioproducts with minimal waste generated.
- Future research on integrated CBP will focus on removing commercial barriers by attaining competitive yields and costs compared with similar products from fossil fuels. The extremophilic microorganisms involved in integrated CBP should be able to degrade lignocellulosic substrates and utilize the organic wastes and residues from upstream steps with low inhibition effects and with high biosynthetic rate and final yield. The development of engineered lignocellulolytic recombinant strains is a worthwhile strategy to improve the performance of integrated CBP and apply the technology to current commercial needs.

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