Chapter 10
Bioenergy: Biofuels Process Technology

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Introduction

There is an increasing interest in the production of chemicals and fuels from renewable resources due to the continuing price increase of fossil resources, the insecurity of the availability of fossil resources in the future, and additionally environmental concerns and legislations (García et al. 2011; Baskar et al. 2012). In recent years, growing attention has been devoted to the conversion of biomass into biofuel such as ethanol, butanol, biodiesel etc. considered the cleanest liquid fuel alternative to fossil fuels (Lin and Tanaka 2006). Moreover, biomass energy can play an important role in reducing greenhouse gas emissions; since CO_2 that arises from biomass wastes would originally have been absorbed from the air, the use of biomass for energy offsets fossil fuel greenhouse gas emissions (Lynd 1996). Currently ethanol is the main bio-fuel used in the world and its use is increasingly widespread, the worldwide prospects are the expansion of the production and consumption of ethanol (Bastos 2007). Fermentation-derived butanol is a possible alternative to ethanol as a fungible biomass-based liquid transportation fuel (Pfromm et al. 2010). The transesterification of vegetable oils (VOs) with short-chain alcohols is used to produce biodiesel or by the esterification of fatty acids. During the past few years

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biodiesel has attracted attention as an environmentally friendly and renewable fuel because of uncertainties concerning petroleum availability and recent increases in petroleum prices (Berchmans and Hirata 2008). Similarly Hydrogen production from biomass conversion plays a very important role in the development of hydrogen economy (Ni et al. 2006). The quality of energy crops, used for biogas production, is determined on the field. Methane production from organic substrates mainly depends on their content of substances that can be degraded to CH_4 and CO_2 (Amon et al. 2007). About 95 % of ethanol produced in the world is from agricultural products (Walter et al. 2008). Ethanol production from sugar crops such as sugarcane and sugar beet account for about 40 % of the total bioethanol produced and nearly 60 % corresponding to starch crops (Biofuels Platform, 2010a, b). Biobutanol is on the agenda of several companies and may be used in the near future as a supplement for gasoline, diesel and kerosene (Antoni et al. 2007). Assuming an oil price of US$60 per barrel, both biodiesel and bioethanol produced from wheat are not profitable in Europe. At the assumed oil price, only bioethanol and biobutanol produced on a large scale from lignocellulose-containing raw materials have the potential to be produced competitively (Festel 2008; Kumar et al. 2012). The U.S. has become the dominant ethanol producer (corn-based), although Brazil has started an ambitious program to increase production by 50 % by 2009 (sugar-based). Biodiesel production has increased at 20–100 % annual rates in recent years, particularly in Germany, France, Italy, Poland, and the United States (Renewables 2005). About half of all the hydrogen as currently produced is obtained from thermo catalytic and gasification processes using natural gas as a starting material, heavy oils and naphtha make up the next largest source, followed by coal. Currently, much research has been focused on sustainable and environmental friendly energy from biomass to replace conventional fossil fuels (Balat and Kırtay 2010). Current total annual worldwide hydrogen consumption is in the range of 400–500 billion Nm^3 (Demirbas 2009a, b). Present utilization of hydrogen is equivalent to 3 % of the energy consumption and with a growth rate estimated at 5–10 % per year (Mohan et al. 2013). Only a fraction of this hydrogen is currently used for energy purposes; the bulk serves as a chemical feedstock for petrochemical, food, electronics and metallurgical processing industries. The global market for hydrogen is already greater than US$40 billion per year (Kraus 2007); including hydrogen used in ammonia production (49 %), petroleum refining (37 %), methanol production (8 %), and miscellaneous smaller-volume uses (6 %) (Konieczny et al. 2008).

Biofuel Feedstocks

Fermentation substrate is an important factor influencing the cost of ethanol, butanol, hydrogen gas etc. production (Qureshi and Blaschek 2000). Lignocellulose is the most abundant renewable resource on the planet, and has great potential as a substrate for fermentation. Hemicelluloses are the second most abundant polysaccharides in nature, and represent about 20 to 35 % of lignocellulosic biomass

(Koukiekolo et al. 2005). Xylan or hemicellulose may contain arabinan, galactan, glucuronic, acetic, ferulic, and rcoumaric acids as well as xylose. The occurrence and quantity of these compounds depend on the sources of xylan (Olsson and Hahn-Hägerdahl 1996; Koukiekolo et al. 2005). The ethanol yield and productivity obtained during fermentation of lignocellulosic hydrolysates is decreased due to the presence of inhibiting compounds, such as weak acids, furans and phenolic compounds produced during hydrolysis (Palmqvist and Hahn-Hägerdal 2000).

Lignocellulosic Feedstocks

Lignocellulosic biomass is generally composed of hemicellulose (25–35 %), cellulose (4–50 %), and lignin (15–20 %), and these structures are illustrated in Fig. 10.1. Cellulose hydrolysis can also be achieved under harsher conditions using solutions of mineral acids (H_2SO_4) at elevated temperatures; however, the harsh conditions required for non-enzymatic deconstruction of cellulose favor the formation of degradation products such as hydroxymethylfurfural (HMF), levulinic acid, and insoluble humins (Alonso et al. 2010; Rinaldi and Schüth 2009).

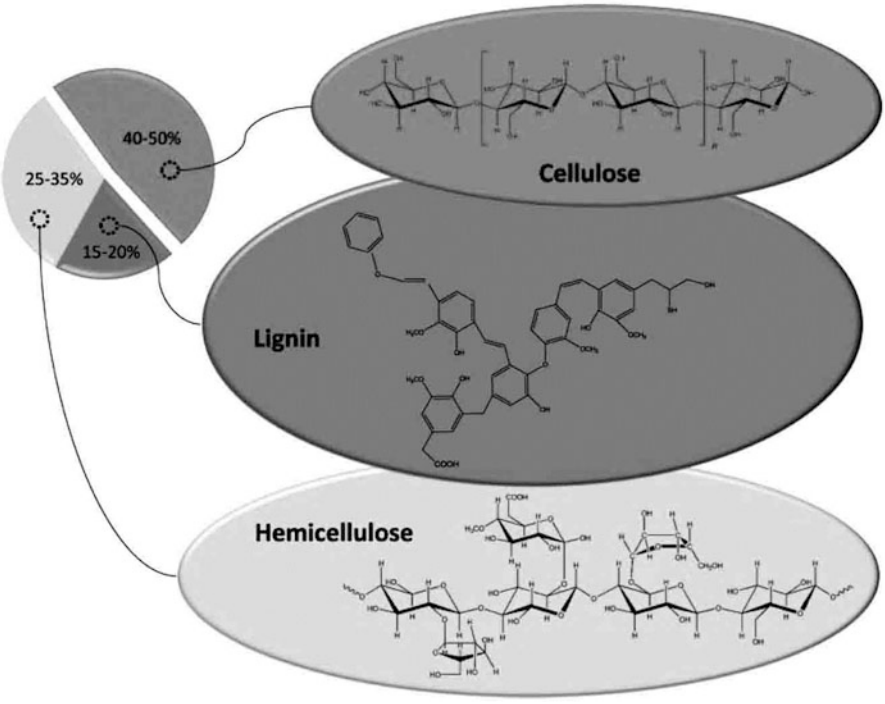


Fig. 10.1 Lignocellulose composition: cellulose, hemicellulose and lignin (Alonso et al. 2010)

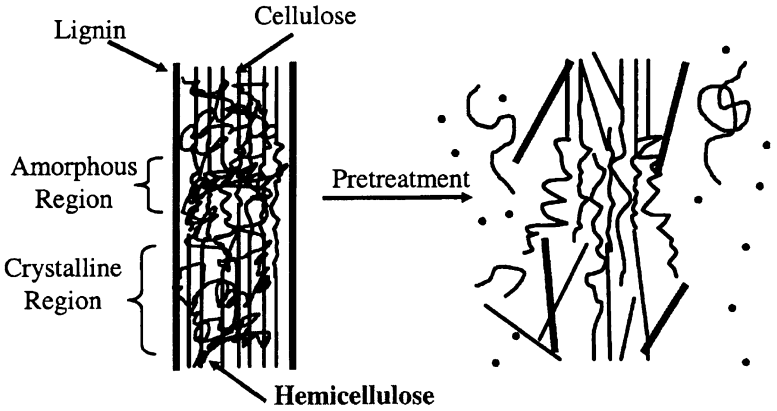


Fig. 10.2 Schematic of goals of pretreatment on lignocellulosic material (Mosier et al. 2005)

Table 10.1 Percent dry weight composition of lignocellulosic feedstock's (Mosier et al. 2005)

Feedstock	Glucan (cellulose)	Xylan (hemicellulose)	Lignin
Corn stover	37.5	22.4	17.6
Corn fiber	14.28	16.8	8.4
Pine wood	46.4	8.8	29.4
Poplar	49.9	17.4	18.1
Wheat straw	38.2	21.2	23.4
Switch grass	31.0	20.4	17.6
Office paper	68.6	12.4	11.3

Note: Because minor components are not listed, these numbers do not sum to 100 %

Pretreatment is necessary to make cellulose more prominent to be attacked by the enzymes which ultimately convert it into fermentable sugars (Fig. 10.2).

Lignocellulosic biomass normally comprises cellulose, hemicellulose and lignin i.e. near about 55–75 % carbohydrates on dry weight basis. Percent dry weight compositions of different lignocellulosic feedstocks were determined by Mosier et al. (2005) as shown in Table 10.1.

Unfortunately, neither commercial ethanol-producing cultures, nor butanol-producing cultures can hydrolyze these substrates. Hence, they need to be hydrolyzed prior to fermentation using a combination of pretreatment (acid, alkali, organosolvent, supercritical extraction or ammonia explosion) and hydrolysis (enzymes: cellulase, β-glucosidase, and xylanase) techniques (Galbe and Zacchi 2002). It should be noted that in contrast to ethanol production by yeasts, hexose and pentose sugars obtained as a result of pretreatment and hydrolysis of these residues can be used by butanol-producing cultures (Qureshi et al. 2008a, b, c).

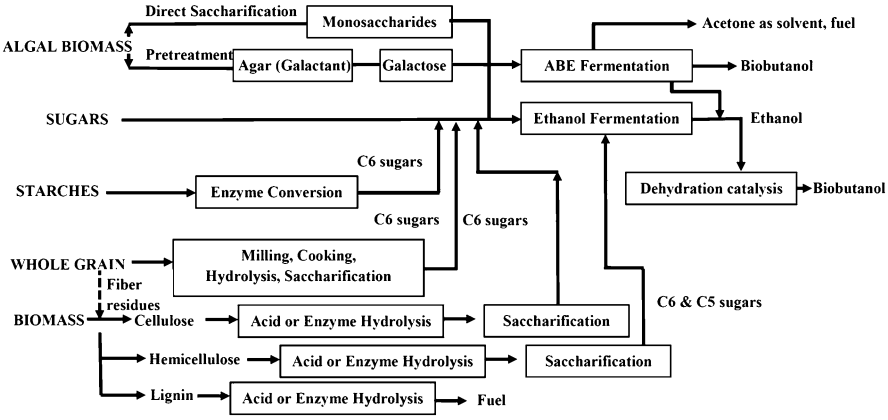


Fig. 10.3 Different feedstocks generally used for ethanol and biobutanol production

Algal Biomass Feedstocks

Algae capable of accumulating high starch/cellulose can serve as an excellent alternative to food crops for bioethanol production, a green fuel for sustainable future. Certain species of algae can produce ethanol during dark-anaerobic fermentation and thus serve as a direct source for ethanol production. Of late, oleaginous microalgae generate high starch/cellulose biomass waste after oil extraction, which can be hydrolyzed to generate sugary syrup to be used as substrate for ethanol production. Macroalgae are also harnessed as renewable source of biomass intended for ethanol production (John et al. 2011; Nguyen et al. 2009). The use of marine algal biomass with high carbohydrate contents of *Ulva lactuca* and other macroalgae like *Saccharina spp*. *Laminaria*, *Durvillaea*, *Ecklonia* and *Homosira* (brown algae) (Figueira et al. 2000) indicates that a more cost effective strategy might be to ferment the carbohydrates like glucose, mannitol and laminarin from these algal species to either ethanol or butane (Potts et al. 2012; Huesemann et al. 2012).

Fate of different feedstocks for ethanol and biobutanol production are shown in Fig. 10.3.

Microbial Modeling of Biofuel Production

A wide variety of biofuels can be produced through the bioconversion of substrates contained in agricultural crops and residues (Fischer et al. 2008). Bioconversion of the sugars, starches, and other organic substrates contained in agricultural residues can be converted to ethanol by a variety of yeasts, to hydrogen by a variety of fermentative bacteria and archae, to methane by a consortium of bacteria and

archae, and to oils for biodiesel production by fungi and algae (Drapcho et al. 2008). All of these microbial processes can be described mathematically to simulate the bioprocess (Kumar and Murthy 2013). First generation biofuels, such as ethanol and biodiesel are already widely used, but they were selected mainly for convenience rather than their properties as fuels. In a microbial biofuel production process, bioengineered microbes are grown inside a reactor in a solution that is rich in cellulose-derived sugar (glucose and pentose) (Turner 2014).

Bioreactor modeling and design based on microbial growth and product formation kinetics may be used to optimize production of high-value biofuels or maximize utilization of feedstock nutrients. Kinetic models are normally divided into two classes: structured and unstructured one. Structured models take metabolic pathways into consideration and are generally complicated. A structured model for acetone–butanol fermentations was established by Votruba et al. (1985).

The Monod model is a widely applied model used to describe microbial growth. Suitable microbial hosts for biofuel production must tolerate process stresses such as end-product toxicity and tolerance to fermentation inhibitors in order to achieve high yields and titers (Fischer et al. 2008)

$$\mu = \frac{\mu_{max} S}{K_s + S} \tag{10.1}$$

where μ = Specific growth rate co-efficient h^{-1}
μ_{max} = Maximum Specific growth rate co-efficient h^{-1}
S = Substrate concentration mg/L
K_s = half-saturation constant, mg/L

Kinetic expressions for product formation must account for growth associated and maintenance-associated production, as in the following equation:

$$r_p = Y_{PX} r_x + m_p X. \tag{10.2}$$

Where r_x is the volumetric rate of biomass formation, Y_{PX} is the theoretical or the true yield of product from biomass, m_p is the specific rate of product formation due to maintenance, and X is biomass concentration (g/L), r_p is the volumetric rate of product formation (Doran 1995).

It is also possible that two or more substrates may simultaneously be growth-limiting, thus, a model that can describe such a system is given by:

$$\mu = \frac{\mu_{max} S_1}{K_1 + S_1} \left(\frac{S_2}{K_2 + S_2} \right) \tag{10.3}$$

Where, μ is the specific growth rate (1/h), μ_m is the maximum specific growth rate (1/h). The specific growth rate could be inhibited by medium constituents such as substrate or product. In a case of substrate inhibition, the term is given by:

$$\mu = \frac{\mu_{max}\, S}{Ks + S + \left[\frac{S^2}{K_I}\right]} \tag{10.4}$$

For a case that exhibits, product inhibition such as ethanol fermentation and ABE fermentation, the specific growth rate is written as:

$$\mu = \frac{\mu_{max}\, S}{Ks + S}\left(\frac{K\,p}{K\,p + P}\right) \tag{10.5}$$

Where K_p is product inhibition constant and P is the concentration of the product

Bioreactor for Biofuel Production

Basic bioreactor designs for suspended growth cultures are batch, continuous (flow) stirred tank reactor (CSTR), and CSTR with external or internal biomass recycle (Fig. 10.4). If the means of cell separation (filtration, centrifugation, settling) removes the compound with the biomass, then the product is considered particulate. Hydrogen and ethanol are examples of soluble, extracellular products, while oils produced by filamentous fungi *Pythium* are intracellular products (Drapcho et al. 2008).

Batch Bioreactor

When plotted on arithmetic paper, batch growth cure assumes a sigmoidal shape, this can be predicted by combing the Monod equation with growth equation (Shuler and Kargi 2002).

$$\frac{dX}{dt} = \frac{\mu_m\, S}{K_s + S} X \tag{10.6}$$

The relationship between microbial growth yield and substrate is

$$X - X_0 = Y_{X/S}(S_0 - S) \tag{10.7}$$

Where X_0 and S_0 are initial values and Yx/s is the cell mass yield based on limiting nutrient.

$$\frac{dX}{dt} = \frac{\mu_m\left(Y_{X/S}S_0 + X_0 - X\right)}{K_s Y_{X/S} + Y_{X/S}S_0} X \tag{10.8}$$

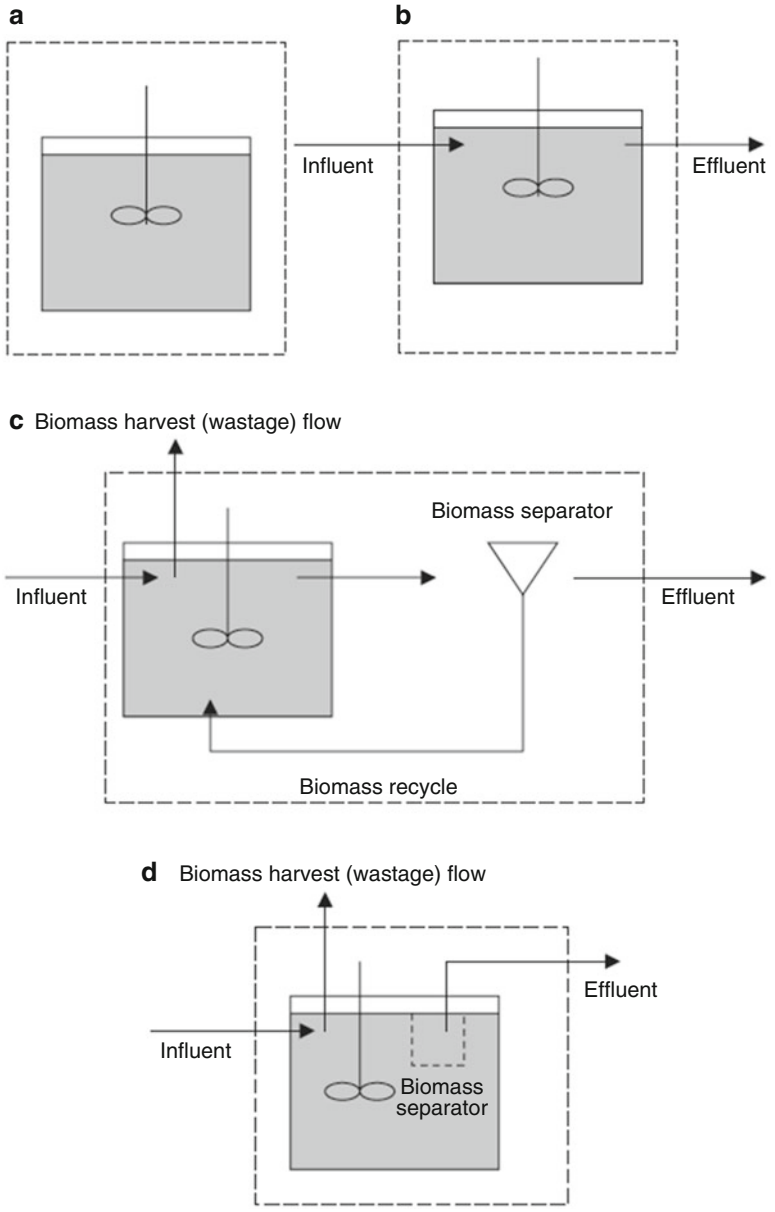


Fig. 10.4 Basic bioreactor types. (**a**) Batch. (**b**) Simple CSTR. (**c**) CSTR with external biomass recycle. (**d**) CSTR with internal biomass recycle. *Dashed lines* indicate system boundary used for developing mass balance equations

Continuous Stirred Tank Reactors

Theoretically, a continuous process can be described with the following equations:

$$\frac{dX}{dt} = (\mu - D)X \tag{10.9}$$

$$D = \frac{F}{V}, \quad \left(\frac{1}{h}\right) \tag{10.10}$$

$$T_R = \frac{V}{F}, \quad (h) \tag{10.11}$$

$$M_S = R_S - \frac{\mu}{Y_{SX}} \tag{10.12}$$

where
μ is the specific growth rate (1/h); D is dilution rate (1/h); X is biomass concentration (g/L); F is the feed flow rate (L/h); V is the volume of the bioreactor (L); T_R is the residence time (h); M_s is the maintenance value (C-mol/C-mol/h); R_s is the rate of substrate consumption (C-mol/C-mol/h); Y_{sx} is the yield of biomass per unit mass of the substrate.

Ethanol Production

Bio-ethanol is ethyl alcohol, grain alcohol, or chemically C_2H_5OH or EtOH. Bio-ethanol and bio-ethanol/gasoline blends have a long history as alternative transportation fuels. Bio-ethanol has a higher octane number (108), broader flammability limits, higher flame speeds and higher heats of vaporization. Disadvantages of bio-ethanol include its lower energy density than gasoline (bio-ethanol has 66 % of the energy that gasoline has), its corrosiveness, low flame luminosity, lower vapor pressure (making cold starts difficult), miscibility with water, toxicity to ecosystems (Spatari et al. 2005) increase in exhaust emissions of acetaldehyde, and increase in vapor pressure (and evaporative emissions) when blending with gasoline. Some properties of alcohol fuels are shown in Table 10.2.

Table 10.2 Some properties of ethanol (alcohol fuel)

S. no.	Fuel property	Ethanol
1	Octane number	108
2	Auto ignition temperature (K)	606
3	Latent heat of vaporization (MJ/Kg)	0.91
4	Lower heating value (MJ/Kg)	26.7

Source: Balat and Balat 2009

Ethanol Production from Sugar, Starch and Lignocellulosic Feedstocks

Bio-ethanol is a fuel derived from biomass sources of feedstock; typically plants such as wheat, sugar beet, corn, straw, and wood. The conversion of lignocellulosic biomass to ethanol is a three step process that involves pretreatment followed by polysaccharide hydrolysis to simple sugars followed by sugar fermentation to ethanol (Mielenz 2001). The presence of lignin in cell walls negatively impacts these conversion steps (Keating et al. 2006; Li et al. 2008).

The effect of pretreatment of lignocellulosic materials has been recognized for a long time (McMillan 1994). Pretreatments for lignocellulosic materials include mechanical comminution, alkali swelling, acid hydrolysis, steam and other fiber explosion techniques, and exposure to supercritical fluids. Mechanical comminution. Waste materials can be comminuted by a combination of chipping, grinding and milling to reduce cellulose crystallinity. Pyrolysis has also been used for pretreatment of lignocellulosic materials. When the materials are treated at temperatures greater than 300 °C, cellulose rapidly decomposes to produce gaseous products and residual char (Sun and Cheng 2002). Mild acid hydrolysis (1 N H_2SO_4, 97 °C, 2.5 h) of the residues from pyrolysis pretreatment has resulted in 80– 85 % conversion of cellulose to reducing sugars with more than 50 % glucose (Sun and Cheng 2002a, b). Steam explosion is the most commonly used method for pretreatment of lignocellulosic materials (McMillan 1994). In this method, chipped biomass is treated with high-pressure saturated steam and then the pressure is swiftly reduced, which makes the materials undergo an explosive decompression. Steam explosion is typically initiated at a temperature of 160–260 °C (corresponding pressure 0.69–4.83 MPa) for several seconds to a few minutes before the material is exposed to atmospheric pressure. The process causes hemicellulose degradation and lignin transformation due to high temperature, thus increasing the potential of cellulose hydrolysis. Ammonia fiber explosion (AFEX) is another type of physico-chemical pretreatment in which lignocellulosic materials are exposed to liquid ammonia at high temperature and pressure for a period of time, and then the pressure is swiftly reduced. Dilute acid hydrolysis such as H_2SO_4 and HCl has been successfully developed for pretreatment of lignocellulosic materials. The dilute sulfuric acid pretreatment can achieve high reaction rates and significantly improve cellulose hydrolysis (Esteghlalian et al. 1997). The mechanism of alkaline hydrolysis is believed to be saponification of intermolecular ester bonds crosslinking xylan hemicelluloses and other components, for example, lignin and other hemicellulose. The porosity of the lignocellulosic materials increases with the removal of the crosslinks (Tarkow and Feist 1969). Dilute NaOH treatment of lignocellulosic materials caused swelling, leading to an increase in internal surface area, a decrease in the degree of polymerization, a decrease in crystallinity, separation of structural linkages between lignin and carbohydrates, and disruption of the lignin structure (Fan et al. 1987).

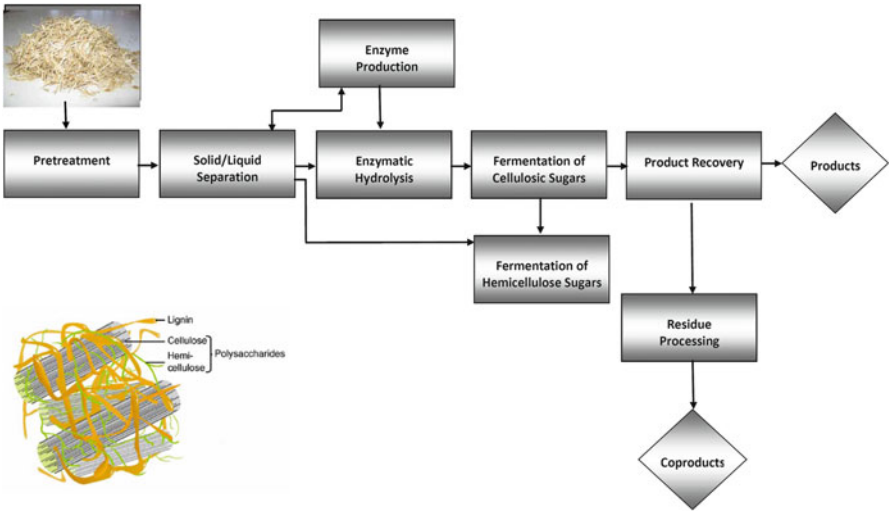


Fig. 10.5 Current process to produce biofuel from lignocellulose

Enzymatic hydrolysis of cellulose is carried out by cellulase enzymes which are highly specific (Beguin and Aubert 1994). The products of the hydrolysis are usually reducing sugars including glucose. Utility cost of enzymatic hydrolysis is low compared to acid or alkaline hydrolysis because enzyme hydrolysis is usually conducted at mild conditions (pH 4.8 and temperature 45–50 °C) and does not have a corrosion problem (Duff and Murray 1996).

Fermentation

Ethanol can be produced from lignocellulosic materials in various ways. The main features of the different ethanol processes are outlined in Fig. 10.5. All processes comprise the same main components: hydrolysis of the hemicellulose and the cellulose to monomer sugars, fermentation and product recovery and concentration by distillation (Galbe and Zacchi 2002).

The most frequently used microorganism for fermenting ethanol in industrial processes is *S. cerevisiae*, *Zymomonas mobilis* can ferment glucose to ethanol with higher yields. Since lignocellulosic hydrolysates contain pentoses, which are not readily fermented by these microorganisms, several attempts to genetically engineer *S. cerevisiae* (Walfridsson et al. 1996; Hahn-Hägerdal et al. 2007), *Z. mobilis* (Panesar et al. 2006) and the bacteria *Escherichia coli* (Decker et al. 2007) have been performed (Fig. 10.6).

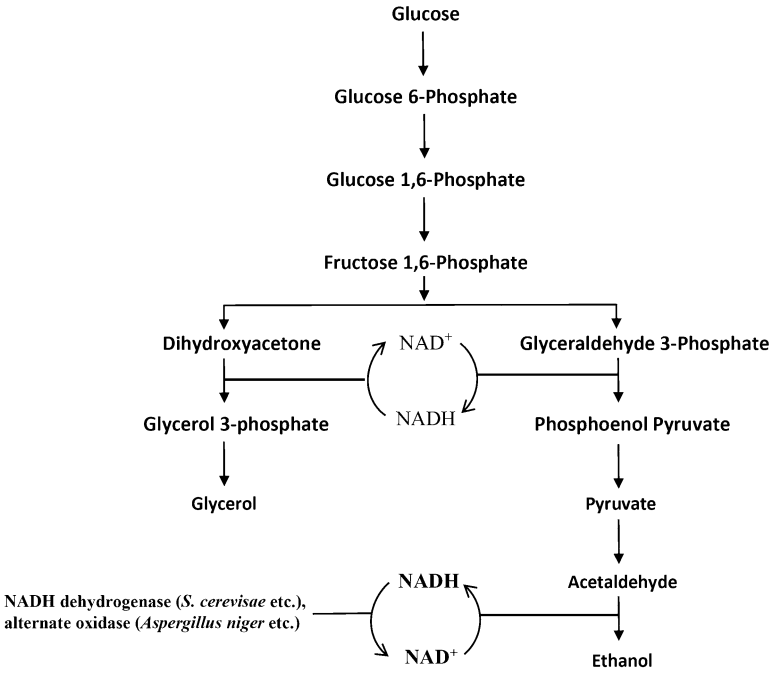


Fig. 10.6 Redox balance in biosynthetic routes to glycerol and ethanol in *S. cerevisiae* (Baskar et al. 2012a, b)

Yeast convert hexose to ethanol and carbon dioxide by glycolysis as shown by the following reaction:

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2$$

Theoretically, 1 kg of glucose will produce 0.51 kg of bio-ethanol and 0.49 kg of carbon dioxide. However, in practice, the microorganisms use some of the glucose for growth and the actual yield is less than 100 % (Demirbas 2009a, b).

Usually by products such as glycerol, succinic acid and acetic acids are produced. Optimum temperature and pH values for yeast are 30–35 °C and 4–5 respectively (Shuler and Kargi 2002). Most notably, *C. thermohydrosulfuricum* strain 39E has the highest reported ethanol yield (1.9 mol of ethanol produced per mol of glucose fermented) of any taxonomically described thermophilic anaerobe (Ng et al. 1981). For thermophilic organisms optimum temperature may range from 50 and 60 °C. Ethanol production is triggered by anaerobic conditions. Glucose concentrations above 100 g/l are inhibitory for yeast. Ethanol and some of the other by-products are inhibitory to yeast above concentration of 5 % (v/v). Ethanol tolerance yeast strains are being developed to avoid ethanol inhibition (Balat and

Balat 2009). Simultaneous removal of ethanol from fermentation broth is another alternative for ethanol inhibitions.

Downstream Processing

Ethanol can be separated from the culture vessels during fermentation using low temperature vacuum distillation, adsorption, or membrane separation.

Butanol Production

Butanol (butyl alcohol and 1-butanol) is a four carbon primary alcohol having the molecular formula of C_4H_9OH (MW 74.12). Butanol is a colorless liquid with a distinct odor. Butanol is completely miscible with organic solvents and partly miscible with water (Lee et al. 2008a, b). Butanol represents a biofuel extender or replacement with properties clearly superior to ethanol (higher mileage, not hygroscopic, usable without engine modifications, not corrosive). In addition, it is a valuable feedstock for the chemical industry (Dürre 2011).

A sustainable bacterial fermentation route to produce biobutanol is poised for re-commercialization. Biobutanol may be produced by the acetone–butanol–ethanol (ABE) fermentation (Kumar and Gayen 2011). Today, biobutanol can compete with synthetic butanol in the chemical market (Green 2011).

Several countries have initiated new alternatives for biobutanol production from renewable feedstocks like sweet sorghum bagasse, rice bran (RB), de-oiled rice bran (DRB), corn stover, and wheat straw (Swana et al. 2011; Al-Shorgani et al. 2012; Zhang et al. 2011). By sustainable harvest based on current yields, these materials can be converted to 8.27 billion gallons of biobutanol replacing 7.55 billion gallons of gasoline annually (Swana et al. 2011). Common feedstocks used for biobutanol fermentation process are mentioned in Table 10.3.

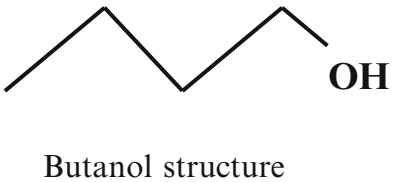
Researchers have been re-directing their interests in biomass based fuels, which currently seem to be the only logical alternative for sustainable development in the context of economic and environmental considerations. Renewable bioresources are available globally in the form of residual agricultural biomass and wastes, which can be transformed into liquid biofuels (Nigam and Singh 2011).

Although research on genetics, fermentation, upstream processing, and downstream processing has progressed significantly, the *Clostridia* are not able to efficiently hydrolyze fiber-rich agricultural residues. For this reason, agricultural biomass must be hydrolyzed to simple sugars using economically developed methods. Dilute sulfuric acid pretreatment can be applied to agricultural residues to bring about hydrolysis. Unfortunately, during acid hydrolysis, a complex mixture of microbial inhibitors is generated. Examples of the inhibitory compounds include

Table 10.3 Feedstocks used for biobutanol fermentation process (Niemisto et al. 2013)

Feedstock source	Examples	Advantages	Disadvantages	Reference
Agricultural residues or byproducts	Bagasse, corn stover/fiber/cobs, straws (e.g. from barley, rice or wheat)	Easier upstream processing to fermentable sugars	Seasonal availability, variations in cultivation yield and quality, land-use change, transport costs (low density)	Soni et al. 1982; Qureshi et al. 2006, 2008a, b, c, 2010a, b; Marchal et al. 1984
Crop biomass	Cassava, corn	Easier upstream processing to fermentable sugars	Edible, seasonal availability, variations in yield and quality, land-use change, water need for irrigation	Thang et al. 2010; Campos et al. 2002; Ezeji et al. 2007a, b, c
Non-food crop biomass	Switchgrass, Jerusalem artichoke	Does not compete with food use	Land-use change possible if fertile land is used, potential water need	Qureshi et al. 2010a, b; Marchal et al. 1985
Wood-based biomass	Wood hydrolysates (e.g. from aspen, pine, beech or hemlock)	Non-food biomass, good availability, lower transport costs	More difficult upstream processing, indirect land-use change possible	Saddler et al. 1983; Yu et al. 1984; Sjolander et al. 1938; Maddox and Murray 1983
Industrial by-products	Apple pomace, cheese whey, distillers dry grain solids (DDGS), potato waste, brans (e.g. from rice or wheat), soy molasses, waste sulfite liquor	Better social acceptance by means of resource use efficiency and waste minimization, no land-use change	Availability and quality of the raw material may vary, additional processing may be needed to separate the feedstock from the main product	Qureshi et al. 2001; Lee et al. 2009; Nimcevic et al. 1998; Gutierrez et al. 1998; Grobben et al. 1993
Biodegradable municipal waste	Food and garden waste, starch-based packing peanuts, sludge from wastewater treatment	Better social acceptance contributes to resource efficiency and waste minimization, no land-use change	(Seasonal) and qualitative variation	Murty and Chandra 1997; Claassen et al. 2000; Jesse et al. 2002; López-Contreras et al. 2000; Kobayashi et al. 2005

Table 10.4 Characteristic properties of butanol

Characteristic	Butanol	
Formula	$CH_3(CH_2)_3OH$	 Butanol structure
Boiling point (°C)	118	
Melting point (°C)	−89.3	
Ignition temperature (°C)	35	
Flash point (°C)	365	
Density at 20 °C (g/mL)	0.8098	
Critical pressure (hPa)	48.4	
Critical temperature (°C)	287	
Heat of vaporization (MJ/kg)	0.43	
Energy density (MJ/L)	29.2	
Motor octane number	78	

furfural, hydroxymethyl furfural (HMF), and acetic, ferulic, glucuronic, rho-coumaric acids, etc. (Varga et al. 2004).

Formerly, ABE fermentation was operated as a batch process followed by distillation to recover the products. Sugars (molasses) or starch (corn, wheat, and potatoes) was used as substrates. In this process the price of the substrate accounts for up to 60 % of the cost, dramatically affecting the economic viability of ABE fermentation (Claassen et al. 1999).

The use of marine algal biomass with high carbohydrate contents of *Ulva lactuca* and other macroalgae like *Saccharina spp. Laminaria*, *Durvillaea*, *Ecklonia* and *Homosira* (brown algae) (Figueira et al. 2000) indicates that a more cost effective strategy might be to ferment the carbohydrates like glucose, mannitol and laminarin from these algal species to either ethanol or butane (Potts et al. 2012; Huesemann et al. 2012).

Important characteristics of butanol are summarized in Table 10.4. The market for biobutanol is currently worth US$5 billion and is estimated to rise to $247 billion by 2020 (Kretzers 2012).

Unfortunately, neither commercial ethanol-producing cultures, nor butanol-producing cultures can hydrolyze these substrates. Hence, they need to be hydrolyzed prior to fermentation using a combination of pretreatment (acid, alkali, organosolvent, supercritical extraction or ammonia explosion) and hydrolysis (enzymes: cellulase, β-glucosidase, and xylanase) techniques (Galbe and Zacchi 2002). It should be noted that in contrast to ethanol production by yeasts, hexose and pentose sugars obtained as a result of pretreatment and hydrolysis of these residues can be used by butanol-producing cultures (Qureshi et al. 2008a, b, c).

Feedstock's biomass pretreatment can be achieved by air dry the biomass, dry, grind, and then hydrolyze with dilute acid such as sulfuric acid with different concentrations (at 0.5, 1.0, 2.0, and 5.0 % by weight) (Potts et al. 2012). After hydrolysis the pH was adjusted to a value deemed suitable for fermentation (approximately 4.5–5). Various lignocellulosic feedstocks have been claimed for maximum solvent production by *Clostridium* (Table 10.5).

Table 10.5 Different feedstocks and strains used along with maximum solvents and productivities achieved (Jurgens et al. 2012)

Substrate	Hydrolysis method	Strain used	Yield (g/g)/ productivity (g/l h)	Total ABE (g/l)	References
Wheat straw	H_2SO_4 + enzyme	*C. beijerinckii* P260	0.60/0.42	25	Qureshi et al. (2007)
Wheat straw	H_2SO_4 + enzyme	*C. beijerinckii* P260	0.41/0.31	21.42	Qureshi et al. (2008a)
Corn fiber	H_2SO_4	*C. beijerinckii* BA101	0.39/0.10	9.3	Qureshi et al. (2008c)
Rice bran and defatted rice bran	HCl + enzyme	*C. beijerinckii* NCIMB 8052	0.31/0.26	16.42	Lee et al. (2009)
Barley straw	H_2SO_4 + enzyme	*C. beijerinckii* P260	0.43/0.39	26.64	Qureshi et al. (2010a)
Corn stover	H_2SO_4 + enzyme	*C. beijerinckii* P260	0.44/0.31	26.27	Qureshi et al. (2010b)
Wheat bran	H_2SO_4	*C. beijerinckii* ATCC 55025	0.32/0.16	11.8	Liu et al. (2010)
Rice straw	H_2SO_4 + enzyme	*C. acetobutylicum* MTCC 481	1.04a/0.017 (Only butanol yield and productivity)	3.0	Ranjan and Moholkar (2011)

In addition, during the last years the use of ionic liquids (ILs) such as [BMIM]Cl, [BMIM][PF6], [BMIM][TFSI], etc. for dissolving lignocellulosics has been examined intensively (García et al. 2011; Holm et al. 2012).

Pretreatment of lignocellulosic biomass in a microwave oven is also a feasible method which uses the higher heating efficiency of a microwave oven and it is also easy to operate (Bjerre et al. 1996). Microwave treatment utilizes thermal and non-thermal effects generated by microwaves in aqueous environments (Sun and Cheng 2002).

ABE (Acetone, Butanol and Ethanol) Fermentation

ABE hetero-fermentation produces acetate, butyrate, ethanol, and acetone, as well as butanol. The metabolism of ABE producing clostridia can be divided into the following two distinct phases: acidogenesis (acid-production) and solventogenesis (solvent-production) during the exponential and stationary phases of growth (Jones and Woods 1986).

Biobutanol is a biofuel that can be produced from renewable resources using special strains of bacteria such as *Clostridium acetobutylicum* or *Clostridium beijerinckii* (Qureshi et al. 2007).

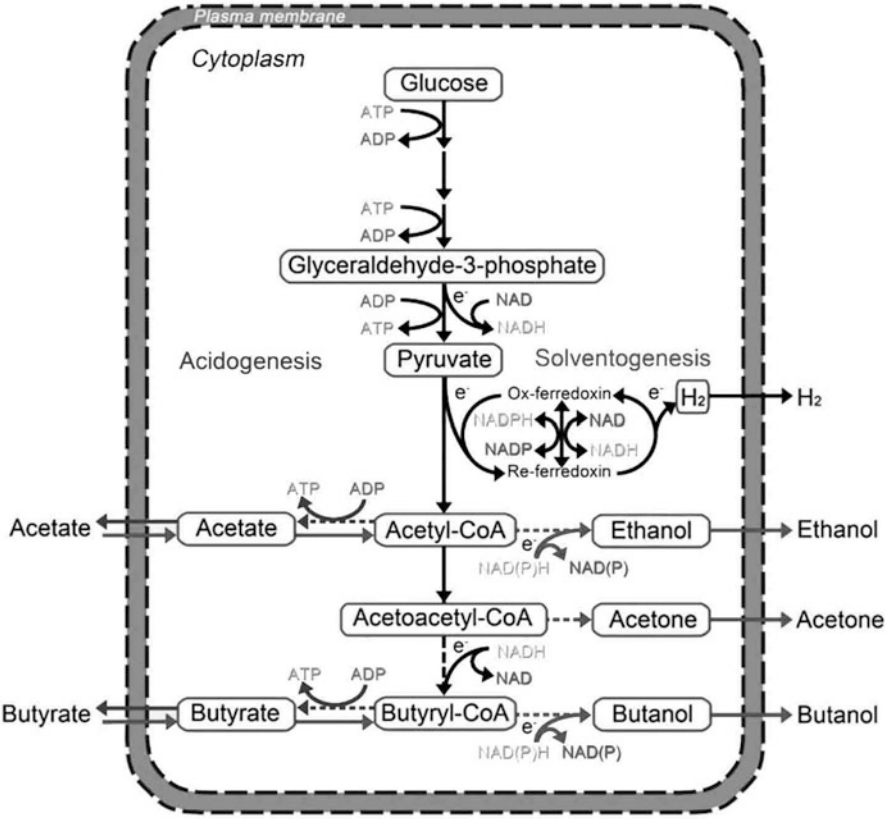


Fig. 10.7 Major redox reactions in acetone–butanol–ethanol fermentation by the bacterium *Clostridium* (Liu et al. 2013)

In a normal batch culture, solvent-producing *Clostridium* species produce hydrogen, carbon dioxide, acetate, and butyrate during the initial growth phase (acidogenic phase), which results in a decrease in the pH of the culture medium. As the culture enters the stationary growth phase, the metabolism of the cells undergoes a shift to solvent production (solventogenic phase). During the second phase of the fermentation the reassimilation of acids, this occurs concomitantly with the continued consumption of carbohydrate, normally results in an increase in the pH of the culture medium. The relationship between the breakpoint in the pH of the fermentation and the onset of solvent production, which occurs at the beginning of the second phase of the fermentation, was identified early on in the development of the industrial fermentation process (Jones and Woods 1986). Major redox reactions in acetone–butanol–ethanol fermentation by the bacterium *Clostridium* is shown in Fig. 10.7.

$$\underset{\text{Glucose}}{12\,C_6H_{12}O_6} \rightarrow \underset{\text{n}-\text{Butanol}}{6CH_3CH_2CH_2CH_2OH} + \underset{\text{Acetone}}{4CH_3COCH_3} + \underset{\text{Ethanol}}{2CH_3CH_2OH} + 18H_2 + 28CO_2 + 2H_2O$$

Acidogenesis

Bacteria grows exponentially in the first phase of fermentation (acidogenesis phase) along the formation of acids (mostly acetate and butyrate), leading to decrease of pH to 4.5 (Gheshlaghi et al. 2009). Two moles each of pyruvate, ATP and NADH are produced from one mole glucose consumed through the glycolytic pathway in the acidogenic phase. In this phase, glycolysis pathway is active to produce pyruvate consuming glucose, which is converted to Acetyl-CoA. Acetyl-CoA is the prime precursor for synthesis of acetate, butyrate, ethanol, butanol and acetone anaerobically. Acetate and butyrate are produced in acid producing phase through two analogous steps from acetyl-CoA and butyryl-CoA respectively (Kumar and Gayen 2011). An update review on key enzymes for butanol production is available (Gheshlaghi et al. 2009). When acids accumulate to sufficiently high levels, cells cannot maintain the pH gradient across membranes, and a dramatic decrease in growth occurs (Huang et al. 2010). Therefore, the shift to solvent production in Clostridia is an adaptive response to toxic effect of acidic metabolites through their re-assimilation and induced expression of genes for the stress response (Grimmler et al. 2011; Grupe and Gottschalk 1992).

Solventogenesis

As intracellular ATP is consumed by biosynthesis, solventogenesis is initiated to consume NAD(P)H accumulated during the acidogenesis (Grupe and Gottschalk 1992). The acetyl-CoA and butyryl-CoA are the key intermediates in synthesizing ethanol and butanol (Sillers et al. 2008). The reduction of acetyl-CoA and butyryl-CoA to acetylaldehyde and butyraldehyde is catalyzed by acetaldehyde dehydrogenase and butyraldehyde dehydrogenase, respectively, followed by the further reduction of acetylaldehyde and butyraldehyde to ethanol and butanol by ethanol dehydrogenase and butanol dehydrogenase (Gheshlaghi et al. 2009; Jones and Woods 1986). In both *C. acetobutylicum* and *C. beijerinckii*, the activity of butanol dehydrogenase was NADPH dependent rather than NADH dependent (Dürre 2008).

The use of excess carbon under nitrogen limitation is required to achieve high levels of solvent production (Madihah et al. 2001). Iron is one of the essential factors for the production of solvent (Kim et al. 1984). When *Clostridium acetobutylicum* was grown in batch culture under iron limitation (0.2 mg l^{-1}) at a pH of 4.8, glucose was fermented, to butanol as the major fermentation end product, and small quantities of acetic acid were produced. The final conversion yield of glucose into butanol could be increased from 20 to 30 % by iron limitation (Junelles et al. 1988). However, if the pH decreases below 4.5 before enough acids are formed, solventogenesis will be brief and unproductive. Increasing the buffering

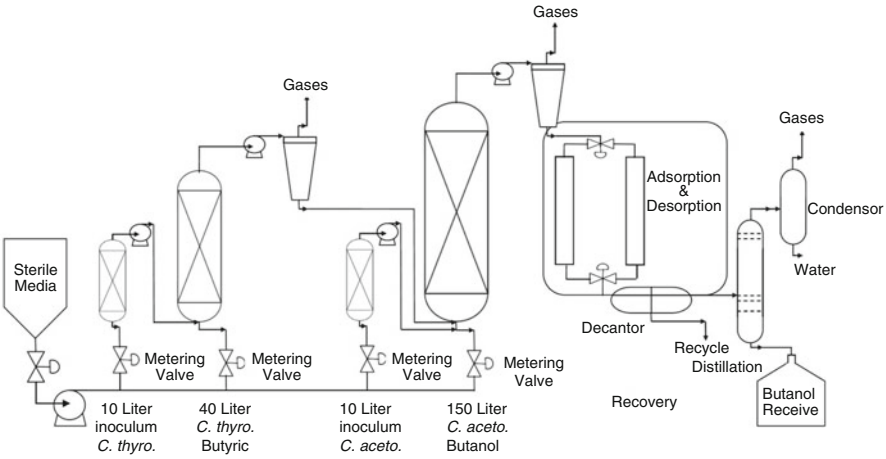


Fig. 10.8 Pilot plant for biobutanol production (*Source*: Butyl fuels, 2010 Korean Institute of Science and Technology)

capacity of the medium is a simple way to increase growth and carbohydrate utilization as well as butanol production (Bryant and Blaschek 1988).

The fermenter is inoculated with a 5 % inoculum from a 24 h culture. The batch fermentation period is usually 2–2.5 days. First rapid growth and production of acetic/butyric acids and carbon dioxide and hydrogen occur. The initial pH of the medium drops from 6.5 to nearly 4.5 during this phase. In a second phase, growth ceases and the organisms convert acetic and butyric acids to neutral acetone and butanol. The acidity of the medium decreases and gas production increases. At the end of the fermentation the pH is approximately 5 (Shuler and Kargi 2002). The final total concentration of solvents produced ranges from 12 to 20 g/L in batch fermentation, which can be separated from the fermentation broth by distillation. Classical fed-batch and continuous cultivation do not seem to be economically feasible, because of solvent toxicity and the biphasic nature of acetone–butanol fermentation, respectively. To overcome this problem, fed-batch culture has been coupled with an in situ recovery process (Ezeji et al. 2004a, b), and multistage continuous fermentation has been conducted (Godin and Engasser 1990). Pilot plant for biobutanol production has been shown in the Fig. 10.8.

Solvent Toxicity is one of the most critical problems in ABE fermentation, which ceases Clostridial cellular metabolism in the presence of 20 g/L or more solvents (Woods 1995). Moreira et al. (1981) and Jones et al. (1982) had attempted to elucidate the mechanism of butanol toxicity in *C. acetobutylicum*.

Downstream Processing

During the past two decades a significant amount of research has been performed on the use of alternative fermentation and product recovery techniques (e.g. adsorption,

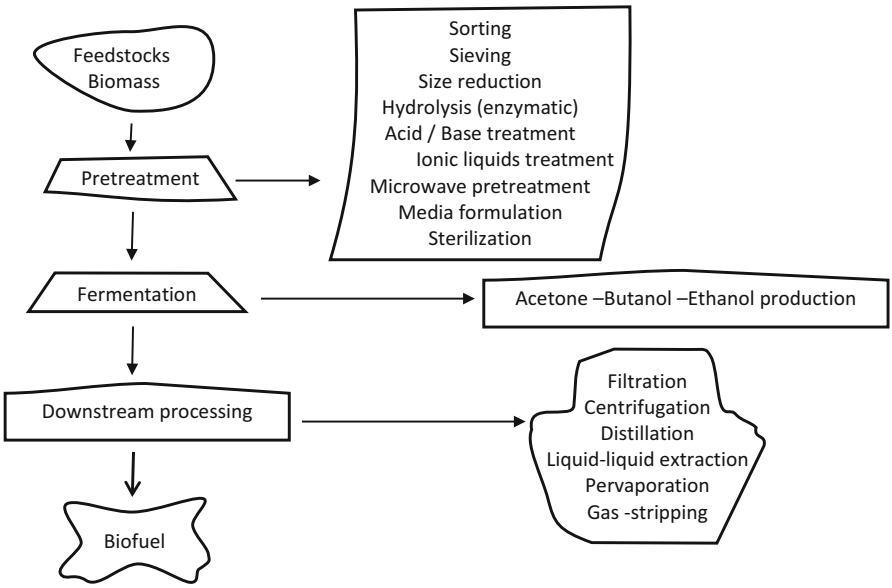


Fig. 10.9 Bioprocess stages and unit operations (Moo-Young and Chisti 1994)

gas stripping, ionic liquids, liquid–liquid extraction, pervaporation, aqueous two-phase separation, supercritical extraction, and perstraction, etc.) for biobutanol production (Ezeji et al. 2007a, b, c). The various bioprocess stages and unit operations along with pretreatment are shown in the Fig. 10.9.

The application of some of these techniques to the ABE fermentation process is described below.

1. Distillation: The cost of recovering butanol by distillation is high because its concentration in the fermentation broth is low due to product inhibition. In addition to the low product concentration, the boiling point of butanol is higher than that of water (118 °C). The usual concentration of total solvents in the fermentation broth is 18–33 g/L (using starch or glucose) of which butanol is only about 13–18 g/L. This makes butanol recovery by distillation energy intensive (Ezeji et al. 2004a, b).
2. Liquid–liquid extraction is another efficient technique to remove solvents from the fermentation broth. This approach takes advantage of the differences in the partition coefficient of the solvents. As butanol is more soluble in the extractant (organic phase) than in the fermentation broth (aqueous phase), it is selectively concentrated in the extractant. Common extractants employed include decanol and oleyl alcohol (Lee et al. 2008a, b).
3. Pervaporation is a membrane-based process that is used to remove solvents from the fermentation broth by using a selective membrane. The liquids or solvents diffuse through a solid membrane, leaving behind nutrients, sugar, and microbial

Table 10.6 Biobutanol recovery (Kumar and Gayen 2011; Heitmann et al. 2012)

Methods	Principle	Advantage	Disadvantage
Distillation	Boiling occurs when the vapor pressure of a liquid exceeds the ambient pressure	Traditional method	Expensive to perform
Gas stripping	Heating of effluent, purging with gas, condensation of solvent/water vapours	Simple to perform, low chance of clogging or fouling	Low selectivity, no complete removal of solvents, more energy required compared to membrane based processes
Liquid-liquid extraction	Contact of water—immiscible solvent with fermentation broth, recovery of acetone/butanol / isopropanol by distillation	High capacity, high selectivity, low chance of clogging or fouling	Expensive to perform, possible formation of emulsions
Pervaporation	Selective diffusion of solvents across a non-porous membrane, recovery of evaporated vapours by applying vacuum or sweep gas	High selectivity compared to membrane evaporation, simple to perform	Lower membrane flux compared to membrane evaporation, possible clogging and fouling

cells. The application of pervaporation to batch butanol fermentation has been described by several investigators (Ezeji et al. 2004a, b).

4. Gas stripping is a simple but efficient way to recover butanol from the fermentation broth. The fermentation gas is bubbled through the fermentation broth, and then passed through a condenser for solvent recovery. The stripped gas is then recycled back to the fermentor and the process continues until all the sugar in the fermentor is utilized (Lee et al. 2008a, b; Ezeji et al. 2003). Butanol recovery is based on the principle along with their advantage and disadvantage is shown in Table 10.6 and integrated systems for fermentation and in situ solvent recovery are shown in Fig. 10.10.

Biodiesel

The transesterification of vegetable oils (VOs) with short-chain alcohols is used to produce biodiesel or by the esterification of fatty acids. During the past few years biodiesel has attracted attention as an environmentally friendly and renewable fuel because of uncertainties concerning petroleum availability and recent increases in petroleum prices. Its chemical structure is that of fatty acid alkyl esters. The production of biodiesel by transesterification employing acid (H_2SO_4, HCl, etc.) or base catalyst (NaOH, KOH, $NaOCH_3$, etc.) has been industrially accepted for its

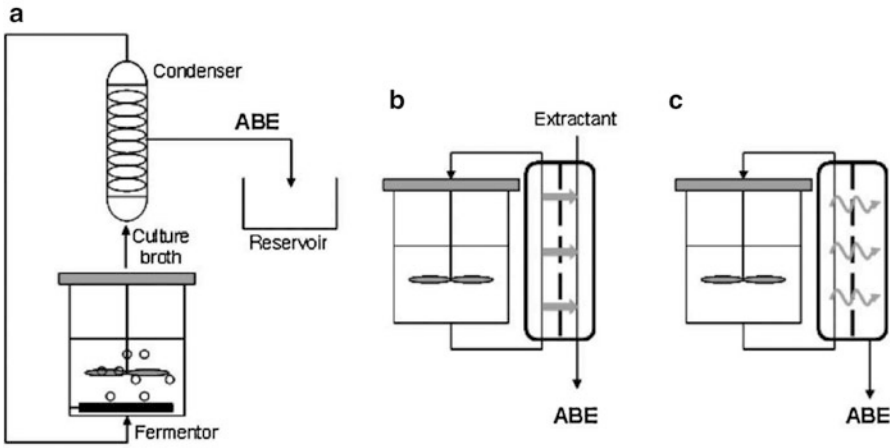


Fig. 10.10 Integrated systems for fermentation and in situ solvent recovery: fermentation coupled with (**a**) gas stripping; (**b**) liquid–liquid extraction (perstraction); (**c**) pervaporation (Lee et al. 2008a, b)

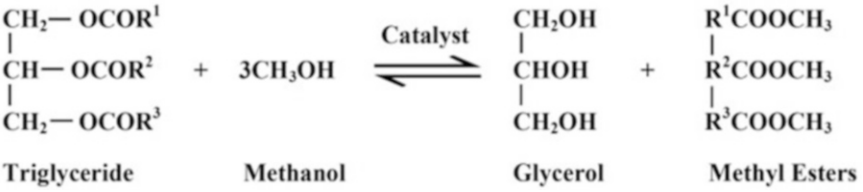


Fig. 10.11 General scheme for transesterification of triglycerides

high reaction and conversion rates. Biological catalyst (lipase) is also sufficient to carry out the reaction at lowest amount, since it is faster.

Biodiesel is defined as fatty acid methyl or ethyl esters (FAME) from vegetable oils or animal fats when they are used as fuel in diesel engines and heating systems (Marchetti and Errazu 2008). Nowadays, it is used as an alternative fuel due to depleting petroleum reserves (Sujan et al. 2009). Fatty acid methyl esters are products of the transesterification (also called methanolysis) of vegetable oils and fats with methanol in the presence of a suitable catalyst to form alkyl esters (biodiesel) and glycerin. The main chemical process to produce biodiesel is the alkaline transesterification with methanol and KOH, where the alcohol reacts in the presence of the catalyst to form alkyl esters (biodiesel) and glycerin (Neha et al. 2013). Beside these products, traces of unreacted glycerides (mono-, di- and tri-acylglycerides) can also be found. If methanol is used in this process it is called methanolysis. Methanolysis of triglyceride is represented in Fig. 10.11.

However; biodiesel has a higher cetane number, no aromatics, and contains 10 %–11 % oxygen by weight. These properties of biodiesel reduce the emissions of carbon monoxide (CO), hydrocarbons (HC), and particulate matter (PM) in the exhaust gas (Math et al. 2010). Preferred methods of production of biodiesel typically consist of reaction of oil sources with alcohols with aid of either acid or base.

Oil Sources and Methods of Biodiesel Production

Biodiesel is usually produced from food-grade vegetable oils using transesterication process. Therefore, it is said that the main obstacle for commercialization of biodiesel is its high cost. Waste cooking oils, restaurant greases, soapstocks and animal fats are potential feedstocks for biodiesel production to lower the cost of biodiesel (Canakci and Sanli 2008). The feed stock for biodiesel production is mainly soybean oil, sunflower oil, jatropha oil, canola oil, rapeseed oil, rubber seed oil and micro-algae etc. (Demirbas 2005). The cost of biodiesel is slightly higher than the petroleum based diesel mainly due to cost of edible oils which makes it more costly than the diesel fuel (Aworanti et al. 2013). Biodiesel obtained from vegetable oils has been considered a promising option but its higher viscosity is major problem which can reduce the fuel atomization (Pratas et al. 2011a, b). The petroleum based diesel fuel emits more carbon dioxide, greenhouse gases and hydrocarbon particulate matter, these are humiliation of the entire environment, regarding environmental concern, biodiesel has received more attention worldwide due to its properties such as clean, biodegradable, safe and eco-friendly (Atadashi et al. 2011). Presently many countries such as Germany, Australia and United State are already using biodiesel in replacement of traditional petroleum based diesel. In the United States, soybean oil is the most common biodiesel feedstock whereas rapeseed and palm oil are the most commonly used in Europe (Singh and Singh 2010). One of the most important disadvantages of using biodiesel is their cost. Biodiesel purification is carried out at the end of the reaction; the glycerin formed is separated from the methyl esters in a decantation funnel. The purification of methyl ester is done by washing with preheated distilled water (at 55 °C for 1 h). The pH of biodiesel should be approximately neutral (Hossain et al. 2010). The less dense phase, composed by esters, are removed and stored for further analysis and purification. The process flow diagram for biodiesel production is given in Figs. 10.12 and 10.13 represents enzymatic production process of biodiesel with immobilized lipase.

ASTM International, recognized from 2001 as the American Society for Testing and Materials, is worldwide standards organization that holds properties values of biodiesel (Table 10.7).

The combined vegetable oil and animal fat production in the United States totals about 35.3 billion pounds per year (Perlack et al. 2005). This production could

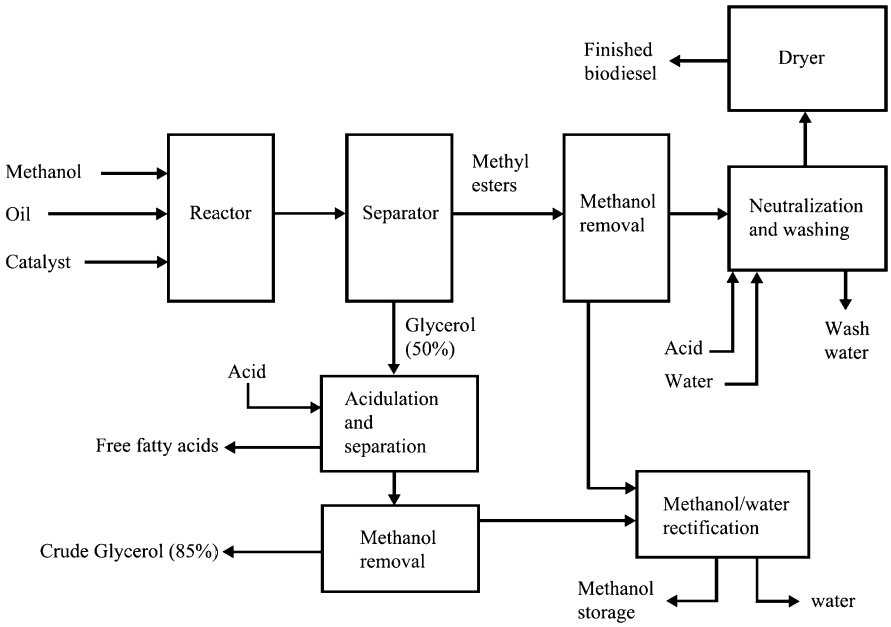


Fig. 10.12 Process flow schematic for biodiesel production

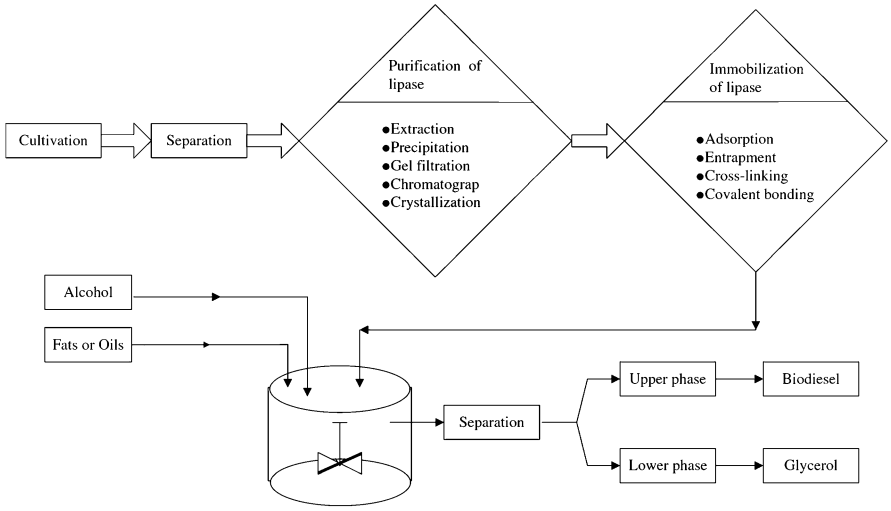


Fig. 10.13 Enzymatic production process of biodiesel with immobilized lipase (*Source*: Zhang et al. 2012)

Table 10.7 Fuel properties of biodiesel from soybean oil

S. no.	Property of biodiesel	ASTM D6751-06 standard	Soybean biodiesel
1	Density	860–890 (kg/m^3)	880 (kg/m^3)
2	Viscosity	–	90 (Redwood second)
3	Flash point	>130 (°C)	162 °C
4	Acid value	0.8 max (mg KOH/g)	0.20 (mg KOH/g)
5	Saponification value	169–280 (mg KOH/g)	137 (mg KOH/g)
6	Cloud point	−3 to 12 (°C)	10 °C

provide 4.6 billion gallons of biodiesel. Methyl ester is analysed by gas chromatography.

Production of Hydrogen

Hydrogen production plays a very important role in the development of hydrogen economy. Biomass and water can be used as renewable resources for hydrogen gas production. Biological production of hydrogen gas has significant advantages over chemical methods (Ni et al. 2006). The major biological processes utilized for hydrogen gas production are bio-photolysis of water by algae, dark and photo-fermentation of organic materials, usually carbohydrates by bacteria (Kapdan and Kargi 2006). Carbohydrate rich, nitrogen deficient solid wastes such as cellulose and starch containing agricultural and food industry wastes and some food industry wastewaters such as cheese whey, olive mill and bakers yeast industry wastewaters can be used for hydrogen production by using suitable bio-process technologies (Ghirardi et al. 2010). Conventional hydrogen gas production methods are steam reforming of methane (SRM), and other hydrocarbons (SRH), non-catalytic partial oxidation of fossil fuels (POX) and autothermal reforming which combines SRM and POX (Kapdan and Kargi 2006). Integrated biohydrogen system is shown in Fig. 10.14.

Hydrogen Production by Fermentation

Biological hydrogen production can be classified into five different groups: (1) direct biophotolysis, (2) indirect biophotolysis, (3) biological water–gas shift reaction, (4) photofermentation and (5) dark fermentation (Levin et al. 2004). Comparative biological hydrogen production process is given in Table 10.8.

Bio-hydrogen production from cellulose/starch containing agricultural wastes and food industry wastewaters is represented in Fig. 10.15.

All processes are controlled by the hydrogen-producing enzymes, such as hydrogenase and nitrogenase. The major components of nitrogenase are MoFe protein and Fe protein. Nitrogenase has the ability to use magnesium adenosine

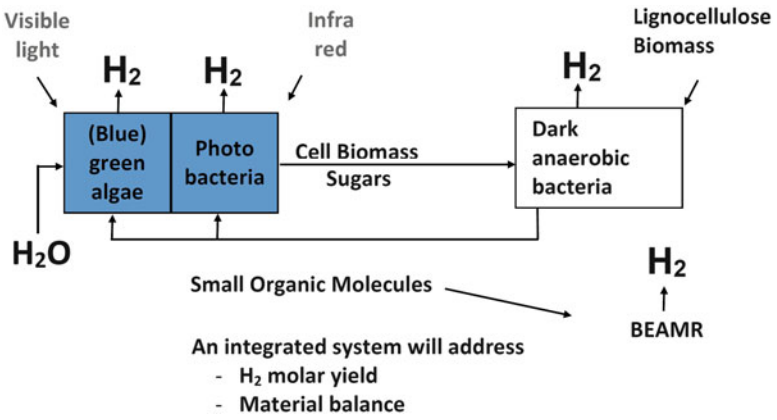


Fig. 10.14 Schematic representation of integrated biohydrogen system

Table 10.8 Major advantages and disadvantages of biological hydrogen production process

Process	Types of microorganism	Advantages	Drawback
Biophotolysis of water	Green algae or Cyanobacteria	Product: $H_2 + O_2$ Substate: $H_2O + CO_2$	Low H_2 production rate O_2 inhibition
Water-gas shift reaction	Photosynthesis or fermentative bacteria	Treatment of CO waste gas	Mass transfer limitation CO substrate limitation
Photodecomposition of organic compounds	Photosynthetic bacteria	High H_2 yield	Light requirement Low H_2 production rate
Fermentation of sugars (dark fermentation)	Fermentative bacteria	Fast rate Treatment of organic wastewater	Low H_2 yield By-product formation

triphosphate (MgATP) and electrons to reduce a variety of substrates (including protons) (Fig. 10.16). This chemical reaction yields hydrogen production by a nitrogenase-based system (Hallenbeck and Benemann 2002):

$$2e^- + 2H^+ + 4ATP \rightarrow H_2 + 4ADP + 4P_i$$

where ADP and P_i refer to adenosine diphosphate and inorganic phosphate, respectively.

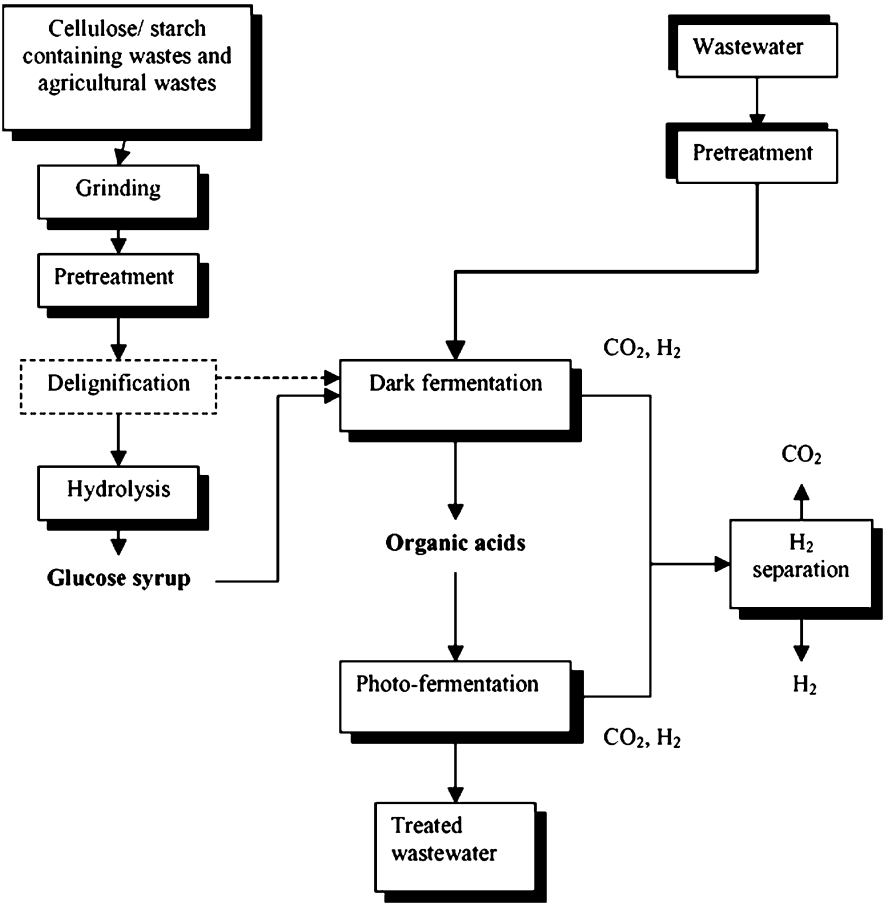


Fig. 10.15 A schematic diagram for bio-hydrogen production from cellulose/starch containing agricultural wastes and food industry wastewaters (*Source*: Kapdan dan Kargi 2006)

Hydrogen Detection and Quantification

The H_2 concentration in the gas phase is commonly measured with gas chromatography (GC) with thermal conductivity detector (TCD), using argon or nitrogen as the carrier gas. Typical GC operating conditions include temperature of 100 °C for the TCD and pressure of 151 kPa (22 psi) for the carrier gas. Silica columns (at 25 °C) or microcapillary columns may be used for separation (Drapcho et al. 2008). Fermentative hydrogen yield by different organisms is reported in Table 10.9.

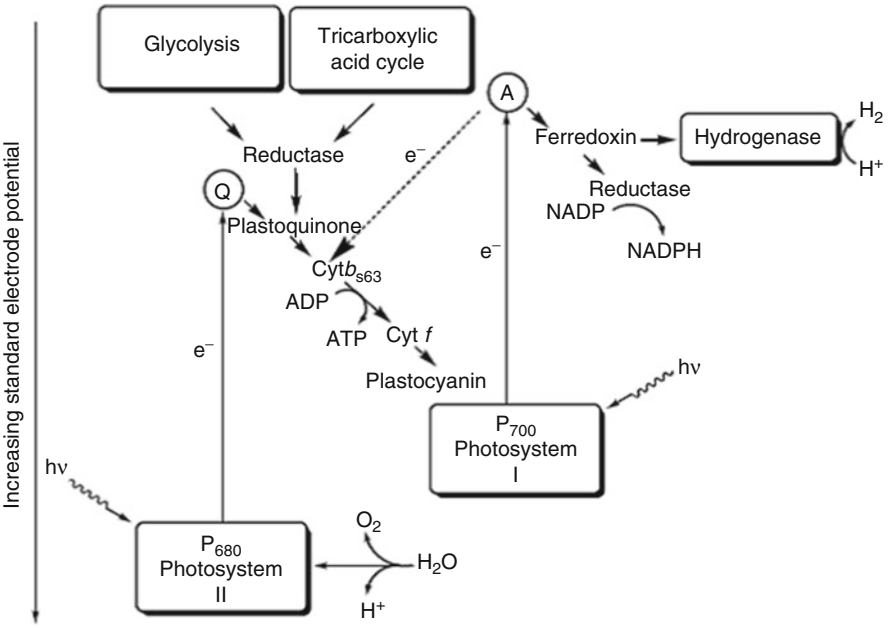


Fig. 10.16 Z scheme of photosynthetic electron flow in green plants and algae showing links to carbon metabolism and hydrogen production: *Q*, *A*, primary electron acceptors in Photosystems II and I, respectively; *dotted arrow* signifies cyclic electron flow (Melis and Happe 2001a, b)

Table 10.9 Reported fermentative hydrogen yield by different organisms

Microorganisms	Culture condition	Carbon source (g/L)	H_2 yield (mol/mol glucose)	Reference
E. coli	Batch	Glucose	0.75	Gottschalk (1986)
E. coli SR15	Batch	Glucose (10)	1.8	Yoshida et al. (2006)
Cl. butyricum strain SC-E1	Continuous	Glucose (10)	1.4	Kataoka et al. (1997)
Clostridium beijerinckii AM21B	Batch	Glucose (10)	1.3–2.0	Taguchi et al. (1992)
C. freundii	Batch	Glucose (7.7)	1.29	Kumar and Vatsala (1989)
Citrobacter intermedim	Continuous	Glucose (7.7)	0.27–1.14	Brosseau and Zajic (1982)
Citrobacter sp Y19	Batch	Glucose (5)	1.4	Oh et al. (2004)
Enterobacter cloacae IIT BT 08	Continuous	Glucose (5)	2.3	Nath and Das (2004)
Enterobacter aerogenes strain E.82005	Batch	Molasses (17 mM)	0.52–1.58	Tanisho et al. (1998)

Microbial Fuel Cells

One of the most exciting technologies for biological production of energy is the microbial fuel cell (MFC). A microbial fuel cell is a mimic of a biological system in which bacteria do not directly transfer their produced electrons to their characteristic electron acceptor. Instead, the transport process is subsequently conducted over an anode, a resistance or power user, and a cathode. Thus way, bacterial energy is directly converted to electrical energy.

Fuel Cell Design and Fabrication

Bacterial reactions can be carried out over several different temperature ranges depending on the tolerance of the bacteria, ranging from moderate or room-level temperatures (15–35 °C) to both high temperatures (50–60 °C) tolerated by thermophiles and low temperatures (<15 °C) where psychrophiles can grow. Virtually any biodegradable organic matter can be used in an MFC, including volatile acids, carbohydrates, proteins, alcohols, and even relatively recalcitrant materials like cellulose (Fig. 10.17) (Logan et al. 2006).

Hydrogen ions (protons, H^+) can accept reducing equivalents (conventionally represented as electrons, e^-) generated either photosynthetically or by the oxidation of organic and inorganic substrates inside microbial cells:

$$2e^- + 2H^+ \rightarrow H_2$$

The terminal electron donor (e.g., reduced ferredoxin) could donate electrons to the anode of a battery. Protons could then, in the presence of O_2, complete the electric circuit at the cathode by the reaction:

$$O_2 + 4e^- + 2H^+ \rightarrow 2H_2O$$

Thus, forming a highly environmentally friendly source of electric power (a battery), fueled by microbial metabolic activity (Logan and Regan 2006). That, in essence, is the definition of a microbial fuel cell (MFC).

The voltage across the external resistor or load in an MFC can be measured using a multimeter. Voltage measurements are converted to current values using Ohm's law:

$$V = IR \quad \text{where } V = \text{Voltage (V)}, \quad I = \text{Current (A)}, \quad R = \text{Resistance},$$

The power output from an MFC is calculated as

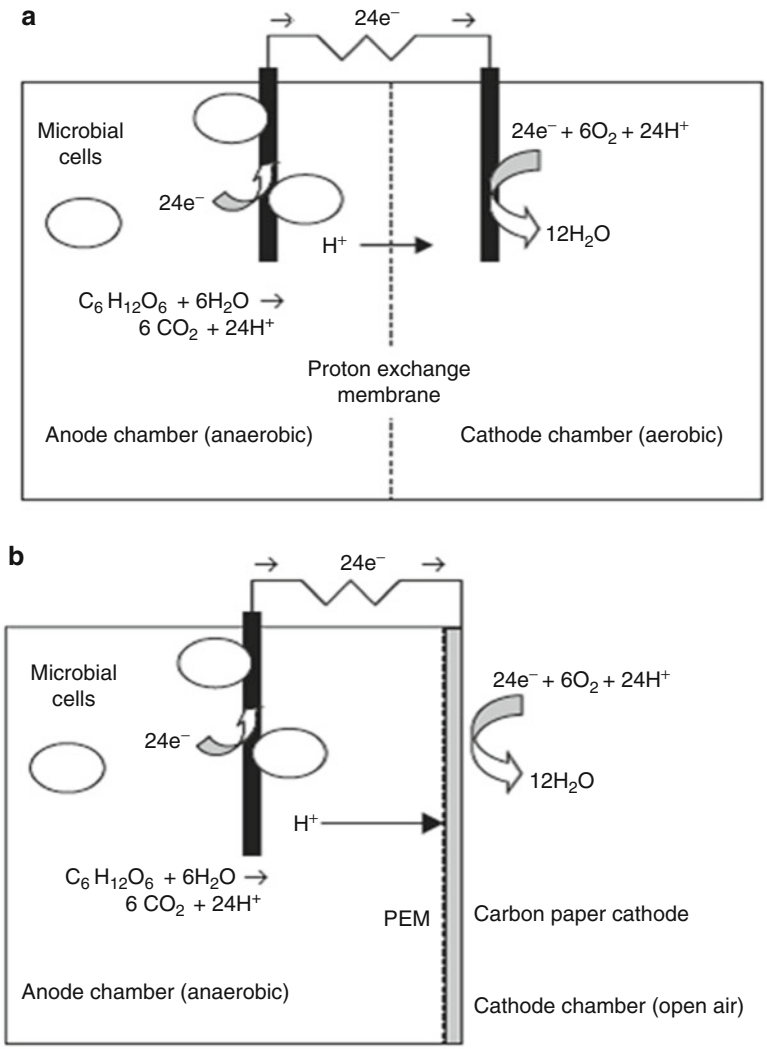


Fig. 10.17 (**a**) Diagram of two-chamber microbial fuel cell with aqueous cathode and anode chambers with solid graphite electrodes. (**b**) Diagram of single-chamber microbial fuel cell with aqueous anode chamber and air cathode chamber. The anode and cathode chambers are separated by a membrane. The bacteria grow on the anode, oxidizing organic matter and releasing electrons to the anode and protons to the solution. The cathode is sparged with air to provide dissolved oxygen for the reactions of electrons, protons and oxygen at the cathode, with a wire (and load) completing the circuit and producing power. The system is shown with a resistor used as the load for the power being generated, with the current determined based on measuring the voltage drop across the resistor using a multimeter hooked up to a data acquisition system (Drapcho et al. 2008)

$$P = IV \quad \text{where } P = \text{Power (W)}$$

Power density is used to relate power output to the anode surface area or anode chamber volume. Power density is calculated based on anode surface area as follows:

$$P\mathrm{D_A} = \frac{IV}{A_A}$$

where $P\mathrm{D_A}$ = power density on area basis, W/m^2; and A_A = anode surface area, m^2.

Methane Production

During anaerobic digestion, organic matter is converted to methane and carbon dioxide by way of a series of interrelated microbial metabolisms, including hydrolysis, acetogenesis, and methanogenesis. The value and stability of the pH in an anaerobic reactor are extremely important because methanogenesis proceeds only at a high rate when the pH is maintained in the neutral range (van Haandel and Lettinga 1994; Zinder 1994). Biogas production from maize along the production process are shown in Fig. 10.18.

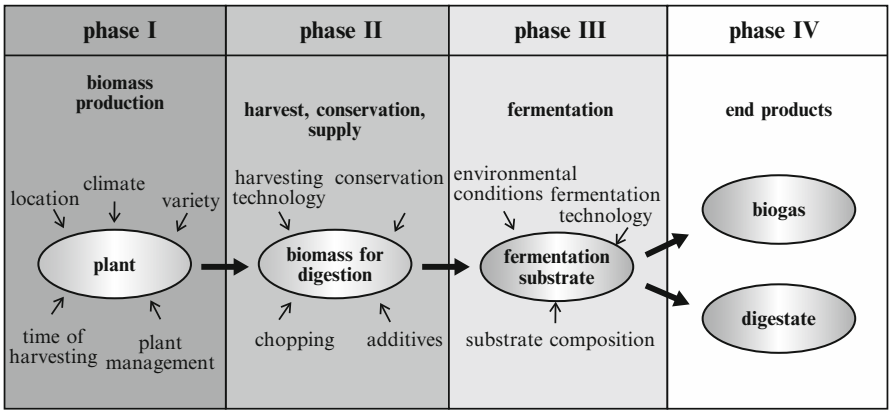


Fig. 10.18 Influences on biogas production from maize along the production process (*Source*: Amon et al. 2007)

Hydrolysis

Many of the potential biomass sources for methane production are high molecular weight, insoluble polymers such as polysaccharides, proteins, and fats that are too large to be transported across bacterial cell membranes. Polysaccharides such as cellulose and hemicellulose are hydrolyzed to glucose and xylose by cellulase and hemicellulase enzymes. Proteins and lipids are hydrolyzed to their constituent amino acids and long-chain fatty acids by proteases and lipases, respectively. The rate of hydrolysis is a function of several factors, such as pH, substrate composition, and particle size.

Fermentation (Acidogenesis)

The second phase of the overall process is fermentation that begins with the conversion of the sugar monomers to pyruvate ($C_3H_4O_3$), ATP, and the electron carrier molecule NADH by central metabolic pathways. The central metabolic pathways found within most bacteria are the Embden-Meyerhof pathway (glycolysis) and the pentose phosphate pathway. Next, these fermentative bacteria convert pyruvate and amino acids to a variety of short-chain organic acids—primarily acetate, propionate, butyrate, and succinate—and alcohols, CO_2, and H_2 through various fermentation pathways. Acid producing organisms are a mixture of facultative anaerobes, such as enteric bacteria and clostridial species which are called acid formers. The optimum temperature and pH values for this step are $T = 35\ ^\circ C$ and $pH = 4–6$ (Shuler and Kargi 2002).

Acetogenesis

The short-chain organic acids produced by fermentation and the fatty acids produced from the hydrolysis of lipids are fermented to acetic acid, H_2, and CO_2 by acetogenic bacteria. Syntrophic bacteria that oxidize organic acids to acetate, H_2, and CO_2 are reliant on the subsequent oxidation of H_2 by the next group, the methanogens, to lower the H_2 concentration and prevent end-product inhibition.

Methanogenesis

In the final phase, methane is produced through two distinct routes by two different microbial groups. Among methanogenic bacteria used for this purpose are

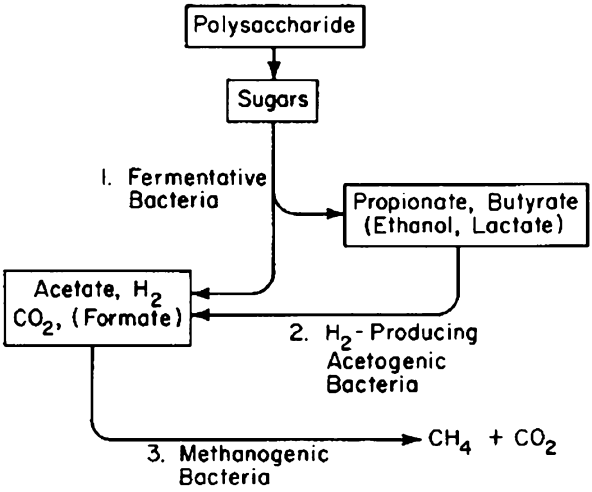


Fig. 10.19 A scheme showing the relationships of the three general metabolic groups of bacteria or stages of fermentation involved in methane production (*Source*: Bryant 1979)

Methanobacterium (nonspore-forming rods), *Methanobacillus* (spore forming rods), and *Methanococcus* and *Methanosarcina*.

The optimum temperature and pH range for methanogenic bacteria are T = 35–40 °C and pH = 7–7.8 (Shuler and Kargi 2002). The relationships of the three general metabolic groups of bacteria or stages of fermentation involved in methane production are shown in Fig. 10.19. One route is by the action of the lithotrophic H_2-oxidizing methanogens that use H_2 as electron donor and reduce CO_2 to produce methane. In the second route, the organotrophic acetoclastic methanogens ferment acetic acid to methane and carbon dioxide.

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$$

$$CH_3COOH \rightarrow CH_4 + CO_2$$

Conclusions and Future Prospects

Bio-fuels are being promoted in the transportation sector. More recently, ethanol produced from sugar and starch-based feedstocks has become another important biofuel. Other biofuels such as lignocellulosic ethanol, biodiesel, biohydrogen, and bioelectricity have been the focus of vigorous research, and the technologies for their production are being developed, although most of these are not quite ready for commercialization. Currently, a large amount of studies regarding the utilization of lignocellulosic biomass as a feedstock for producing fuel ethanol is being carried out worldwide (Balat and Balat 2009). Bioconversion of lignocellulosic biomass to ethanol is significantly hindered by the structural and chemical complexity of biomass, which makes these materials a challenge to be used as feedstocks for cellulosic ethanol production (Zheng et al. 2009). But in addition to that, the

technology of recombinant DNA will provide important advances for the development of fuel ethanol industry. The development of genetically modified microorganisms capable of converting starch or biomass directly into ethanol and with a proven stability under industrial conditions will allow the implementation of the consolidated bioprocessing of the feedstocks (Cardona and Sánchez 2007). The willingness of mankind to pay high prices for energy in the future is a great uncertainty. Hence, the biobutanol production can aid in extending the life of petroleum oil reserves and diminish environmental concerns (Kumar and Gayen 2011). The acetone-butanol fermentation was the first large-scale fermentation process developed which is sensitive to contamination. Therefore this fermentation contributed much to the knowledge of how to run sterile processes on an industrial scale. As an alternative plan, many research projects have been initiated for the efficient use of lignocellulosic biomass, algal biomass, etc. which should be accomplished in the future (Mosier et al. 2005; Kumar et al. 2009). Sucrose from sugar cane is also an excellent substrate in certain regions of the world. An optimal Bioprocess for butanol production can be developed by integrating the fermentation and downstream processes with strain development (Lee et al. 2008a, b). Researchers are also attempting an aerobic production of biobutanol using genetically engineered organisms like *E. coli, S. cerevisiae* etc. (Atsumi and Liao 2008; Steen et al. 2008). It will be the milestone to attract the attention of government, commercial, and research organizations for further support in implementing the innovative fermentation and extraction technology. The study on biodiesel synthesis showed that the quantity of catalyst, the temperature and reaction time are the main factors affecting the production of methyl esters both for short chain methyl esters and long chain methyl esters (Riadi et al. 2014). Biological methods offer distinct advantages for hydrogen production such as operation under mild conditions and specific conversions. However, raw material cost is one of the major limitations for bio-hydrogen production. Utilization of some carbohydrate rich, starch or cellulose containing solid wastes and/or some food industry wastewaters is an attractive approach for bio-hydrogen production (Kapdan and Kargi 2006). Microbial production of electricity may become an important form of bioenergy in future because MFCs offer the possibility of extracting electric current from a wide range of soluble or dissolved complex organic wastes and renewable biomass. A large number of substrates have been explored as feed. The major substrates that have been tried include various kinds of artificial and real wastewaters and lignocellulosic biomass (Pant et al. 2010). Currently, biogas production from energy crops is mainly based on the anaerobic digestion of maize. Among them, methane produced by anaerobic digestion has been used by the human race for hundreds, if not thousands, of years. In the near future, biogas production from energy crops will increase and it has to be considered that energy crops are grown in versatile, sustainable crop rotations (Strauß et al. 2012).

References

Alonso, D. M., Bond, J. Q., & Dumesic, J. A. (2010). Catalytic conversion of biomass to biofuels. *Green Chemistry, 12*(9), 1493–1513.

Al-Shorgani, N. K. N., Kalil, M. S., & Yusoff, W. M. W. (2012). Biobutanol production from rice bran and de-oiled rice bran by *Clostridium saccharoperbutylacetonicum* N1-4. *Bioprocess and Biosystems Engineering, 35*(5), 817–826.

Amon, T., Amon, B., Kryvoruchko, V., Zollitsch, W., Mayer, K., & Gruber, L. (2007). Biogas production from maize and dairy cattle manure—Influence of biomass composition on the methane yield. *Agriculture, Ecosystems & Environment, 118*(1), 173–182.

Antoni, D., Zverlov, V. V., & Schwarz, W. H. (2007). Biofuels from microbes. *Applied Microbiology and Biotechnology, 77*(1), 23–35.

Atadashi, I. M., Aroua, M. K., & Aziz, A. A. (2011). Biodiesel separation and purification: A review. *Renewable Energy, 36*(2), 437–443.

Atsumi, S., & Liao, J. C. (2008). Metabolic engineering for advanced biofuels production from *Escherichia coli*. *Current Opinion in Biotechnology, 19*(5), 414–419.

Aworanti, O. A., Agarry, S. E., & Ajani, A. O. (2013). Statistical optimization of process variables for biodiesel production from waste cooking oil using heterogeneous base catalyst. *British Biotechnology Journal, 3*(2), 116–132.

Balat, M., & Balat, H. (2009). Recent trends in global production and utilization of bio-ethanol fuel. *Applied Energy, 86*(11), 2273–2282.

Balat, H., & Kırtay, E. (2010). Hydrogen from biomass – Present scenario and future prospects. *International Journal of Hydrogen Energy, 35*(14), 7416–7426.

Baskar, C., Baskar, S., & Dhillon, R. S. (2012a). *Biomass conversion: The interface of biotechnology, chemistry and materials sceince*. London: Springer. ISBN 978-3-642-28417-5.

Baskar, C., Baskar, S., & Dhillon, R. S. (2012b). *Biomass conversion: The interface of biotechnology, chemistry and materials science*. New York, NY: Springer.

Bastos, V. D. (2007). Etanol, alcoolquímica e biorrefinarias. *BNDES Setorial, 25*, 5–38.

BEguin, P., & Aubert, J. P. (1994). The biological degradation of cellulose. *FEMS Microbiology Reviews, 13*(1), 25–58.

Berchmans, H. J., & Hirata, S. (2008). Biodiesel production from crude *Jatropha curcus* L. Seed oil with a high content of free fatty acids. *Bioresource Technology, 99*, 1716–1721.

Bjerre, A. B., Olesen, A. B., & Fernqvist, T. (1996). Pretreatment of wheat straw using combined wet oxidation and alkaline hydrolysis resulting in convertible cellulose and hemicellulose. *Biotechnology and Bioengineering, 49*(5), 568–577.

Bramucci, M. G., Flint, D., Miller, E. S., Nagarajan, V., Sedkova, N., Singh, M., et al. (2008). *US20080274526*.

Brosseau, J. D., & Zajic, J. E. (1982). Hydrogen-gas production with Citrobacter intermedim and Clostridium pasteurianum. *Journal of Chemical Technology and Biotechnology, 32*(3), 496–502.

Bryant, M. P. (1979). Microbial methane production—Theoretical aspects. *Journal of Animal Science, 48*(1), 193–201.

Bryant, D. L., & Blaschek, H. P. (1988). Buffering as a means for increasing growth and butanol production by *Clostridium acetobutylicum*. *Journal of Industrial Microbiology, 3*(1), 49–55.

Campos, E. J., Qureshi, N., & Blaschek, H. P. (2002). Production of acetone butanol ethanol from degermed corn using *Clostridium beijerinckii* BA101. *Applied Biochemistry and Biotechnology, 98–100*, 553–561.

Canakci, M., & Sanli, H. (2008). Biodiesel production from various feedstocks and their effects on the fuel properties. *Journal of Industrial Microbiology & Biotechnology, 35*(5), 431–441.

Cardona, C. A., & Sánchez, Ó. J. (2007). Fuel ethanol production: Process design trends and integration opportunities. *Bioresource Technology, 98*(12), 2415–2457.

Claassen, P. A. M., Budde, M. A. W., & López-Contreras, A. M. (2000). Acetone, butanol and ethanol production from domestic organic waste by solventogenic clostridia. *Journal of Molecular Microbiology and Biotechnology, 2*, 39–44.

Claassen, P. A. M., Van Lier, J. B., Contreras, A. L., Van Niel, E. W. J., Sijtsma, L., Stams, A. J. M., et al. (1999). Utilisation of biomass for the supply of energy carriers. *Applied Microbiology and Biotechnology, 52*(6), 741–755.

Decker, S. R., Sheehan, J., Dayton, D. C., Bozell, J. J., Adney, W. S., Hames, B., et al. (2007). Biomass conversion. In J. A. Kent (Ed.), *Kent and Riegel's handbook of industrial chemistry and biotechnology* (pp. 1449–1548). New York, NY: Springer.

Demirbas, A. (2005). Biodiesel production from vegetable oils via catalytic and non-catalytic supercritical methanol transesterification methods. *Progress in Energy and Combustion Science, 31*(5), 466–487.

Demirbas, A. (2009a). *Biohydrogen for future engine fuel demands*. London: Springer.

Demirbas, A. (2009b). *Biorenewable liquid fuels. Biofuels: Securing the planet's future energy needs* (pp. 103–230). New York, NY: Springer.

Doran, P. M. (1995). *Bioprocess engineering principles*. Waltham, MA: Academic.

Drapcho, C. M., Nhuan, N. P., & Walker, T. H. (2008). *Biofuels engineering process technology* (pp. 334–335). New York, NY: McGraw-Hill.

Duff, S. J., & Murray, W. D. (1996). Bioconversion of forest products industry waste cellulosics to fuel ethanol: A review. *Bioresource Technology, 55*(1), 1–33.

Dürre, P. (2008). Fermentative butanol production. *Annals of the New York Academy of Sciences, 1125*(1), 353–362.

Dürre, P. (2011). Fermentative production of butanol – The academic perspective. *Current Opinion in Biotechnology, 22*(3), 331–336.

Esteghlalian, A., Hashimoto, A. G., Fenske, J. J., & Penner, M. H. (1997). Modeling and optimization of the dilute-sulfuric-acid pretreatment of corn stover, poplar and switchgrass. *Bioresource Technology, 59*(2), 129–136.

Ezeji, T. C., Qureshi, N., & Blaschek, H. P. (2003). Production of acetone, butanol and ethanol by *Clostridium beijerinckii* BA101 and in situ recovery by gas stripping. *World Journal of Microbiology and Biotechnology, 19*(6), 595–603.

Ezeji, T. C., Qureshi, N., & Blaschek, H. P. (2004a). Acetone butanol ethanol (ABE) production from concentrated substrate: Reduction in substrate inhibition by fed-batch technique and product inhibition by gas stripping. *Applied Microbiology and Biotechnology, 63*(6), 653–658.

Ezeji, T. C., Qureshi, N., & Blaschek, H. P. (2004b). Butanol fermentation research: Upstream and downstream manipulations. *The Chemical Record, 4*(5), 305–314.

Ezeji, T., Qureshi, N., & Blaschek, H. P. (2007a). Butanol production from agricultural residues: Impact of degradation products on *Clostridium beijerinckii* growth and butanol fermentation. *Biotechnology and Bioengineering, 97*(6), 1460–1469.

Ezeji, T., Qureshi, N., & Blaschek, H. P. (2007b). Production of acetone-butanol-ethanol (ABE) in a continuous flow bioreactor using degermed corn and *Clostridium beijerinckii*. *Process Biochemistry, 42*, 34–39.

Ezeji, T. C., Qureshi, N., & Blaschek, H. P. (2007c). Bioproduction of butanol from biomass: From genes to bioreactors. *Current Opinion in Biotechnology, 18*(3), 220–227.

Fan, L. T., Gharpuray, M. M., & Lee, Y. H. (1987). Enzymatic hydrolysis. In L. T. Fan, M. M. Gharpuray, & Y. H. Lee (Eds.), *Cellulose hydrolysis* (pp. 21–119). Berlin: Springer.

Festel, G. W. (2008). Biofuels – Economic aspects. *Chemical Engineering & Technology, 31*(5), 715–720.

Figueira, M. M., Volesky, B., Ciminelli, V. S. T., & Roddick, F. A. (2000). Biosorption of metals in brown seaweed biomass. *Water Research, 34*(1), 196–204.

Fischer, C. R., Klein-Marcuschamer, D., & Stephanopoulos, G. (2008). Selection and optimization of microbial hosts for biofuels production. *Metabolic Engineering, 10*(6), 295–304.

Galbe, M., & Zacchi, G. (2002). A review of the production of ethanol from softwood. *Applied Microbiology and Biotechnology, 59*(6), 618–628.

García, V., Päkkilä, J., Ojamo, H., Muurinen, E., & Keiski, R. L. (2011). Challenges in biobutanol production: How to improve the efficiency? *Renewable and Sustainable Energy Reviews, 15* (2), 964–980.

GHeshlaghi, R. E. Z. A., Scharer, J. M., Moo-Young, M., & Chou, C. P. (2009). Metabolic pathways of clostridia for producing butanol. *Biotechnology Advances, 27*(6), 764–781.

Ghirardi, M. L., Kosourov, S., Maness, P., Smolinski, S., & Seibert, M. (2010). *Algal hydrogen production. Encyclopedia of Industrial Biotechnology*. New York: Wiley.

Godin, C., & Engasser, J. M. (1990). Two-stage continuous fermentation of *Clostridium acetobutylicum*: Effects of pH and dilution rate. *Applied Microbiology and Biotechnology, 33*(3), 269–273.

Gottschalk, G. (1986). Bacterial fermentations. In G. Gottschalk (Ed.), *Bacterial metabolism* (pp. 208–282). New York, NY: Springer.

Green, E. M. (2011). Fermentative production of butanol – The industrial perspective. *Current Opinion in Biotechnology, 22*(3), 337–343.

Grimmler, C., Janssen, H., Krauβe, D., Fischer, R. J., Bahl, H., Dürre, P., et al. (2011). Genome-wide gene expression analysis of the switch between acidogenesis and solventogenesis in continuous cultures of Clostridium acetobutylicum. *Journal of Molecular Microbiology and Biotechnology, 20*(1), 1.

Grobben, N. C., Eggink, G., Cuperus, F. P., & Huizing, H. J. (1993). Production of acetone, butanol and ethanol (ABE) from potato wastes; fermentation with integrated membrane extraction. *Applied Microbiology and Biotechnology, 39*, 494–498.

Grupe, H., & Gottschalk, G. (1992). Physiological events in *Clostridium acetobutylicum* during the shift from acidogenesis to solventogenesis in continuous culture and presentation of a model for shift induction. *Applied and Environmental Microbiologyl, 58*(12), 3896–3902.

Gutierrez, N. A., Maddox, I. S., Schuster, K. C., Swoboda, H., & Gapes, J. R. (1998). Strain comparison and medium preparation for the acetone-butanol-ethanol (ABE) fermentation process using a substrate of potato. *Bioresource Technology, 66*, 263–265.

Hahn-Hägerdal, B., Karhumaa, K., Fonseca, C., Spencer-Martins, I., & Gorwa-Grauslund, M. F. (2007). Towards industrial pentose-fermenting yeast strains. *Applied Microbiology and Biotechnology, 74*(5), 937–953.

Hallenbeck, P. C., & Benemann, J. R. (2002). Biological hydrogen production; fundamentals and limiting processes. *International Journal of Hydrogen Energy, 27*(11), 1185–1193.

Heitmann, S., Krings, J., Kreis, P., Lennert, A., Pitner, W. R., Górak, A., et al. (2012). Recovery of n-butanol using ionic liquid-based pervaporation membranes. *Separation and Purification Technology, 97*, 108–114.

Holm, J., Lassi, U., Romar, H., Lahti, R., Kärkkäinen, J., & Lajunen, M. (2012). Pretreatment of fibre sludge in ionic liquids followed by enzyme and acid catalysed hydrolysis. *Catalysis Today, 196*(1), 11–15.

Hossain, A. B. M. S., Nasrulhaq, B. A., Salleh, A., & Chandran, S. (2010). Biodiesel production from waste soybean oil biomass as renewable energy and environmental recycled process. *African Journal of Biotechnology, 9*(27), 4233–4240.

Huang, H., Liu, H., & Gan, Y. R. (2010). Genetic modification of critical enzymes and involved genes in butanol biosynthesis from biomass. *Biotechnology Advances, 28*(5), 651–657.

Huesemann, M. H., Kuo, L. J., Urquhart, L., Gill, G. A., & Roesijadi, G. (2012). Acetone-butanol fermentation of marine macroalgae. *Bioresource Technology, 108*, 305–309.

Jesse, T. W., Ezeji, T. C., Qureshi, N., & Blaschek, H. P. (2002). Production of butanol from starch-based waste packing peanuts and agricultural waste. *Journal of Industrial Microbiology and Biotechnology, 29*(3), 117–123.

John, R. P., Anisha, G. S., Nampoothiri, K. M., & Pandey, A. (2011). Micro and macroalgal biomass: A renewable source for bioethanol. *Bioresource Technology, 102*(1), 186–193.

Jones, D. T., Van der Westhuizen, A., Long, S., Allcock, E. R., Reid, S. J., & Woods, D. R. (1982). Solvent production and morphological changes in Clostridium acetobutylicum. *Applied and Environmental Microbiology, 43*(6), 1434–1439.

Jones, D. T., & Woods, D. R. (1986). Acetone-butanol fermentation revisited. *Microbiological Reviews, 50*(4), 484.

Junelles, A. M., Janati-Idrissi, R., Petitdemange, H., & Gay, R. (1988). Iron effect on acetone-butanol fermentation. *Current Microbiology, 17*(5), 299–303.

Jurgens, G., Survase, S., Berezina, O., et al. (2012). Butanol production from lignocellulosics. *Biotechnology Letters, 34*(8), 1415–1434.

Kapdan, I. K., & Kargi, F. (2006). Bio-hydrogen production from waste materials. *Enzyme and Microbial Technology, 38*(5), 569–582.

Kataoka, N., Miya, A., & Kiriyama, K. (1997). Studies on hydrogen production by continuous culture system of hydrogen-producing anaerobic bacteria. *Water Science and Technology, 36*(6), 41–47.

Keating, J. D., Panganiban, C., & Mansfield, S. D. (2006). Tolerance and adaptation of ethanologenic yeasts to lignocellulosic inhibitory compounds. *Biotechnology and Bioengineering, 93*(6), 1196–1206.

Kim, B. H., Bellows, P., Datta, R., & Zeikus, J. G. (1984). Control of carbon and electron flow in Clostridium acetobutylicum fermentations: Utilization of carbon monoxide to inhibit hydrogen production and to enhance butanol yields. *Applied and Environmental Microbiology, 48*(4), 764–770.

Kobayashi, G., Eto, K., Tashiro, Y., Okubo, K., Sonomoto, K., & Ishizaki, A. (2005). Utilization of excess sludge by acetone–butanol–ethanol fermentation employing Clostridium saccharoperbutylacetonicum N1-4 (ATCC 13564). *Journal of Bioscience and Bioengineering, 99*(5), 517–519.

Konieczny, A., Mondal, K., Wiltowski, T., & Dydo, P. (2008). Catalyst development for thermocatalytic decomposition of methane to hydrogen. *International Journal of Hydrogen Energy, 33*, 264e72.

Koukiekolo, R., Cho, H. Y., Kosugi, A., Inui, M., Yukawa, H., & Doi, R. H. (2005). Degradation of corn fiber by Clostridium cellulovorans cellulases and hemicellulases and contribution of scaffolding protein CbpA. *Applied and Environmental Microbiology, 71*(7), 3504–3511.

Kraus, T. (2007). Hydrogen fuel – An economically viable future for the transportation industry. *Duke Journal of Economics, 19*, 39.

Kretzers, I. (2012). Conference report: Euroscicon conference on innovations in renewable energies. *Biofuels, 3*(4), 375–376.

Kumar, P., Barrett, D. M., Delwiche, M. J., & Stroeve, P. (2009). Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Industrial & Engineering Chemistry Research, 48*(8), 3713–3729.

Kumar, M., & Gayen, K. (2011). Developments in biobutanol production: New insights. *Applied Energy, 88*(6), 1999–2012.

Kumar, M., Goyal, Y., Sarkar, A., & Gayen, K. (2012). Comparative economic assessment of ABE fermentation based on cellulosic and non-cellulosic feedstocks. *Applied Energy, 93*, 193–204.

Kumar, D., & Murthy, G. S. (2013). Stochastic molecular model of enzymatic hydrolysis of cellulose for ethanol production. *Biotechnology for Biofuels, 6*(1), 1–20.

Kumar, G. R., & Vatsala, T. M. (1989). Hydrogen production from glucose by Citrobacter freundii. *Indian Journal of Experimental Biology, 27*(9), 824–825.

Lee, S. Y., Park, J. H., Jang, S. H., Nielsen, L. K., Kim, J., & Jung, K. S. (2008). Fermentative butanol production by Clostridia. *Biotechnology and Bioengineering, 101*(2), 209–228.

Lee, S. Y., Park, J. H., & Papoutsakis, E. (2008). *WIPO Patent 2008072921*.

Lee, J., Seo, E., Kweon, D. H., Park, K., & Jin, Y. S. (2009). Fermentation of rice bran and defatted rice bran for butanol production using *Clostridium beijerinckii* NCIMB 8052. *Journal of Microbiology and Biotechnology, 19*, 482–490.

Levin, D. B., Pitt, L., & Love, M. (2004). Biohydrogen production: Prospects and limitations to practical application. *International Journal of Hydrogen Energy, 29*(2), 173–185.

Li, X., Weng, J. K., & Chapple, C. (2008). Improvement of biomass through lignin modification. *The Plant Journal, 54*(4), 569–581.

Lin, Y., & Tanaka, S. (2006). Ethanol fermentation from biomass resources: Current state and prospects. *Applied Microbiology and Biotechnology, 69*(6), 627–642.

Liu, C. G., Xue, C., Lin, Y. H., & Bai, F. W. (2013). Redox potential control and applications in microaerobic and anaerobic fermentations. *Biotechnology Advances, 31*(2), 257–265.

Liu, Z., Ying, Y., Li, F., Ma, C., & Xu, P. (2010). Butanol production by Clostridium beijerinckii ATCC 55025 from wheat bran. *Journal of Industrial Microbiology & Biotechnology, 37*(5), 495–501.

Logan, B. E., Hamelers, B., Rozendal, R., Schröder, U., Keller, J., Freguia, S., et al. (2006). Microbial fuel cells: Methodology and technology. *Environmental Science & Technology, 40* (17), 5181–5192.

Logan, B. E., & Regan, J. M. (2006). Electricity-producing bacterial communities in microbial fuel cells. *Trends in Microbiology, 14*(12), 512–518.

López-Contreras, A. M., Claassen, P. A. M., Mooibrock, H., & De Vos, W. M. (2000). Utilisation of saccharides in extruded domestic organic waste by *Clostridium acetobutylicum* ATCC 824 for production of acetone, butanol and ethanol. *Applied Microbiology and Biotechnology, 54*, 162–167.

Lynd, L. R. (1996). Overview and evaluation of fuel ethanol from cellulosic biomass: Technology, economics, the environment, and policy. *Annual Review of Energy and the Environment, 21* (1), 403–465.

Maddox, I. S., & Murray, A. E. (1983). Production of n-butanol by fermentation of wood hydrolysate. *Biotechnology Letters, 5*, 175–178.

Madihah, M. S., Ariff, A. B., Sahaid, K. M., Suraini, A. A., & Karim, M. I. A. (2001). Direct fermentation of gelatinized sago starch to acetone–butanol–ethanol by Clostridium acetobutylicum. *World Journal of Microbiology and Biotechnology, 17*(6), 567–576.

Marchal, R., Blanchet, D., & Vandecasteele, J. P. (1985). Industrial optimization of acetone-butanol fermentation: A study of the utilization of Jerusalem artichokes. *Applied Microbiology and Biotechnology, 23*, 92–98.

Marchal, R., Rebeller, M., & Vandecasteele, J. P. (1984). Direct bioconversion of alkali-pretreated straw using simultaneous enzymatic hydrolysis and acetone butanol production. *Biotechnology Letters, 6*, 523–528.

Marchetti, J. M., & Errazu, A. F. (2008). Comparison of different heterogeneous catalysts and different alcohols for the esterification reaction of oleic acid. *Fuel, 87*(15), 3477–3480.

Math, M. C., Kumar, S. P., & Chetty, S. V. (2010). Technologies for biodiesel production from used cooking oil. A review. *Energy for Sustainable Development, 14*(4), 339–345.

McMillan, J. D. (1994). Pretreatment of lignocellulosic biomass. In M. E. Himmel, J. O. Baker, & R. P. Overend (Eds.), *Enzymatic conversion of biomass for fuels production, ACS symposium series (USA)*. Washington, DC: American Chemical Society.

Melis, A., & Happe, T. (2001). Hydrogen production. Green algae as a source of energy. *Plant Physiology, 127*(3), 740–748.

Mielenz, J. R. (2001). Ethanol production from biomass: Technology and commercialization. *Current Opinion in Microbiology, 4*(3), 324–329.

Mohan, S. V., Srikanth, S., Velvizhi, G., & Babu, M. L. (2013). Microbial fuel cells for sustainable bioenergy generation: Principles and perspective applications. In V. K. Gupta & M. G. Tuohy (Eds.), *Biofuel technologies: Recent developments* (pp. 335–368). Berlin: Springer.

Moo-Young, M., & Chisti, Y. (1994). Biochemical engineering in biotechnology. *Pure and Applied Chemistry, 66*, 117.

Moreira, A. R., Ulmer, D. C., & Linden, J. C. (1981). Butanol toxicity in the butylic fermentation. *Biotechnology & Bioengineering Symposium, 11*, 567–579.

Mosier, N., Wyman, C., Dale, B., et al. (2005). Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology, 96*(6), 673–686.

Murty, M. V. S., & Chandra, T. S. (1997). Fermentability of hemicelluloses extracted from municipal waste and commercial xylans by *Clostridium* sp. *Applied Microbiology and Biotechnology, 47*, 212–217.

Nath, K., & Das, D. (2004). Improvement of fermentative hydrogen production: Various approaches. *Applied Microbiology and Biotechnology, 65*(5), 520–529.

Neha, P., Chintan, B., Pallavi, D., & Neha, T. (2013). Use of sunflower and cottonseed oil to prepare biodiesel by catalyst assisted transesterification. *Research Journal of Chemical Sciences, 2231*, 606X.

Ng, T. K., Ben-Bassat, A., & Zeikus, J. G. (1981). Ethanol production by thermophilic bacteria: fermentation of cellulosic substrates by cocultures of Clostridium thermocellum and Clostridium thermohydrosulfuricum. *Applied and Environmental Microbiology, 41*(6), 1337–1343.

Nguyen, M. T., Choi, S. P., Lee, J., Lee, J. H., & Sim, S. J. (2009). Hydrothermal acid pretreatment of Chlamydomonas reinhardtii biomass for ethanol production. *Journal of Microbiology and Biotechnology, 19*(2), 161–166.

Ni, M., Leung, D. Y., Leung, M. K., & Sumathy, K. (2006). An overview of hydrogen production from biomass. *Fuel Processing Technology, 87*(5), 461–472.

Niemisto, J., Saavalainen, P., Pongrácz, E., & Keiski, R. L. (2013). Biobutanol as a potential sustainable biofuel-assessment of lignocellulosic and waste-based feedstocks. *Journal of Sustainable Development of Energy, Water and Environment Systems, 1*(2), 58–77.

Nigam, P. S., & Singh, A. (2011). Production of liquid biofuels from renewable resources. *Progress in Energy and Combustion Science, 37*(1), 52–68.

Nimcevic, D., Schuster, M., & Gapes, J. R. (1998). Solvent production by *Clostridium beijerinckii* NRRL B592 growing on different potato media. *Applied Microbiology and Biotechnology, 50*, 426–428.

Oh, Y. K., Kim, S. H., Kim, M. S., & Park, S. (2004). Thermophilic biohydrogen production from glucose with trickling biofilter. *Biotechnology and Bioengineering, 88*(6), 690–698.

Olsson, L., & Hahn-Hägerdal, B. (1996). Fermentation of lignocellulosic hydrolysates for ethanol production. *Enzyme and Microbial Technology, 18*(5), 312–331.

Palmqvist, E., & Hahn-Hägerdal, B. (2000). Fermentation of lignocellulosic hydrolysates. I: inhibition and detoxification. *Bioresource Technology, 74*(1), 17–24.

Panesar, P. S., Marwaha, S. S., & Kennedy, J. F. (2006). Zymomonas mobilis: An alternative ethanol producer. *Journal of Chemical Technology and Biotechnology, 81*(4), 623–635.

Pant, D., Van Bogaert, G., Diels, L., & Vanbroekhoven, K. (2010). A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production. *Bioresource Technology, 101*(6), 1533–1543.

Perlack, R. D., Wright, L. L., Turhollow, A. F., Graham, R. L., Stokes, B. J., & Erbach, D. C. (2005). *Biomass as feedstock for a bioenergy and bioproducts industry: The technical feasibility of a billion-ton annual supply*. Oak Ridge, TN: Oak Ridge National Lab.

Pfromm, P. H., Amanor-Boadu, V., Nelson, R., Vadlani, P., & Madl, R. (2010). Bio-butanol vs. bio-ethanol: A technical and economic assessment for corn and switchgrass fermented by yeast or Clostridium acetobutylicum. *Biomass and Bioenergy, 34*(4), 515–524.

Potts, T., Du, J., Paul, M., May, P., Beitle, R., & Hestekin, J. (2012). The production of butanol from Jamaica bay macro algae. *Environmental Progress & Sustainable Energy, 31*(1), 29–36.

Pratas, M. J., Freitas, S. V., Oliveira, M. B., Monteiro, S. C., Lima, Á. S., & Coutinho, J. A. (2011a). Biodiesel density: Experimental measurements and prediction models. *Energy & Fuels, 25*(5), 2333–2340.

Pratas, M. J., Freitas, S., Oliveira, M. B., Monteiro, S. C., Lima, Á. S., & Coutinho, J. A. (2011b). Densities and viscosities of minority fatty acid methyl and ethyl esters present in biodiesel. *Journal of Chemical & Engineering Data, 56*(5), 2175–2180.

Qureshi, N., & Blaschek, H. P. (2000). Economics of butanol fermentation using hyper-butanol producing *Clostridium Beijerinckii* BA101. *Food and Bioproducts Processing, 78*(3), 139–144.

Qureshi, N., Ezeji, T. C., Ebener, J., Dien, B. S., Cotta, M. A., & Blaschek, H. P. (2008a). Butanol production by Clostridium beijerinckii. Part I: Use of acid and enzyme hydrolyzed corn fiber. *Bioresource Technology, 99*(13), 5915–5922.

Qureshi, N., Li, X. L., Hughes, S., Saha, B. C., & Cotta, M. A. (2006). Butanol production from corn fiber xylan using Clostridium acetobutylicum. *Biotechnology Progress, 22*(3), 673–680.

Qureshi, N., Lolas, A., & Blaschek, H. P. (2001). Soy molasses as fermentation substrate for production of butanol using *Clostridium beijerinckii* BA101. *Journal of Industrial Microbiology and Biotechnology, 26*, 290–295.

Qureshi, N., Saha, B. C., Dien, B., Hector, R. E., & Cotta, M. A. (2010a). Production of butanol (a biofuel) from agricultural residues: Part I—Use of barley straw hydrolysate. *Biomass and Bioenergy, 34*(4), 559–565.

Qureshi, N., Saha, B. C., Hector, R. E., Hughes, S. R., & Cotta, M. A. (2008b). Butanol production from wheat straw by simultaneous saccharification and fermentation using Clostridium beijerinckii: Part I—Batch fermentation. *Biomass and Bioenergy, 32*(2), 168–175.

Qureshi, N., Saha, B. C., Hector, R. E., Dien, B., Hughes, S., Liu, S., et al. (2010b). Production of butanol (a biofuel) from agricultural residues: Part II—Use of corn stover and switchgrass hydrolysates. *Biomass and Bioenergy, 34*(4), 566–571.

Qureshi, N., Saha, B. C., & Cotta, M. A. (2007). Butanol production from wheat straw hydrolysate using *Clostridium beijerinckii*. *Bioprocess and Biosystems Engineering, 30*, 419–427.

Qureshi, N., Saha, B. C., & Cotta, M. A. (2008c). Butanol production from wheat straw by simultaneous saccharification and fermentation using *Clostridium beijerinckii*: part II—Fedbatch fermentation. *Biomass and Bioenergy, 32*, 176–183.

Ranjan, A., & Moholkar, V. S. (2011). Comparative study of various pretreatment techniques for rice straw saccharification for the production of alcoholic biofuels. *Fuel*. doi:10.1016/j.fuel.2011.03.030.

Riadi, L., Purwanto, E., Kurniawan, H., & Oktaviana, R. (2014). Effect of bio-based catalyst in biodiesel synthesis. *Procedia Chemistry, 9*, 172–181.

Rinaldi, R., & Schüth, F. (2009). Acid hydrolysis of cellulose as the entry point into biorefinery schemes. *ChemSusChem, 2*(12), 1096–1107.

Saddler, J. N., Ernest, K. C., Mes-Hartree, M., Levitin, N., & Brownell, H. H. (1983). Utilization of enzymatically hydrolyzed wood hemicelluloses by microorganisms for production of liquid fuels. *Applied and Environmental Microbiology, 45*(1), 153–160.

Shuler, M. L., & Kargi, F. (2002). *Bioprocess engineering*. Upper Saddle River, NJ: Prentice Hall.

SillersR, C. A., Tracy, B., et al. (2008). Metabolic engineering of the non-sporulating, non-solventogenic *Clostridium acetobutylicum* strain M5 to produce butanol without acetone demonstrate the robustness of the acid-formation pathways and the importance of the electron balance. *Metabolic Engineering, 10*(6), 321–332.

Singh, S. P., & Singh, D. (2010). Biodiesel production through the use of different sources and characterization of oils and their esters as the substitute of diesel: A review. *Renewable and Sustainable Energy Reviews, 14*(1), 200–216.

Sjolander, N. O., Langlykke, A. F., & Peterson, W. H. (1938). Butyl alcohol fermentation of wood sugar. *Industrial and Engineering Chemistry, 30*, 1251–1255.

Sonderegger, M., Jeppsson, M., Larsson, C., Gorwa-Grauslund, M. F., Boles, E., Olsson, L., et al. (2004). Fermentation performance of engineered and evolved xylose-fermenting Saccharomyces cerevisiae strains. *Biotechnology and Bioengineering, 87*(1), 90–98.

Soni, B. K., Das, K., & Ghose, T. K. (1982). Bioconversion of agro-wastes into acetone butanol. *Biotechnology Letters, 4*, 19–22.

Spatari, S., Zhang, Y., & MacLean, H. L. (2005). Life cycle assessment of switchgrass-and corn stover-derived ethanol-fueled automobiles. *Environmental Science & Technology, 39*(24), 9750–9758.

Steen, E. J., Chan, R., Prasad, N., Myers, S., Petzold, C. J., Redding, A., et al. (2008). Metabolic engineering of Saccharomyces cerevisiae for the production of n-butanol. *Microbial Cell Factories, 7*(1), 36.

Strauß, C., Vetter, A., & Von Felde, A. (2012). Biogas production and energy crops biogas energy crops. In R. A. Meyers (Ed.), *Encyclopedia of sustainability science and technology* (pp. 1097–1145). New York, NY: Springer.

Sujan, S. A., Hirata, S., & Minowa, T. (2009). An approach to produce biodiesel from non-edible Jatropha curcus oil through dual step process: Preesterification and transesterification. *Bangladesh Journal of Scientific and Industrial Research, 44*(3), 347–352.

Sun, Y., & Cheng, J. (2002). Hydrolysis of lignocellulosic materials for ethanol production: A review. *Bioresource Technology, 83*(1), 1–11.

Swana, J., Yang, Y., Behnam, M., & Thompson, R. (2011). An analysis of net energy production and feedstock availability for biobutanol and bioethanol. *Bioresource Technology, 102*(2), 2112–2117.

Taguchi, F., Takiguchi, S., & Morimoto, M. (1992). Efficient hydrogen production from starch by a bacterium isolated from termites. *Journal of Fermentation and Bioengineering, 73*(3), 244–245.

Tanisho, S., Kuromoto, M., & Kadokura, N. (1998). Effect of CO_2 removal on hydrogen production by fermentation. *International Journal of Hydrogen Energy, 23*(7), 559–563.

Tarkow, H., & FEIST, W. C. (1969). *A mechanism for improving the digestibility of lignocellulosic materials with dilute alkali and liquid ammonia*.

Thang, V. H., Kanda, K., & Kobayashi, G. (2010). Production of acetone–butanol–ethanol (ABE) in direct fermentation of cassava by *Clostridium saccharoperbutylacetonicum* N1-4. *Applied Biochemistry and Biotechnology, 161*, 157–170.

Turner, W. J. (2014). *Understanding and improving microbial biofuel tolerance as a result of efflux pump expression through genetic engineering and mathematical modeling* (Doctoral dissertation). The University of Vermont

Van Haandel, A. C., & Lettinga, G. (1994). *Anaerobic sewage treatment: A practical guide for regions with a hot climate*. New York, NY: John Wiley & Sons.

Varga, E., Réczey, K., & Zacchi, G. (2004). Optimization of steam pretreatment of corn stover to enhance enzymatic digestibility. *Applied Biochemistry and Biotechnology, 114*(1–3), 509–523.

Votruba, J., Volesky, B., & Yerushalmi, L. (1985). Mathematical model of a batch acetone-butanol fermentation. *Biotechnology and Bioengineering, 28*, 247.

Walfridsson, M., Bao, X., Anderlund, M., Lilius, G., Bülow, L., & Hahn-Hägerdal, B. (1996). Ethanolic fermentation of xylose with Saccharomyces cerevisiae harboring the Thermus thermophilus xylA gene, which expresses an active xylose (glucose) isomerase. *Applied and Environmental Microbiology, 62*(12), 4648–4651.

Walter, A., Rosillo-Calle, F., Dolzan, P., Piacente, E., & Borges da Cunha, K. (2008). Perspectives on fuel ethanol consumption and trade. *Biomass and Bioenergy, 32*(8), 730–748.

Woods, D. R. (1995). The genetic engineering of microbial solvent production. *Trends in Biotechnology, 13*, 259–264.

Worldwatch Institute. (2005). *Renewables, 2005: Global status report*. Washington, DC: Worldwatch Institute.

Yoshida, N., Nakasato, M., Ohmura, N., Ando, A., Saiki, H., Ishii, M., & Igarashi, Y. (2006). Acidianus manzaensis sp. nov., a novel thermoacidophilic archaeon growing autotrophically by the oxidation of H_2 with the reduction of Fe^{3+}. *Current Microbiology, 53*(5), 406–411.

Yu, E. K. C., Deschatelets, L., & Saddler, J. N. (1984). The bioconversion of wood hydrolyzates to butanol and butanediol. *Biotechnology Letters, 6*, 327–332.

Zhang, T., Du, N., & Tan, T. (2011). Biobutanol production from sweet sorghum bagasse. *Journal of Biobased Materials and Bioenergy, 5*(3), 331–336.

Zhang, B., Weng, Y., Xu, H., & Mao, Z. (2012). Enzyme immobilization for biodiesel production. *Applied Microbiology and Biotechnology, 93*(1), 61–70.

Zheng, Y., Pan, Z., & Zhang, R. (2009). Overview of biomass pretreatment for cellulosic ethanol production. *International Journal of Agricultural and Biological Engineering, 2*(3), 51–68.
Zinder, S. H. (1994). Syntrophic acetate oxidation and "reversible acetogenesis". In H. L. Drake (Ed.), *Acetogenesis* (pp. 386–415). New York, NY: Springer.