Chapter 14
Lignocellulosic Biomass for Bioethanol Production Through Microbes: Strategies to Improve Process Efficiency

Ajay Kumar, Joginder Singh and Chinnappan Baskar

Abstract Lignocellulosic biomass can be a potential source of bioethanol by a microorganism such as yeast and bacteria. Hydrolysis of cellulose resulted in reducing sugars and fermentation of sugar produces bioethanol. Fermentable sugar can be obtained by pretreatment of lignocellulosic biomass which involves physic-chemical techniques along with biological pretreatment. Many fungal organisms such as white fungus and enzymes obtained from them have been reported to carry out the pretreatment process. Several models have been proposed to validate the hydrolysis of cellulose and hemicellulose. Tools of metabolic engineering and genetic engineering are used for the modification of microorganism so that they can utilize the different forms of carbon and perform the fermentation process at a wide range of pH and temperature. Process optimization and kinetic studies of microorganism can help in enhancing the productivity of bioethanol. Monod model and its modifications are used to describe the growth kinetics whereas Leudeking–Piret model for product formation kinetics. Different kinds of unit operations as a tool of downstream processing can be coupled with fermenter to prevent the product toxicity and increase the yield of the ethanol. Thus fuelling the future, the engineered microorganism can be explored for the production of next-generation lignocellulosic bioethanol.

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14.1 Introduction

The global energy requirement is fulfilled by fuel which represents about 70% of the total energy demands (Gouveia and Oliveira 2009). The global energy runs on energy. The high cost of the fossil fuel and conservation of fossil fuel resources forced to produce biofuels via microbial fermentation of biomass (Wargacki et al. 2012). An economic growth and rising population compel for high energy demand. The need of energy will be drastically increased by almost 60% more than today in 2030 by the world of this 45% will be accounted for by India and China together (Patil et al. 2008). Thermochemical conversion and biochemical conversion are primarily used for the conversion of lignocellulosic biomass into simple sugars. In industries the biochemical conversion process produces ethanol. The first generation ethanol can be produced by fermentation of sugars or starch while second-generation ethanol is produced by lignocellulogic biomass which can be converted into sugars. Bioethanol is used in spark ignition engine alternative to petrol as blended fuel E85 (85% bioethanol and 15% gasoline) in most of the developed countries like Brazil, Indonesia, and USA (Jayed et al. 2011; Mussatto et al. 2010). Several developed and developing countries like Brazil, the United States (USA), Australia, Canada, Colombia Japan, India, China, and Europe are interested in economic development by their internal major biofuel markets. Such interests are developed by

(I) increasing the oil prices,
(II) concern about greenhouse gas (GHG) emissions measured by carbon footprint,
(III) the requirements of the "Paris Agreement".

These days biofuels are the favorable choice of fuel consumption due to generating an acceptable quantity of exhaust gases (Demirbas 2008).

Lignocellulosic biomass such as agricultural residue, forest residue, non-feed energy crops, and municipal solid waste (MSW) are used by lignocellulosic refineries (Chandel et al. 2018). The main constituents of lignocellulosic biomass are cellulose (32–54%), hemicelluloses (11–37%), and lignin (17–32%). Cellulose which is a polymer of glucose formed via β,1 $\rightarrow$ 4 glycosidic bond and hemicelluloses is made up of xylopyranose units linked through β,1 $\rightarrow$ 4 glycosidic bonds are chain polysaccharides. Lignin is heteropolymer arranged by cross-linked three dimension phenolic polymers formed from the oxidative combinatorial coupling of three monolignol monomers such as (p-coumaryl alcohol [$C_9H_{10}O_2$], coniferyl alcohol [$C_{10}H_{12}O_3$] and sinapyl alcohol [$C_{11}H_{14}O_4$]) (Cao et al. 2017). Figure 14.1 shows lignocellulosic biomass components and their degradable products.

Lignocellulosic biomass pretreatment is used to remove cellulose, hemicellulose, and lignin which enhances cellulose hydrolysis to produce reducing sugars (Sun and Cheng 2002). The effective utilization of both cellulose and hemicellulose consisting of C_6 and C_5 carbon respectively is required for the production of biofuels and fine chemicals. Figure 14.2 shows the comparative analysis of ethanol production as 1st and 2nd generation biofuel.

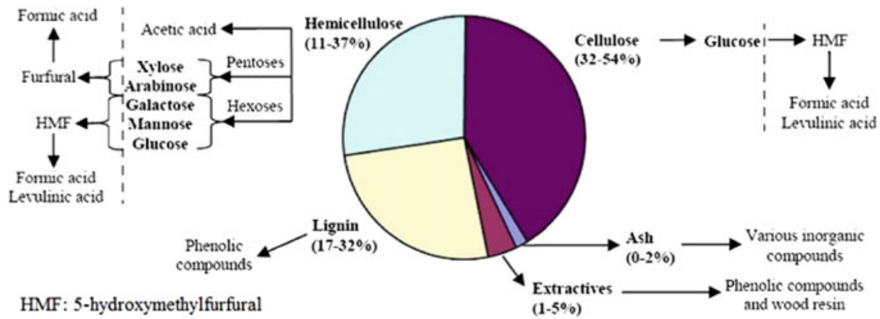


Fig. 14.1 Lignocellulosic biomass components and their degradable products. Dashed line denotes the secondary degradation products (Zabed et al. 2017)

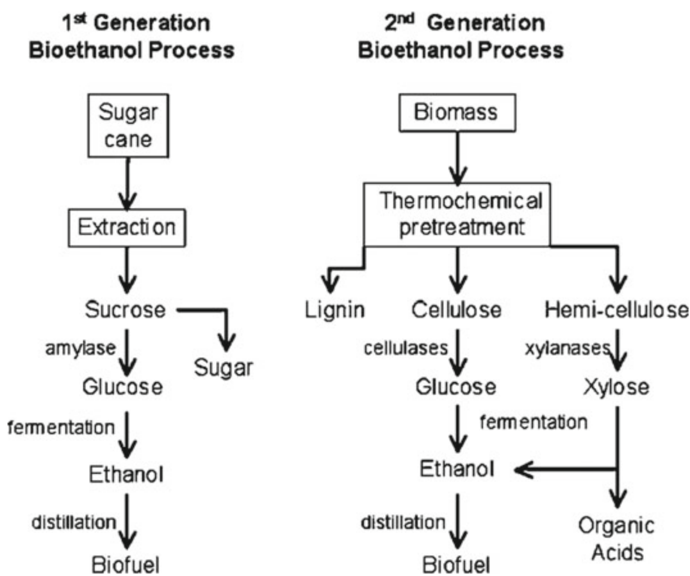


Fig. 14.2 Schematic representation of the biofuel production process (Bugg et al. 2011)

14.2 Kinetics of Solubilization

The mechanism of hydrolysis of cellulose by cellulose has been actively studied over the past 70 years. Bansal et al (2009) described the cellulose hydrolysis kinetic model. Figure 14.3 shows the steps in cellulose hydrolysis.

The hydrolysis of cellulose involved the following critical steps:

1. Cellulases get adsorbed on the substrate with the help of binding domain.
2. The bonds susceptible to hydrolysis on the substrate surface are localized.
3. The enzyme-substrate complex is formed.

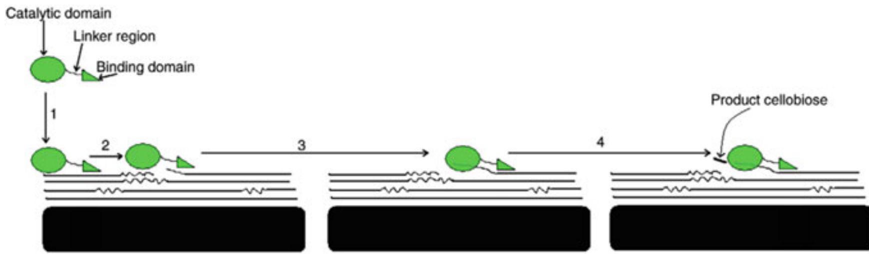
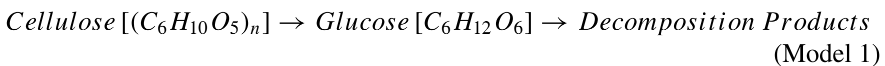


Fig. 14.3 Cellobiohydrolase acting on a cellulosic substrate (Bansal et al. 2009)

4. The β-glycosidic bonds present on the cellulose chain are hydrolyzed by the action of the enzyme and simultaneous forward sliding of the enzyme.
5. Cellulases desorption from the substrate
6. Cellobiose hydrolysis by the action of β-glucosidase for the formation of glucose.

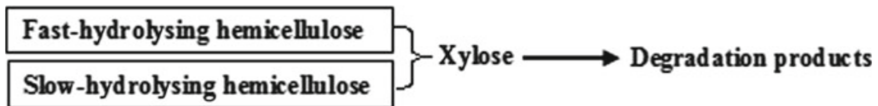
Several kinetics models have been studies, which proposed the hydrolysis of cellulose and hemicelluloses (Shi et al. 2017a, b). dos Santos Rocha et al. (2017) summarized the models as follows:

Model 1: Cellulose hydrolysis (Saeman 1945).
The kinetics model of lignocellulosic material hydrolysis such as wood was initially proposed by Saeman (1945) at high temperature and in the presence of dilute acid. This model was designed for cellulose hydrolysis to glucose.



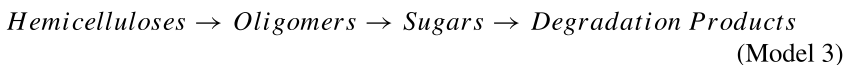
$$Cellulose\,[(C_6H_{10}O_5)_n] \rightarrow Glucose\,[C_6H_{12}O_6] \rightarrow Decomposition\ Products \quad \text{(Model 1)}$$

Model 2: Hemicellulose hydrolysis (Conner 1984).
Conner (1984) proposed a model to show the degradation of hemicellulose.



(Model 2)

Model 3: Hemicellulose degradation into xylooligomers and monomers (Pronyk and Mazza 2010).
A model proposed by Pronyk and Mazza (2010) describes the formation of xylooligomers and sugars by the degradation of hemicelluloses.



$$Hemicelluloses \rightarrow Oligomers \rightarrow Sugars \rightarrow Degradation\ Products \quad \text{(Model 3)}$$

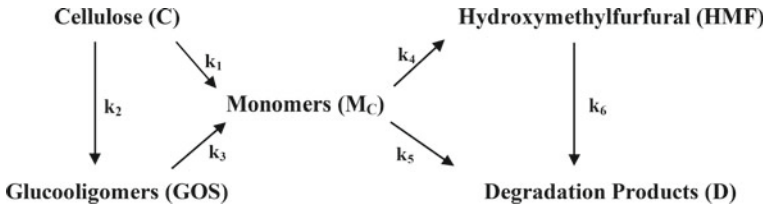


Fig. 14.4 The degradation of cellulose

14.2.1 Kinetics of Cellulosic Solubilization

The release of sugar from cellulosic biomass is one of the expensive operation (Shi et al. 2017a, b). The sequential steps in the degradation of cellulose are described in Fig. 14.4.

A first-order sequential reactions was proposed to describe the cellulose degradation, by the following equations:

$$\frac{d(C)}{dt} = -(k_1 + k_2) \cdot C \tag{14.1}$$

$$\frac{d(GOS)}{dt} = k_2C - k_3GOS \tag{14.2}$$

$$\frac{d(M_C)}{dt} = k_1C + k_3GOS - (k_4 + k_5) \cdot M_C \tag{14.3}$$

$$\frac{d(HMF)}{dt} = k_4M_C - k_6HMF \tag{14.4}$$

$$\frac{d(D)}{dt} = k_5M_C - k_6HMF \tag{14.5}$$

where

k_1 rate of solubilization for cellulosic fractions in monomers,
k_2 rate of solubilization for cellulosic fractions in glucooligomers,
k_3 rate of solubilization of glucooligomers to monomers,
k_4 rate of transformation of glucose monomers degradation to hydroxymethylfurfural
k_5 rate of solubilization of monomers to final degradable products,
k_6 rate of solubilization of hydroxymethylfurfural to final degradable products.

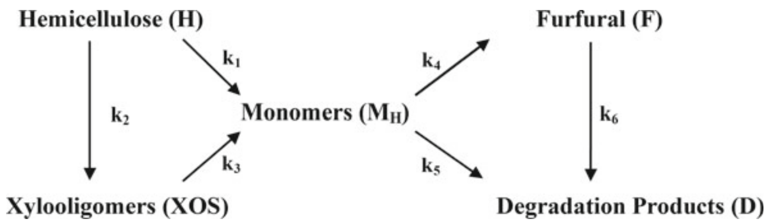


Fig. 14.5 The degradation of hemicelluloses

14.2.2 Kinetics of Hemicellulosic Solubilization

The degradation of hemicellulosic fraction during hydrothermal pretreatment can be described in Fig. 14.5.

A first-order sequential reactions steps are proposed to describe the degradation of a hemicellulosic fraction by the following equations:

$$\frac{d(H)}{dt} = -(k_1 + k_2)H \tag{14.6}$$

$$\frac{d(XOS)}{dt} = k_2 - k_3 XOS \tag{14.7}$$

$$\frac{d(M_H)}{dt} = k_1 H + k_3 XOS - (k_4 + k_5)M_H \tag{14.8}$$

$$\frac{d(F)}{dt} = k_4 M_H - k_6 F \tag{14.9}$$

$$\frac{d(D)}{dt} = k_5 M_H + k_6 F \tag{14.10}$$

where

k_1 rate of solubilization for hemicellulose into monomeric fractions,
k_2 rate of solubilization for hemicellulose into xylooligomers,
k_3 rate of solubilization of xylooligomers to monomers,
k_4 rate of transformation of xylose monomers to furfural,
k_5 rate of solubilization of xylose to final degradable products,
k_6 rate of solubilization of furfural to final degradable products.

14.3 Pretreatment Methods

Several physical, chemical, physicochemical, and biological methods have been developed for the pretreatment of lignocellulosic biomass to get fermentable sugars which have been briefly summarized as follows (Larsen et al. 2018; Tian et al. 2018).

14.3.1 Milling

Milling (Mechanical grinding) which involves size reduction of biomass to increase the surface area is generally treated as the first step of the pretreatment process. Different milling methods such as ball milling (to reduce cellulose crystallinity), two-roll milling, hammer milling, vibro energy milling, colloid milling, and disk milling are used in bioethanol production processes which resultant in the particles size reduction to 0.2–2 mm. High energy requirement is one of the most important drawbacks of this process (Veluchamy et al. 2018)

14.3.2 Steam Explosion Pretreatment

Steam explosion is the most widely and commonly used physicochemical method of biomass pretreatment. Biomass is usually treated with high-pressure saturated steam at temperatures 160–240 °C, and pressures 0.7–4.8 MPa, which resulted into digestibility of the lignocellulosic biomass (Agbor et al. 2011; Chiaramonti 2012).

14.3.3 Liquid Hot Water Treatment (LHW)

Liquid hot water (LHW) which is used in hydrothermal pretreatment is used to reduce cell wall rigidity of lignocellulosic biomass. In addition, LHW pretreatment which maintains water in the liquid state at elevated temperatures (160–240 °C) is a green approach, does not need any chemicals (Zhuang et al. 2016).

14.3.4 Ammonia Fiber Expansion (AFEX) Pretreatment

Ammonia-based pretreatment method uses liquid ammonia in a batch reactor under pressure (1.72–2.06 MPa) and moderate temperature (60–120 °C) for several minutes (30–60 min) followed by rapid pressure release is used for lignocellulosic biomass pretreatment. AFEX treatment process resulted in cleavage of carbohydrate and lignin complex (Mood et al. 2013; Yang and Wyman 2008).

14.3.5 CO_2 Explosion Pretreatment

Supercritical carbon dioxide (SC–CO_2) explosion method uses inexpensive CO_2 which acts as a green solvent at critical temperature (T_c) of 31 °C and critical pressure

(*Pc*) of 7.4 MPa, is used for the pretreatment of wet lignocellulosic biomass (Brodeur et al. 2011).

14.3.6 Wet Oxidation Technology

Wet oxidation technology includes water and oxygen or air as a catalyst which is carried out at a temperature above 120 °C and pressures (0.5–2 MPa) for about 30 min. Formation of inhibitors such as furfural and hydroxymethylfurfural (HMF) is lower in the wet oxidation pretreatment (Talebnia et al. 2010).

14.3.7 Acid and Base Pretreatment

Concentrated and dilute acids such as sulphuric acid (H_2SO_4), hydrochloric acid (HCl), phosphoric acid (H_3PO_4), nitric acid (HNO_3), etc., are used for the pretreatment of lignocellulosic biomass. The process of enzymatic hydrolysis can be improved with the pretreatment of acids to release fermentable sugars (Kumar et al. 2009). Some bases such as sodium hydroxide (NaOH), potassium hydroxide (KOH), calcium hydroxide [$Ca(OH)_2$], ammonium hydroxide (NH_4OH), etc., has been reported for the hydrolysis of biomass which is less harsh as compared to other pretreatment methods can be carried out at lower temperature and pressure. The effect of alkaline treatment depends on the content of lignin present in the biomass. It has been observed that alkaline pretreatment causes less sugar degradation as compared to the acid treatment (Hendriks and Zeeman 2009).

14.3.8 Ozonolysis Pretreatment

Ozonolysis pretreatment includes ozone gas as an effective oxidant in order to break down lignin and hemicelluloses complex and increase cellulose biodegradability and sugar yield (Chaturvedi and Verma 2013).

14.3.9 Organosolvation

Organosolvation process uses an organic acid such as oxalic, acetylsalicylic, and salicylic acids as catalysts or aqueous organic solvents such as methanol, ethanol, acetone, ethylene glycol, triethylene glycol, and tetrahydrofurfuryl alcohol mixture with inorganic acid catalysts (HCl or H_2SO_4) for lignin and hemicelluloses bond

breakage during lignocellulosic biomass pretreatment (Zhu and Pan 2010; Kumar et al. 2009).

14.3.10 Biological Pretreatment

Biological pretreatment methods include either pure or crude enzyme for hydrolysis of different lignocellulosic biomass. Brown, white, and soft rot fungi have been reported for the degradation of lignin and hemicelluloses and very little cellulose. Several white-rot fungi such as *Phanerochaete chrysosporium, Ceriporia lacerata, Cyathus stercolerus, Ceriporiopsis subvermispora, Pycnoporus cinnarbarinus* and *Pleurotus ostreaus* has been reported for their lignin degradation efficiency (Alvira et al. 2010). The main advantages of biological treatment are low energy requirement and mild environment conditions (Taherzadeh and Karimi 2008; Sindhu et al. 2016). Table 14.1 shows the pros and cons of lignocellulosic biomass pretreatment methods.

14.4 Microbes for Bioethanol Production

Microorganism such as *Saccharomyces cerevisiae, Schizosaccharomyces pombe, Zymomonas mobilis, Fusariumoxys porum,* etc., plays a vital role during ethanol fermentation.

In ethanol fermentation, glucose can be utilized via oxidative metabolism (leads to cell growth) and fermentative metabolism (leads to ethanol fermentation) which are the two different energy producing pathways (Ji et al. 2016). Combined aerobic and anaerobic fed-batch operations are recommended to enhance the ethanol production. Table 14.2 shows the comparison among *Zymomonas mobilis*, *Escherichia coli,* and *Saccharomyces cerevisiae*.

Yeast is most commonly used for the ethanol fermentation due to the utilization of a different range of substrate (Mansouri et al. 2016). The rate of glycolysis is regulated by dissolved oxygen concentration.

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \rightarrow \underset{\text{Ethanol}}{2C_2H_5OH} + \underset{\text{Carbon dioxide}}{CO_2} \tag{14.11}$$

The theoretical ethanol yield over glucose is 0.15 g/g and growth yield over glucose is 0.12 g/g. Optimum temperature and pH values for yeast are 30 °C to 35 °C and 4–6 respectively. Production of ethanol from C_5 carbon such as xylose is described as follows (Tri and Kamei 2018).

$$\underset{\text{Xylose}}{3C_5H_{10}O_5} \rightarrow \underset{\text{Ethanol}}{5C_2H_5OH} + \underset{\text{Carbon dioxide}}{5CO_2} \tag{14.12}$$

Table 14.1 Pros and cons of lignocellulosic biomass pretreatment methods (Maurya et al. 2015)

Pretreatment method	Advantages	Disadvantages
Milling	• The decrease of cellulose crystallinity and degree of polymerization • Reduction of particle size to increase specific surface area and pore size	• High power and energy consumption
Steam explosion	• Causes lignin transformation and hemicellulose solubilization • Lower cost • Higher yield of glucose and hemicellulose in the two-step method	• Generation of toxic compounds • Partial hemicellulose degradation
Liquid hot water	• Size reduction of the biomass is not needed • No chemicals are generally required • No requirement of corrosion-resistant materials	• High energy and high water requirement • Formation of toxic compounds
Ammonia fiber expansion (AFEX)	• Increases accessible surface area • Less inhibitors formation • Does not require small particle size of biomass	• Not very effective for the biomass with high lignin content • The high cost of a large amount of ammonia
CO_2 explosion	• Increase accessible surface area • Availability at relatively low cost • Do not form inhibitory compounds • Nonflammability • Easy recovery after extraction and environmental acceptability	• Very high-pressure requirements
Wet oxidation	• High degree of solubilization of hemicellulose and lignin • Avoid formation of degradation compounds	• The high cost of oxygen and alkaline catalyst

(continued)

Table 14.1 (continued)

Pretreatment method	Advantages	Disadvantages
Concentrated acid	• High glucose yield • Ambient temperatures	• The high cost of acid and need to be recovered • Corrosion-resistant equipments are required • Concentrated acids are toxic and hazardous
Diluted acid	• High recovery of sugars at the end of the process • Low formation of toxic products	• The concentration of reducing sugars is relatively low • Generation of degradation products
Alkali	• The decrease in the degree of polymerization and crystallinity of cellulose • Disruption of lignin structure	• High cost • Not used for large-scale plant
Ozonolysis	• Effectively removes lignin content • Does not produce toxic residues • The reaction is carried out at room temperature and pressure	• The high cost of a large amount of ozone
Organosolv	• Causes lignin and hemicellulose hydrolysis	• Solvents need to be drained and recycled • High cost
Biological	• Low energy requirements • Delignification • Reduction in the degree of polymerization of cellulose • Partial hydrolysis of hemicelluloses • No chemical requirements • Mild environmental conditions	• Slow process rate • The very low treatment rate • Not very effective for commercial application

Recently, thermophilic microorganism is in practice for ethanol production at elevated temperature (Shuler and Kargi 2002).

The cellulose and hemicelluloses fraction of lignocellulosic feedstocks can be converted to ethanol either by

(i) simultaneous saccharification and fermentation (SSF)
(ii) separate enzymatic hydrolysis and fermentation (SSF) process and
(iii) consolidated bioprocessing (CBP)

Binod et al. (2010) describe the various ethanol processes as shown in Fig. 14.6.

Table 14.2 Comparison among *Zymomonas mobilis*, *Escherichia coli* and *Saccharomyces cerevisiae* (Wang et al. 2018)

Categories	*Zymomonas mobilis*	*Escherichia coli*	*Saccharomyces cerevisiae*
Growth condition	Facultative anaerobic	Facultative aerobic	Facultative aerobic
Taxonomy	Gram-negative bacterium	Gram-negative bacterium	Eukaryotic microorganism
Energy metabolism	ED pathway (1 ATP per glucose)	EMP pathway (2 ATP per glucose) and TCA	EMP pathway (2 ATP per glucose) and TCA
Ethanol productivity (g/g/h)	5.67	0.60	0.67
Respiratory chain	Uncoupled energetics and cellular growth, high rate O_2 consumption	Coupled with cell growth, ATP accumulation inhibits PFK	Coupled with cell growth, ATP accumulation inhibits PFK
Safety status	GRAS	Not GRAS	GRAS
Theoretical yield of ethanol	98%	88% (recombinant *E. coli* (pLPA102))	90–93%
Ethanol tolerance (v/v) (%)	16	6	15
pH range	3.5–7.5	4.0–8.0	2.0–6.5
N2 utilization	Yes	No report	No report
Median genome size (Mb)	2.14	5.15	12.12

ED Entner-Doudoroff pathway, *EMP* Embden-Meyerhof-Parnas pathway, *TCA* tricarboxylic acid cycle, *GRAS* generally recognized as safe, *PFK* phosphofructokinase

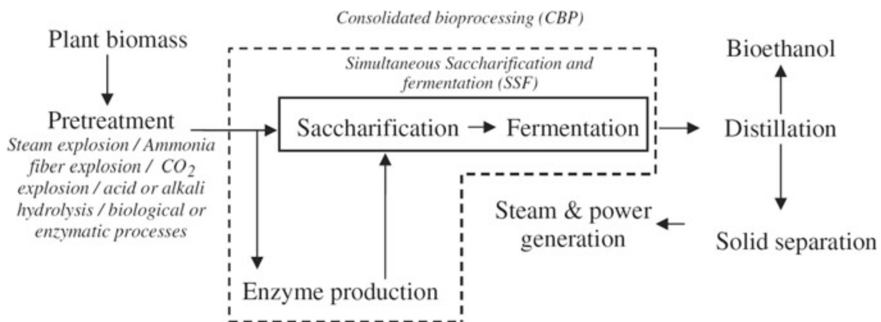


Fig. 14.6 Various methods of bioethanol production from lignocellulosic feedstocks (Nigam and Singh 2011)

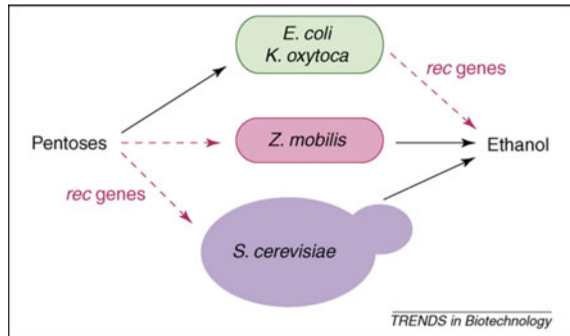


Fig. 14.7 Metabolically engineered strains for ethanol production from pentose sugars. *Abbreviation rec* recombinant (Hahn-Hägerdal et al. 2006)

Microbial consortium which may consist of a strain such as *Trichoderma reesei*, for enzyme production to hydrolyse lignocellulosic biomass and *Saccharomyces cerevisiae*, and *Scheffersomyces stipitis*, to utilize hexose and pentose sugars respectively could be used to perform consolidated bioprocessing (CBP) rather than a single microbe to increase the ethanol product yield (Rastogi, and Shrivastava 2017). Figure 14.7 shows the various metabolically engineered strains for ethanol production from pentose sugars.

Microorganisms like *Saccharomyces cerevisiae, Candida shehatae, Zymomonas mobilis, Pichia stiplis, Pachysolen tannophilus, Escherichia coli*, *Kluveromyces marxianus*, *Thermophilic bacteria, Thermoanaero bacterium saccharolyticum, Thermoanaerobacter ethanolicus* and *Clostridium thermocellum* have been reviewed for the production of bioethanol. The advantages and drawbacks of organisms used in lignocellulosic refinery have been depicted in Table 14.3.

14.5 Kinetics Models in Bioethanol Fermentation

Microbial growth kinetics is described by a logistic equation which is a common unstructured growth model. It deals with inhibition of growth which occurs in a batch process (Sewsunker-Sukai and Kana 2018).

$$\frac{dX}{dt} = \mu X \tag{14.13}$$

Specific growth rate μ is given by Monod model

$$\mu = \frac{\mu \max s}{k_s + s} \tag{14.14}$$

$$\frac{dX}{dt} = \mu_m X\left(1 - \frac{X}{X_m}\right) \tag{14.15}$$

Table 14.3 Advantages and drawbacks of organisms used in lignocellulosic refinery (Limayem et al. 2012)

Species	Characteristics	Advantage	Drawbacks
Saccharomyces cerevisiae	Facultative anaerobic yeast	• Naturally adapted to ethanol fermentation • High alcohol yield (90%) • High tolerance to ethanol (up to10% v/v) and chemical inhibitors • Amenability to genetic modifications	• Not able to ferment xylose and arabinose sugars • Not able to survive high temperature of enzyme hydrolysis
Candida shehatae	Micro-aerophilic yeast	• Ferment xylose	• Low tolerance to ethanol • Low yield of ethanol • Require micro-aerophilic conditions • Does not ferment xylose at low pH
Zymomonas mobilis	Ethanologenic Gram-negative bacteria	• Ethanol yield surpasses *S. cerevisiae* (97% of the theoretical) • High ethanol tolerance (up to 14% v/v) • High ethanol productivity (five-fold more than *S. cerevisiae* volumetric productivity) • Amenability to genetic modification • Does not require additional oxygen	• Not able to ferment xylose sugars • Low tolerance to inhibitors • Neutral pH range
Pichia stiplis	Facultative anaerobic yeast	• Best performance xylose fermentation • Ethanol yield (82%) • Able to ferment most of cellulosic-material sugars including glucose, galactose, and cellobiose • Possess cellulase enzymes favorable to SSF process	• Intolerant to a high concentration of ethanol above 40 g/L • Does not ferment xylose at low pH • Sensitive to chemical inhibitors. • Requires micro-aerophilic conditions to reach peak performance • Re-assimilates formed ethanol

(continued)

Table 14.3 (continued)

Species	Characteristics	Advantage	Drawbacks
Pachysolen tannophilus	Aerobic fungus	• Ferment xylose	• Low yield of ethanol • Require micro-aerophilic conditions • Does not ferment xylose at low pH
Escherichia coli	Mesophilic Gram-negative bacteria	• Ability to use both pentose and hexose sugars • Amenability for genetic modifications	• Repression catabolism interfere to co-fermentation • Limited ethanol tolerance • Narrow pH and temperature growth range • Production of organic acids • Genetic stability not proven yet • Low tolerance to inhibitors and ethanol
Kluveromyces marxianus	Thermophilic yeast	• Able to grow at a high temperature above 52 °C • Suitable for SSF/CBP process • Reduces cooling cost • Reduces contamination • Ferments a broad spectrum of sugars. • Amenability to genetic modifications	• Excess of sugars affect its alcohol yield • Low ethanol tolerance • Fermentation of xylose is poor and leads mainly to the formation of xylitol
Thermophilic bacteria: Thermoanaerobacterium saccharolyticum Thermoanaerobacter ethanolicus Clostridium thermocellum	Extreme anaerobic bacteria	• Resistance to an extremely high temperature of 70 °C • Suitable for SSCombF/CBP Processing • Ferment a variety of sugars • Display cellulolytic activity • Amenability to genetic modification	• Low tolerance to ethanol

where

X the biomass concentration (g/l),
X_m the maximum biomass concentration which is identical to carrying capacity (g/l),
μ_m the maximum growth rate (h^{-1}),
t the time (h).

The integration of the Eq. (14.15) with the boundary condition at $t = 0$, $X = X_0$ gives logistic curve.

$$X = \frac{X_0 e^{\mu_m t}}{1 - \frac{X_0}{X_m}(1 - e^{\mu_m t})} \tag{14.16}$$

Product formation kinetic is described by the following equation:

$$\frac{dp}{dt} = Y_{P/S}\frac{dX}{dt} \tag{14.17}$$

where $Y_{P/S}$ is yield coefficient.

In a batch process, substrate consumption kinetic is described by the following equation (Doran 1995):

$$-\frac{dS}{dt} = \frac{1}{Y_{X/S}}\frac{dX}{dt} + mX \tag{14.18}$$

where $Y_{X/S}$ is yield coefficient and m is maintenance coefficient.

$$S = S_0 - \frac{1}{Y_{X/S}}\left[\frac{X_0 X_m e^{\mu_m t}}{X_m - X_0 + e^{\mu_m t}} - X_0\right] - \frac{X_m m}{\mu_m}\ln\frac{X_m - X_0 + X_0 e^{\mu_m t}}{X_m} \tag{14.19}$$

Monod model is generally used to describe the growth of the cells. Excess substrate concentration often leads to poor product formation (the 'Crabtree effect'). Monod equation that includes a substrate and product inhibition is described as follows (Kashid and Ghosalkar 2018).

$$\mu = \frac{\mu_m S}{K_s + S + \frac{S^2}{K_I}}\left(1 - \frac{P}{P_{\max}}\right)^n \tag{14.20}$$

$$\mu = \frac{\mu_m S}{K_s + S + \frac{S^2}{K_I}}\left[1 - \left(\frac{P}{P_{\max}}\right)^n\right] \tag{14.21}$$

$$\mu = \frac{\mu_m S}{K_s + S + \frac{S^2}{K_I}}\frac{K_P}{K_P + P} \tag{14.22}$$

where

P ethanol concentration (g/l),
S substrate concentration (g/l),
μ specific growth rate (h^{-1}),
μ_{max} the maximum specific growth rate (h^{-1}),
K_s saturation constant (g/l),
K_I inhibition parameter for sugar,
P_{max} inhibition parameter for ethanol,
K_p a constant representing the inhibitory effect due to product,
n exponents governing ethanol inhibition of growth.

$$Y_{P/S} = \frac{P_f - P_0}{S_0 - S_f} \tag{14.23}$$

$$Y_{X/S} = \frac{X_f - X_0}{S_0 - S_f} \tag{14.24}$$

where $Y_{p/s}$ is the yield coefficient for ethanol on the substrate used for ethanol formation,

$$q_p = \frac{1}{X}\frac{dP}{dt} \tag{14.25}$$

The value of substrate concentration at which the specific growth rate is maximum is given by the following equation (Rao 2010):

$$S_{\max} = \sqrt{K_I K_S} \tag{14.26}$$

Substrate inhibition can overcome by fed-batch operation (Lin and Tanaka 2006).

$$\frac{dx}{dt} = \mu x - \frac{F}{V}x \tag{14.27}$$

where

F feed rate (m^3/h),
V liquid volume (m^3),
x cell concentration (g/l),
D dilution rate (h^{-1}),
μ the specific growth rate (h^{-1}).

$$\frac{dx}{dt} = x(\mu - D) \tag{14.28}$$

$$D = \frac{F}{V} \tag{14.29}$$

$$\frac{dp}{dt} = q_p x - \frac{F}{V}p \tag{14.30}$$

$$\frac{dS}{dt} = D(S_F - S) - \left(\frac{\mu}{Y_{X/S}} + \frac{q_p}{Y_{P/S}} + m_s\right)x \tag{14.31}$$

It is a differential equation for the rate of change of cell and substrate concentration in a fed-batch reactor. Where

μ specific growth rate (h^{-1}),
q_p the specific rate of product formation (h^{-1}),
S_F feed concentration of glucose (g/l),
$Y_{X/S}$ true biomass yield from the substrate (g/g),
$Y_{p/s}$ true product yield from the substrate (g/g),
m_s maintenance coefficient ($g\ g^{-1}h^{-1}$).

Substituting $\mu = D$, Monod equation is changed

$$D = \frac{\mu\text{max}\, S}{K_s + S} \tag{14.32}$$

Rearrangement of Eq. (14.32) gives an expression of substrate concentration as a function of the dilution rate.

$$S = \frac{DK_s}{\mu\text{max} - D} \tag{14.33}$$

$$\mu = D \tag{14.34}$$

$$X = (S_i - S)Y_{X/S} \tag{14.35}$$

$$X = \left(S_i - \frac{DK_S}{\mu\text{max} - D}\right)Y_{X/S} \tag{14.36}$$

Reciprocal plot (1/D vs. 1/S) is used to find out the value of Ks and μ_{max} by interpreting the slope and intercept (Srimachai et al. 2015).

$$\frac{1}{D} = \frac{Ks}{\mu\text{max}\, S} + \frac{1}{\mu\text{max}} \tag{14.37}$$

$$\frac{D}{S} = \frac{\mu\text{max}}{K_S} - \frac{D}{K_S} \tag{14.38}$$

$$\frac{S}{D} = \frac{Ks}{\mu\text{max}} + \frac{S}{\mu\text{max}} \tag{14.39}$$

In chemostat culture with $\mu = D$, a plot of $\frac{1}{Y_{X/S}^{obs}}$ verses $\frac{1}{D}$ gives a straight line with slope m_s and intercept $\frac{1}{Y_{X/S}^{true}}$

$$\frac{1}{Y_{X/S}^{obs}} = \frac{1}{Y_{X/S}^{true}} + \frac{m_s}{D} \tag{14.40}$$

where

$\frac{1}{Y_{X/S}^{obs}}$ the observed biomass yield from the substrate,
$\frac{1}{Y_{X/S}^{true}}$ the true biomass yield from the substrate,
m_s maintenance coefficient.

The formation of ethanol by microbes can be represented by Leudeking and Piret model (Mansouri et al. 2016).

$$q_p = \alpha\mu + \beta \tag{14.41}$$

Ethanol production rate in batch mode is represented by the following equation:

$$\frac{dP}{dt} = \alpha\frac{dX}{dt} + \beta X \tag{14.42}$$

where

q_p specific product formation rate,
μ specific growth rate,
α growth-associated product formation coefficient,
β nongrowth-associated product formation coefficient,
P bioethanol as product concentration,
X cell biomass concentration.

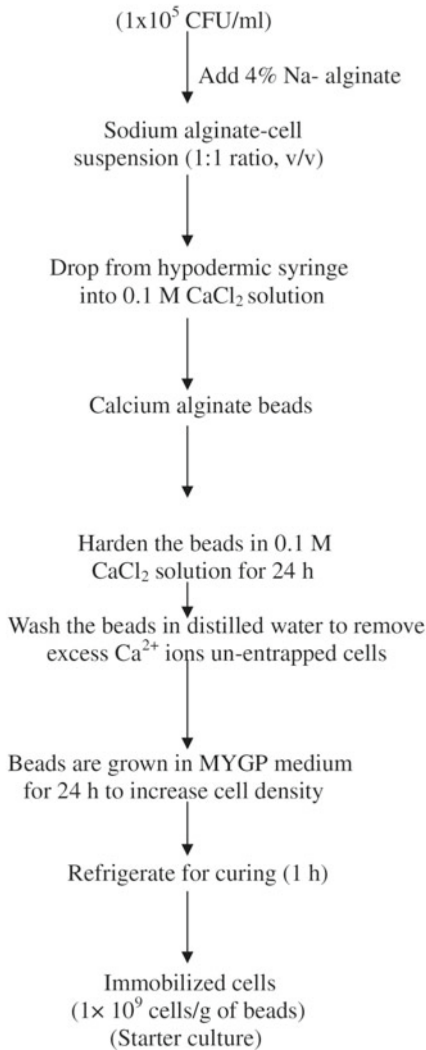
Immobilization of yeast within porous or polymeric matrices results in high cell concentrations in the reactor and therefore, high ethanol productivities. Immobilized cells reactors may be in the form of packed columns or fluidized beds. The immobilization kinetic has been given in the equation (Ariyajaroenwong et al. 2016).

$$D_e\left(\frac{d^2S}{dr^2}r^2 + 2r\frac{dS}{dr}\right) - \frac{\mu\max S}{K_S + S}r^2 = 0 \tag{14.43}$$

where,

D_e effective diffusivity of the substrate,
$\mu_{\max}$ the specific growth rate of the organism (h^{-1}),
K_S the saturation constant (kg/m^{-3})
S the concentration of the limiting substrate (kg/m^{-3})
r the distance measured radially from the center.

Figure 14.8, shows the method of immobilization of yeast cells. The action of microbes on lignocellulosic feedstocks and optimization parameters for growth conditions is listed in Table 14.4.



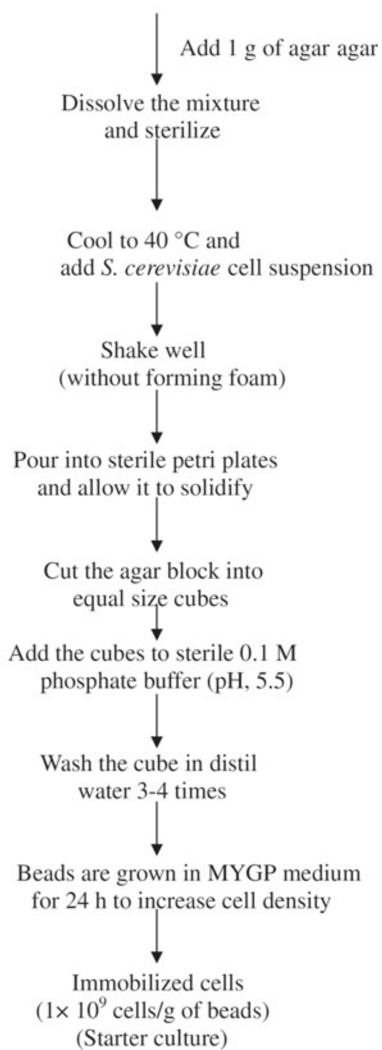


Fig. 14.8 The methods of immobilization of yeast cells in **a** calcium alginate beads and **b** agar agar cubes (Behera et al. 2010)

Table 14.4 Ethanol production from lignocellulosic biomass by microbes

Biomass	Organism	Fermentation condition	Ethanol production (g/L)	References
Rice straw	Sestc engineered *Aspergillus niger* with Sestc engineered *Saccharomyces cerevisiae*	Temp 30 °C	31.9	Yang et al. (2018)
Pomegranate peel	*Saccharomyces cerevisiae*, *Pichia stipitis*	Temp 30 °C, pH 5	5.58	Demiray et al. (2018)
Banana stem	*Aspergillus niger, Trichoderma reesei, Zymomonas mobilis*	Temp 30 °C, pH 5	3.493	Mustofa (2018)
Dioscorea rotundata	*Saccharomyces cerevisiae* strain LC 269108	Temp 40 °C, pH 5.5	46.6	Nwuche et al. (2018)
Banana peels hydrolysate	*Zymomonas mobilis* CCT 4494, *Pachysolen tannophilus* CCT 1891	Temp 30 °C, pH 4.5–5.5	11.32	Ferreira et al. (2018)
Mango pulp	*Saccharomyces cerevisiae*	Temp 30 °C, pH 4.5	5.81	Barbosa et al. (2018)
Rice husk	*Escherichia coli* KO11	Temp 37 °C,	2.7	Tabata et al. (2017)
Wheat straw	*Saccharomyces cerevisiae*, *Lipomyces starkeyi*, and *Rhodotorula babjevae*	Temp 30 °C, pH 5	23.85	Brandenburg et al. (2018)
Wheat Bran	*Saccharomyces cerevisiae* MTCC 174	Temp 30 °C and pH 5.0	4.12	Sharma et al. (2018)
Bamboo biomass	*Saccharomyces cerevisiae* SR8u	Temp 30 °C and pH 5.5	46	Yuan et al. (2018)

14.6 Technologies Used for Development of Strains

14.6.1 CRISPR-Cas9 Genome Editing Technology

Saccharomyces cerevisiae genome can be edited by the CRISPR-Cas9 technology for the utilization of xylose for lignocellulosic ethanol production. This technology has made the genome editing easier in diploid organisms and enable the engineering of 5-10 pathways in yeast genome simultaneously (Jansen et al. 2017; Wang 2015; Löbs, et al 2017). Figure 14.9 shows CRISPR-Cas9-mediated genome editing.

14.6.2 Protein Engineering

Protein engineering has improved the pentose uptake kinetics in yeast by the modification of amino acid sequences in proteins (Ko and Lee 2018). Figure 14.10, shows the role of protein engineering for fuel production.

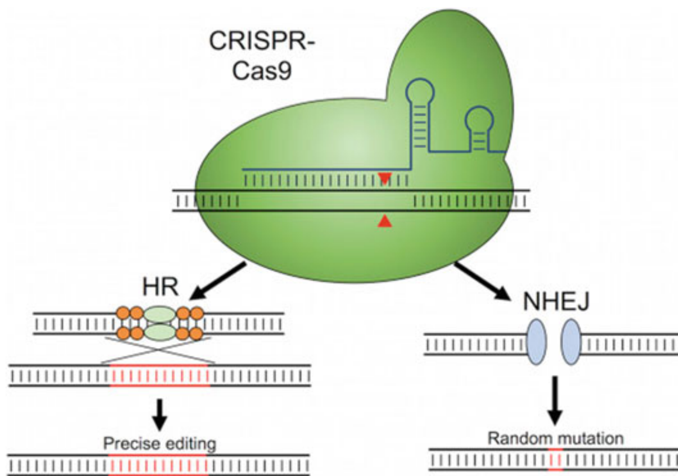


Fig. 14.9 CRISPR-Cas9-mediated genome editing [*HR* Homologous recombination; *NHEJ* Non-homologous end-joining] (*Source* Löbs et al. 2017)

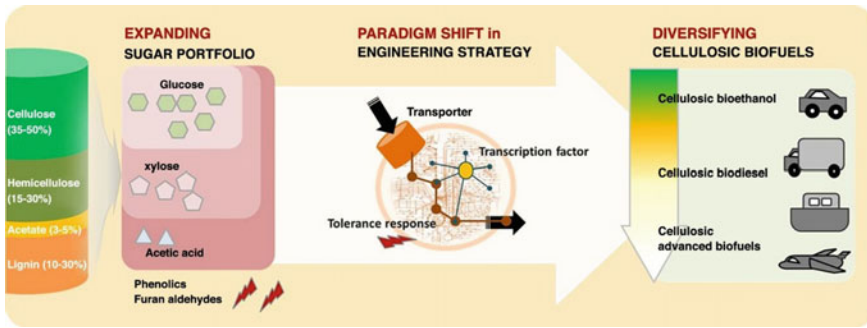


Fig. 14.10 Protein engineering for fuel production (Ko and Lee 2018)

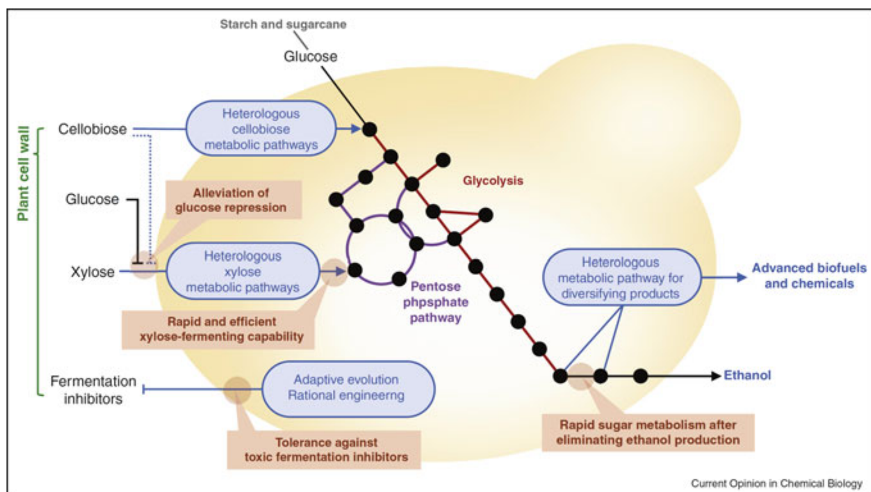


Fig. 14.11 Metabolic engineering of yeast for biofuels production (Jin and Cate 2017)

14.6.3 Metabolic Engineering

Tools of system biology as metabolic engineering have improved the production of ethanol in nonconventional yeast by the modification of the pathways as shown in Fig. 14.11 (Löbs et al. 2017).

14.6.4 Evolutionary Engineering

Evolutionary engineering is used to improve the traits of the organisms. It uses adaptive laboratory evolution for relevant industrial traits selection (Mans et al. 2018).

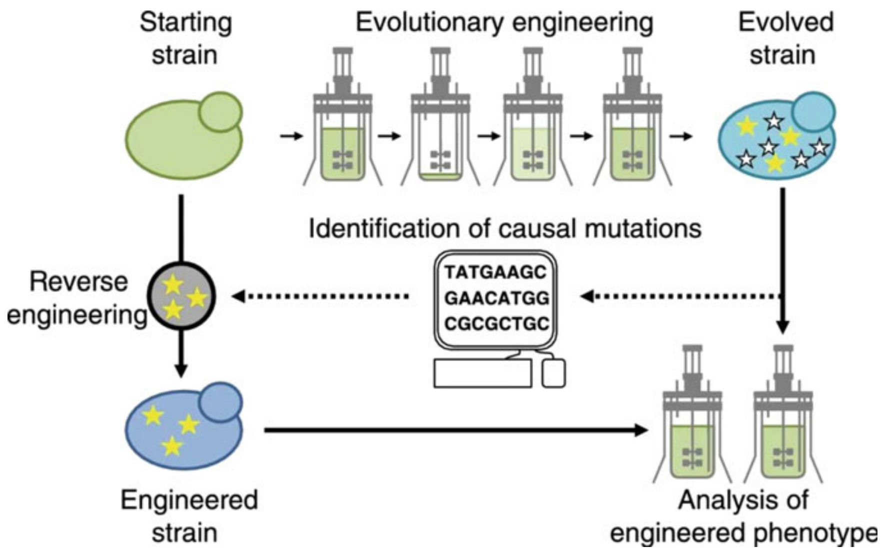


Fig. 14.12 Evolutionary engineering for strain improvement (Mans et al. 2018)

Through adaptive laboratory evolution, yeast strain has been improved which can be grown on pentose sugar to enhance the yield of ethanol (Fig. 14.12).

14.7 Downstream Processing of Ethanol from Fermentation Broth

Conventional distillation is commonly used for ethanol purification. Vacuum fermentation with cell recycling is used for volatile ethanol extraction which enhances the overall process productivity of ethanol (Cardona and Sánchez 2007). Ethanol can be recovered from fermentation broth through gas stripping. Pervaporation which is membrane-based technology is used for ethanol removal and keeping the ethanol concentration below the inhibitory level of the microorganism when coupled with fermentation (Chovau et al. 2011). Extractive fermentation is another promising technique for ethanol recovery. Figure 16.13, shows different modes of ethanol recovery from the fermentation broth.

Furthermore fuelling the future, the engineered microorganism can be used for next-generation bioethanol production depending upon lignocellulosic biomass utility by bacteria and fungi (Liao et al. 2016). A portion of hemicellulose can be hydrolyzed through the pretreatment method such as acid pretreatment. The main industrial ethanol producer such as conventional yeast (*Saccharomyces cerevisiae*)

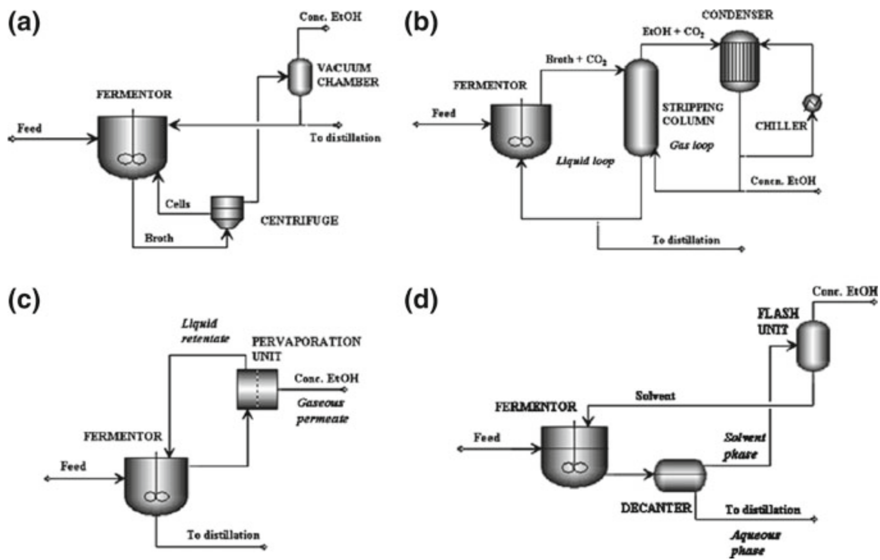


Fig. 14.13 Different modes of ethanol recovery from the fermentation broth. **a** Vacuum fermentation with cell recycling. **b** Fermentation coupled with gas stripping. **c** Fermentation coupled with pervaporation. **d** Extractive fermentation (Cardona and Sánchez 2007)

and *Zymomonas mobilis* cannot utilize xylose (major pentose sugar) as a source of carbon. In an attempt to circumvent this problem, a group of yeast and bacteria have been engineered to utilize xylose with varying degree of success (Fig. 14.14).

14.8 Conclusions and Future Prospect

Bioethanol production from lignocellulosic feedstocks by means of microbes is an alternative to renewable energy. But the development of an economically viable process and optimization of pretreatment methods are still required for lignocellulosic feedstocks to enhance the yield of ethanol. Bioethanol production has some major obstacles such as pretreatment process, enzymatic hydrolysis, fermentation, and distillation which are required to overcome by means of efficient technology. Production of fermentable sugars in high concentration by hydrolysis process is yet to be achieved as biomass processing is a major challenging task. Fermentation process requires both pentose and hexose sugars in presence of engineered microbial strains. However much work is still required to bring ethanol production by engineered

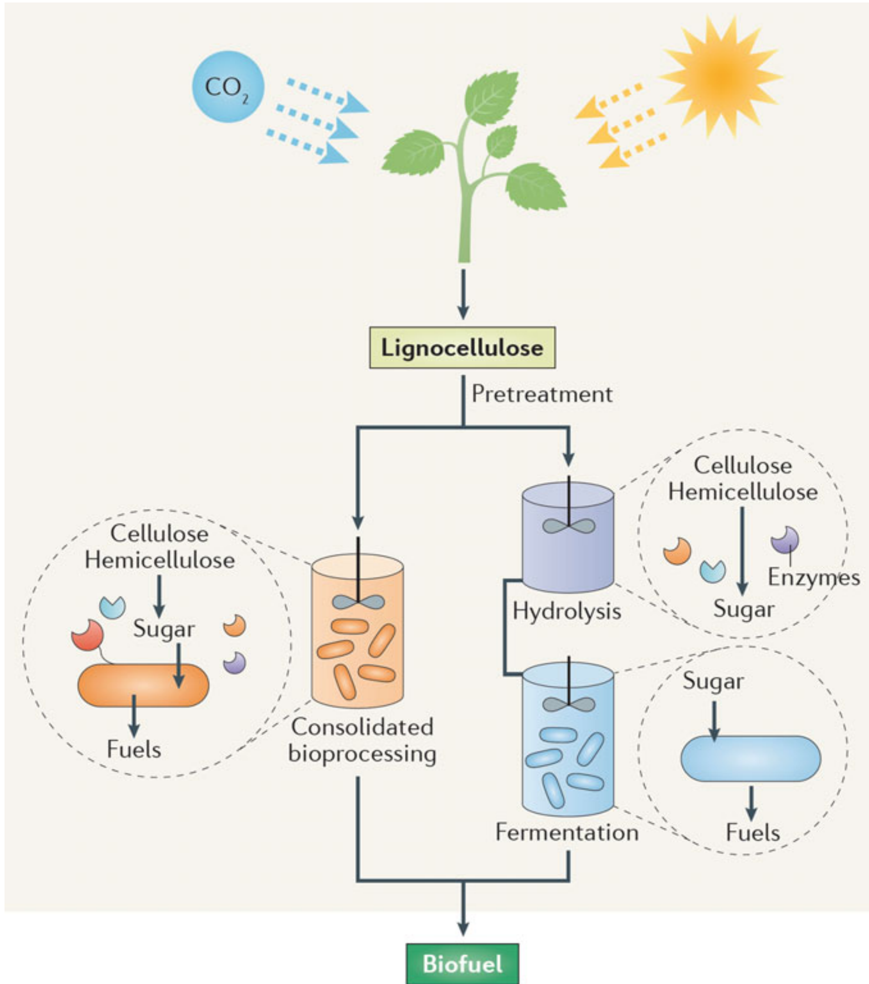


Fig. 14.14 Overview of biofuel production from lignocellulosic biomass (Liao et al. 2016)

microorganisms to an industrial level. Distillation is an energy-consuming process, an alternative green process such as pervaporation should be commercialized on industrial scale. Thus, in near future different types of biomass can be effectively utilized and optimized for bioethanol production with the improvement of technologies.

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