Biofuel and Biorefinery Technologies 4

Sachin Kumar
Rajesh K. Sani *Editors*

Biorefining of Biomass to Biofuels

Opportunities and Perception

Springer

Biofuel and Biorefinery Technologies

Volume 4

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Preface

Energy is one of the major elements for the sustenance of life. It is the driver of life and can be obtained from different sources-conventional and non-conventional and renewable and non-renewables. There are several renewable energy resources including biomass, solar, wind, tidal, hydro, geothermal, etc., which can be exploited to meet the energy demand of the burgeoning global population. Biomass is one of the renewable resources which is available abundantly and is almost evenly distributed across the globe. Biomass resources can partially offset the ever-increasing energy demand for power, process steam, home-heating and transportation. However, the use of biomass for a particular purpose or for the production of a single product may not be economical and viable. The harnessing of biomass and its utilization for multiple uses such as energy, chemicals/solvents/intermediates and other products can make the process economically viable.

Biorefinery, based on the concept of the conventional grass-roots crude oil refinery and petrochemicals complex, may open up multiple options for the production of various forms and classes of fuels, platform chemicals, chemical intermediates for downstream processing, and heat and power generation from a myriad of biomass materials.

Biomass can be transformed and converted into a number of products through various routes. Such routes may be physical, biochemical, chemical, thermal and their combinations. These processes can be divided based on the steps involved in the process. For example, if microbial or biological entities are involved in the process, the process is called the biochemical conversion process. Likewise, if high temperature and pressure are involved, we call it the thermochemical conversion process. If a catalyst is involved, it is called a catalytic conversion process. The application generally dictates the selection of the biomass feedstock and the appropriate conversion process.

The biochemical conversion processes can be aerobic, anaerobic and facultative depending on the kind of microbial strains and the environmental conditions prevailing in the system. For example, we can process the biomass to produce biofuels such as ethanol, butanol, biogas, and hydrogen through fermentation

and/or anaerobic digestion. However, the complex structure of the biomass makes its biochemical processing difficult as the microbial species involved in the fermentation processes are unable to decompose the complex molecular structure of the biomass into simple sugars which can then be converted into desired products biochemically. The breakage of the complex structure can be achieved by using cellulolytic enzymes, which themselves are produced by microorganisms. However, the process is beset with the low rate and low activity of lignocellulolytic enzymes on the raw biomass. This necessitates the pretreatment or preprocessing of the biomass, which may break the complex structure of the biomass molecules, increase the surface area and make the cellulosic polymers accessible to enzymes and amenable to enzymatic attack. This enhances the rate and the efficiency of the breaking down of the complexity of the biomass molecules into fermentable sugars. The pretreatment can be physical, thermal, biological, chemical, and their combinations.

Thermochemical conversion processes comprise combustion, gasification and pyrolysis of the biomass to produce thermal energy/electrical power, liquid fuel, gas, and char. These processes generally do not require much pretreatment. However, the use of densified biomass propels up the process efficiency. Densified biomass such as wood pellets, briquette, etc. can be used. Thermochemical gasification of biomass produces gas which can be used in the gas turbine to produce power or it may be upgraded through various operations downstream for use as the syngas for the production of various chemicals downstream. Chemical conversion processes involve the catalytic conversion of biomass including vegetable oils (non-edible to be used due to food security concern) and lignocellulosics to produce biodiesel and green diesel through trans-esterification and hydrothermal liquefaction.

Other than biofuels, platform chemicals from cellulosic/ hemicellulosic fractions and lignin can be produced using biochemical, thermochemical and/or chemical processes. The products may include polyols, organic acids, polymers, cyclic compounds, etc.

The present book is an attempt to make a reader familiar with biomass characteristics, treatment and conversion processes and the challenges one faces in exploiting various biomass materials. It is a comprehensive book dealing with different aspects of processing of the biomass materials for the production of biofuels and other chemicals and to tackle technical challenges associated with the processes. The book is the joint effort of the contributing experts and researchers and covers different areas including Biorefinery in General, Thermochemical, Chemical and Biochemical Conversion Processes, Algal Biorefinery, Techno-economic Assessment, Modelling, and Simulation.

The first chapter describes the general biorefinery concept. The authors have focused on the characteristics of the biomass, global distribution of biomass, conversion technologies and challenges, and the biorefinery concept. The authors have also highlighted the importance and classification of biorefineries based on feedstocks.

The second chapter deals with the biomass, its potential and applications. The authors discussed the liberal spectrum of biomass available in general and in India, in particular. This chapter also deals with the utilization of biomass under different technology pathways for various applications and operational issues. The data provided herein will be helpful in arriving at the correct technology for the use of a given biomass.

The third chapter deals with biomass gasification and sustainability assessment of biomass utilization. This chapter discusses the implication of gasification technology for all three pillars of sustainability. Section 1 discusses gasification in brief and its types. Section 2 covers discussion on sustainability and about three pillars—environmental sustainability, social sustainability and economic sustainability, through relevant studies. Section 3 provides a summary of the discussion while Sect. 4 provides the conclusion of the chapter.

The fourth chapter describes the advancement in transformation of lignocellulosic biomass to carbohydrate derived fuel precursors. The authors in this chapter have focused on carbohydrate transformation to monomers and the monomers to furanic chemical fuel precursors.

The fifth chapter deals with biodiesel synthesis using activated carbon as support of the catalysts. This chapter provides the comparison of the homogeneous, and heterogeneous catalysis and biocatalysis for biofuel production, taking into account the types of catalysts and the price factor.

The sixth chapter describes the utilization of biodiesel in compression ignition engines. The first section deals with fuel quality of biodiesel in comparison to the base diesel The effect of biodiesel on the engine performance (power and torque, brake thermal efficiency) and emission characteristics (CO, HC, NOx, and smoke) of diesel engines are also discussed.

The seventh chapter deals with the potential role of halophiles in crude glycerol based biorefinery. This Chapter provides a comprehensive summary of the recent research on the microbial assimilation of glycrerol. The use of halophiles as the viable alternatives for valorization of crude glycerol is also discussed.

The eighth chapter describes the advent of bio-jet fuel in the aviation sector. The author has addressed the emerging challenges to meet the stringent specifications of aviation fuels and to the utilization of bio-jet fuel as fuel sustainable, cost effective, green aviation fuel.

The ninth chapter deals with the pretreatment of lignocellulosic biomass for biofuel production. The Authors have described different pretreatment processes for the down steam operations and processes for the conversion of biomass materials.

The tenth chapter describes the operational strategies for enzymatic hydrolysis. This chapter gives an overview of the enzymatic hydrolysis process, the effect of pretreatment on enzymatic hydrolysis, operational strategies, the reactor design and operation as well as the recent advances.

The eleventh chapter describes an overview of the butanol tolerant microbes, their solvent survival strategies, and the techniques to overcome the problem associated with high concentration of butane in the fermentation media.

The twelfth chapter describes the simultaneous saccharification and fermentation of lignocellulosic biomass. This chapter emphasizes on various aspects of SSF viz. lignocellulosic substrates for SSF, biological agents involved and the factors effecting the process, different modes of operation for commercialization, constraints in SSF, their mitigation strategies and the major commercial products generated during fermentation in SSF.

The thirteenth chapter deals with bioalkanes as an ecofriendly and alternate fuel in bioenergy research. This chapter discusses the conversion strategies of biomass to bioalkanes and bioalkenes with special emphasis on metabolic engineering approaches along with the bottlenecks which hinder their commercial scale production as well as the possible solutions to overcome these hurdles.

The fourteenth chapter describes the algal biorefineries for biofuels and other value-added products. This chapter describes the general characteristics of microalgae, and their potential to be used as a raw material in the biorefinery process. It also focuses on the products, mainly biofuels obtained from microalgae, and different pathways employed in the biomass fractionation for other valuable products.

The fifteenth chapter describes the economic and technical viability of biodiesel production in India. This chapter discusses availability of oil bearing plants/crops, biodiesel production technologies, and the current status of technology in India.

The sixteenth chapter deals with the kinetic modeling of ethanol production for substrate-microbe system. The kinetic model proposed in this chapter provides good predictions for growth of biomass, substrate consumption and ethanol production for all types of substrate-microbe systems.

This comprehensive volume provides a holistic view of biomass as a valuable resource for energy and chemicals, and will help readers in understanding the broad fundamental principles involved in the exploitation of biomass and various operations and processes involved in the production of various chemicals. The readers are encourages to point out any error which might have crept in during the process of revision/typesetting, etc.

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Biorefining of Lignocelluloses: An Opportunity for Sustainable Biofuel Production

Pratibha Dheeran and Lalini Reddy

Abstract The depleting fossil fuel reservoirs, over-dependency of developing countries on fossil fuels to meet the day to day rising demands, global climate change by increased carbon foot prints have compelled countries to take discernible initiatives towards the use of renewable bioresources for their sustainable development. The trilema of E's (Energy, Environment and Economy) lead the global scientific community to develop policies to move from fossil-based economy to bio-based economy which is baptised as Biorefinery. Biorefineries integrate eco-friendly and more efficient technologies to cut down the rate of harmful emissions that contribute to the deteriorating environmental conditions. Though renewable lignocellulosic biomass generated via photosynthesis has the inherent potential to satiate the rising energy demands, there are technological challenges associated with the structural complexity of lignin, cellulose and hemicelluloses. From this perspective, the need of the hour is to develop a lignocellulose biorefinery platform equipped with advanced technologies to combat the challenges in unfolding of biomolecules for biofuel, power and value added chemical production. The focus of the chapter is therefore on understanding biomass structures and characteristics; distribution of biomass globally; conversion technologies and challenges; and the emerging biorefinery concept.

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1 Introduction

Energy independence has become a major issue for most nations around the globe in recent years. Each country has a unique profile in terms of energy production, consumption and its impact on the environment. The emergence of the biorefinery concept proposes the utilisation of every single component of the agro-forestry biomass for fuels and value added by-products. Like oil refineries, biorefineries involve the production of fuels, heat, power and value added chemicals from crude agro-forestry biomass.

The last two decades witnessed the extensive use of fossil fuels to meet the per capita demands of energy which ignited the debate on the challenges related to: (i) exhaustion of fossil fuel reservoirs, (ii) energy crisis in subsequent years, (iii) carbon emission and climate change. This sparked much the interest in biomass based energy production and laid out the future of bioenergy with the introduction of first generation biofuel comprising of liquid fuel production using corn, sugar beet, sugarcane juice and vegetable oils.

The first generation biofuel created the long standing controversies around the globe about the utilization of the food commodities for fuel production during 2007 and 2008 when some of the nations were battling with hiked food prices. Jean Ziegler (The United Nations Special Rapporteur) called "biofuels a crime against humanity" at the Thirtieth Regional Conference of the Food and Agriculture Organization at Brasilia (Pedro 2008; HLPE 2013). The worldwide food crisis especially in the developing countries led policies prohibiting food crops or fertile land use for biofuel productions. The food versus fuel conflict scrutinised the potential of lignocellulosic biomass (produced via photosynthesis) as renewable yet inexpensive resource.

Lignocellulose often referred to as a plant dry matter is a complex polymer consisting lignin, cellulose and hemicelluloses. It not only offers the potential for being ideal feedstock for liquid biofuels (ethanol, butanol) but has tremendous potential in gaseous fuel production as well as value-added products. Lignocellulose became the 'renewable gold' after the introduction of 'biorefinery' concept to deal with renewable energy and production of value-added chemicals. Biorefining as defined by the International Energy Agency Bioenergy Task 42 is "the sustainable processing of biomass into a spectrum of bio-based products (food, feed, chemicals, materials) and bioenergy (biofuels, power and/or heat)" (IEA Bioenergy 2009; Jungmeier et al. 2015). The biorefinery concept originated from the conventional oil refinery, where crude oil is refined into fuel, electricity and value-added chemicals.

The concept of lignocellulose-based biorefinery is gaining momentum world-wide for providing a wide spectrum of bio-based fuel and chemical productions which are conventionally produced from petroleum or petroleum feedstocks. The advantage of biorefining of lignocellulosic biomass for sustainable development of any nation is that it involves the processing of all biomass components into useful products with no wastage of unused fractions. Despite the huge potential of this niche area of refinery, the challenge is to develop efficient technologies to harness

the potential of lignocellulosic biomass. The aim of this chapter is to evaluate the potential of lignocellulose-based biorefinery strategies and challenges.

2 Availability of the Lignocellulosic Biomass: Global Scenario

The discovery of fire (symbolically representing the source of energy) and its maintenance using wood propelled the wheel of ancient human civilization towards the modern civilization and laid the foundation of industrial revolution switching from burning of wood biomass to coal. Though utilization of biomass (traditional fuel wood and agriculture residues) for heat, cooking and other purposes is not new to mankind, the impetus on harnessing the trapped biomolecules from lignocellulosic biomass for sustainable, efficient and renewable energy production has put forth a new paradigm towards the wise exploitation of available biomass (Erakhrumen 2011; Nakada et al. 2014).

Technically, biomass may be divided into forest products (fuel wood from trees or shrubs), agriculture residues (non-woody biomass such as straw, husk, stover), energy crops and animal waste (dung, etc.) (Demirbas 2009; Nakada et al. 2014). The distribution and abundance of biomass varies globally and depends on the geo-climatic conditions and utility rate in a particular region. Every continent has diverse geographic conditions which contribute in the different kind of vegetations,

Table 1 Forest biomass production

Region	Types of biomass					
	Wood fuel coniferous (m^3)	Wood fuel non-coniferous (m^3)	Wood residues (m^3)	Wood chips and particles (m^3)	Wood charcoal (tonnes)	Wood Pellets (tonnes)
Africa	17,907,160	647,635,974	1,071,205	2,246,599	32,403,254	31,000
Asia	87,626,589	641,905,519	110,299,570	57,158,251	8,845,332	1,986,100
Australia & New Zealand	29,000	4,716,064	2,577,000	12,487,000	24,269	153,000
Caribbean	166,352	4,663,056	600	500	177,774	0
Central America	27,449,375	54,553,826	799,000	260,085	182,272	27,675
Europe	53,275,699	93,400,095	72,960,207	72,767,993	634,485	16,348,716
Northern America	9,748,442	39,236,144	22,574,000	77,880,000	982,260	9,300,000
Southern America	9,956,711	167,802,456	21,765,400	20,613,112	8,896,750	113,000
Oceania	29,000	10,605,646	2,577,000	12,697,000	37,996	153,000

Source Forestry Production and Trade (2015)

Table 2 Global pattern of agricultural residues generation (Kummamuru 2016)

Crops	Region wise crop residues (Exa Joule EJ)				
	Africa	America	Asia	Europe	Oceania
Maize	4.55	30.89	17.85	6.62	0.03
Rice	0.99	1.2	21.14	0.12	0.02
Wheat	0.34	1.46	4.10	3.24	0.33
Sorghum	1.13	1.04	0.37	0.05	0.05
Barley	0.06	0.16	0.20	0.96	0.10
Sugarcane	0.74	7.68	5.78	0.00	0.24
Cassava	1.09	0.24	0.67	0.00	0.00
Coconut	0.05	0.12	1.14	0.00	0.07
Rapeseed/canola	0.01	0.46	0.56	0.78	0.10

Table 3 Total forest area continent wise

Continent	Units 1000 hectare (Ha)
Africa	626,938.53
Asia	592,570.36
Australia & New Zealand	134,595.26
Caribbean	7,104.34
Central America	86,533.92
Europe	1,015,100.39
Northern America	656,939.64
Oceania	173,219.2
South America	844,035.13

Source Land Use (2014)

agriculture patterns and forests products. (Tables 1 and 2) (Forestry Production and Trade 2015; Kummamuru 2016).

Fuel wood, wood charcoal and wood pellet are often considered as traditional source of bioenergy used by the rural sector of developing countries in cooking and heating purpose. The percentage of wood charcoal production is high in Africa, Asia and Southern America due to its consumption in domestic chores which has four-times higher energy potential as compare to wood pellets (Kummamuru 2016). As evident from the Table 3 that the largest share of wood fuel also comes from Africa and Asia. However, African continent has faced the annual decrease of 0.5% in forest area since 2000–2014 while the increment of 0.09 and 0.34% has estimated in European and Asian forest area, respectively. Among the top 5 largest forest resources possessing countries, Russian forest reserve estimates were found considerably higher in comparison to Brazil, Canada, USA and China with the total forest area 815,013 (1000 Ha) (Kummamuru 2016).

Forest biomass has been considered as the largest contributor in bioenergy sector, whereas, agricultural residues contribute only 9% of the total biomass supply with high potential of 123 EJ in biofuel production (Kummamuru 2016; World Energy Resources, Bioenergy 2016; Kummamuru 2017). Modern

technologies made it possible to switch over from traditional use of biomass to modern biomass utilization which emphasize on sustainability of biomass and less carbon emission such as biomass conversion to liquid biofuels or combined heat and power generation (World Energy Resources, Bioenergy 2016).

The studies on traditional biomass versus modern biomass utilization reflect that the developing countries from Sub-Sahara African regions, Southern Asia and South-Eastern Asia predominantly utilize the traditional biomass and the reason is obvious that the poor rural parts of these countries heavily rely on the fuel wood and wood charcoal as these are easily accessible at nominal cost (Table 4). Kenya largely depends on biomass (68%, includes crop residues), where fuel wood provides almost 90% of the energy in rural areas, in approximately equal shares traded as wood and charcoal. Sustainable wood yields meet only 43% of the total demand. In Malawi, biomass accounts for 97% of total primary energy supply, of which 59% is used in its primary form as firewood (52%) or residues (7%), and 41% are converted into charcoal. More than 80% of the wood consumption goes into private households and 98% of all households depend on it (Black et al. 2010).

Agro-forestry biomass comprising of lignocelluloses has tremendous potential for the production sustainable biofuel to avoid the food versus fuel conflict. Despite of this fact, every year thousands of tons of biomass are generated which is used in cooking by residential sector of rural areas or in generating heat for boilers by small-scale industries and the leftover biomass is either dumped at the landfill sites or burnt down in the fields (World Energy Resources, Bioenergy 2016). The strategic planning with adequate data assessment on biomass generation, utilization and leftover surplus biomass will be an advantageous approach for the formulation and implementation of lignocellulose-based biorefinery.

Table 4 The percentage share of traditional biomass versus modern biomass (World Energy Resources, Bioenergy 2016)

Region	Traditional biomass use (%)	Modern biomass use (%)
Africa		
Sub-Sahara region	65.3	9
Northern Africa	2.5	1
Asia		
Central Asia	0.4	0.4
Eastern Asia	10.4	0
South Eastern Asia	23.4	6
Southern Asia	26.7	6
Western Asia	0	2
Europe	0.3	6
Northern America	0	3
Oceania	4.3	5
Southern America and Caribbean	5.1	12

3 Chemical Characterization of Lignocellulosic Biomass

It's important to understand the peculiar physical and chemical structure of lignocellulosic biomass to have an insight for designing the bioprocess. Lignocellulose is a complex structure consisting of lignin (complex of organic polymers phenylpropanoid), cellulose (an unbranched homopolysaccharide consisting of D-glucopyranosyl units) and hemicelluloses (heteropolymer consisting of xylan, glucuronoxylan, arabinoxylan, glucomannan, and xyloglucan) (Narayanaswamy et al. 2013). Agriculture (wheat straw, rice straw, corn stalk, sugarcane bagasse, etc.) and forestry (wood) biomass consist lignocelluloses in varied ratios. The general composition of lignocelluloses in agro-forestry biomass is accounted as 40–50% cellulose, 20–30% hemicellulose and 10–25% lignin (Anwar et al. 2014). Each component has its unique structure which makes them resistant against degradation.

Cellulose is a building block of plant cell walls which provides the mechanical strength. This is the most abundant biopolymer available in the nature having a molecular formula $(C_6H_{10}O_5)_n$. It's a linear homoploymer consist of glucose units linked by β(1→4) glycosidic bonds with high degree of polymerization (approximate native degree of polymerization of 10,000–15,000) (Yang et al. 2011). Naturally, cellulose occurs in two forms i.e. crystalline form and amorphous form (Narayanaswamy et al. 2013). The cellulose molecules are held together in plant cell wall by intermolecular hydrogen bonding, however, its tendency to form inter as well as intramolecular hydrogen bonding contributes to the rigidity and make it resistant against cellulolysis (break down of cellulose into basic units and cellodextrins) in organic solvents and water (Fig. 1).

Hemicellulose is the second most abundant renewable biopolymer available in agro-forestry biomasses. It's a branched heteropolymer comprises of pentose (D-xylose, D-arabinose) and hexose (D-glucose, D-mannose, and D-galactose) sugars and their acidified derivatives such as glucuronic and galacturonic acids (Fig. 2) (Narayanaswamy et al. 2013; Yang et al. 2011). As compare to cellulose, degree of polymerization in hemicelluloses is 500–3000 sugar units. The composition of hemicelluloses varies from trees to grass. The hardwood consists mainly of xylan and glucomannan, while, softwood contains small fraction of xylan and predominantly rich in galactoglucomannan (Narayanaswamy et al. 2013; Agbor et al. 2011). Among the hemicellulosic components, xylan is important substrate for bioenergy and bio-based chemicals production. Xylan composed of a backbone chain that consists of a varying number of β-1,4-D-xylopyranosyl residues (70–130 in softwood xylan and 150–200 in hardwood xylan) (Dheeran et al. 2012).

Next to cellulose and hemicelluloses, lignin is the third largest heteropolymer occurs in all dryland plant cell walls. It provides the mechanical strength to the plants and protects them in water conduction. The unique feature of lignin which differentiates it from cellulose and hemicelluloses is the presence of aromatic monomers. Lignin is devoid of sugar monomers and composed of three different phenyl propane monomers: (a) coniferyl alcohol, (b) coumaryl alcohol and

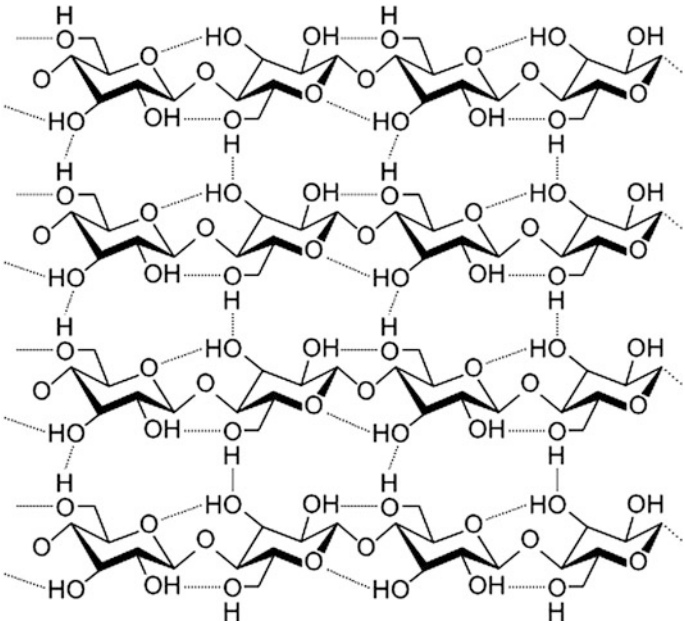

Fig. 1 A cellulose strand showing inter and intra-molecular hydrogen bonding (dashed line)

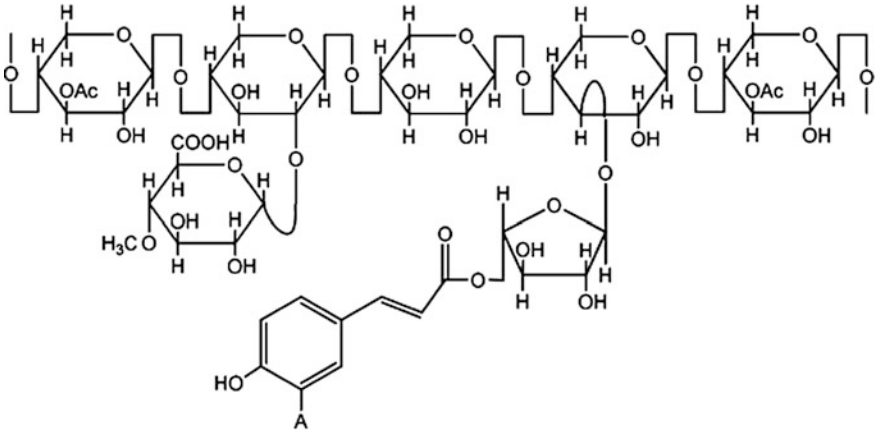

Fig. 2 A representative part of hemicellulose structure

(c) syringyl alcohol (Fig. 3). The distribution of these monomers differs species to species for example hardwood trees contains syringyl alcohol, whereas, the coniferyl alcohol predominantly is found in the conifers (softwood trees) (Narayanaswamy et al. 2013; Anwar et al. 2014). Lignin is an important constituent of plant cell wall to provide the strength to the plants but it has become the biggest

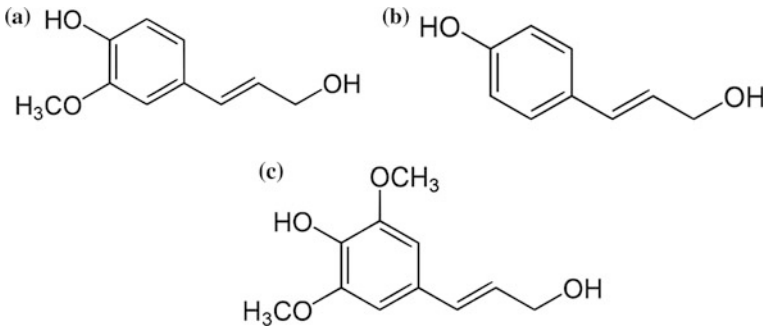

Fig. 3 Structure of three phenyl propane monomers. **a** Coniferyl alcohol, **b** coumaryl alcohol and **c** syringyl alcohol

hindrance in accessing the sugars trapped in cellulosic and hemicellulosic complex for biofuel processes (Narayanaswamy et al. 2013; Chapple et al. 2007).

4 An Overview of Selective Conversion Technologies for Biomass Processing

Lignocellulose-based biorefinery is an integrated approach of upstream, midstream, and downstream processing of lignocelluloses biomass into a range of products. It can utilize all kind of forestry and agriculture biomass. Biofuel and value-added chemical production from lignocellulosic waste is technically more challenging as compared to first generation biofuel which was produced mainly from corn, sorghum grains, sugarcane juice and beet. Production of biofuels and biochemicals includes an intensive process of pretreatment, hydrolysis and microbial fermentation. Lignocellulose is a complex structure as discussed in previous section which needs effective pretreatment in order to release the sugar monomers. The aim of pretreatment is to alter the structure to have the access to cellulose and hemicellulosic fractions. Several physical, chemical and physico-chemical approaches have been applied to develop the cost-effective pretreatment technologies but none of the technology can be considered as the cost effective and environment friendly. Some preferably selective pretreatment methods are discussed here which are frequently used for lignocellulosic biomass degradation.

4.1 Physical Methods

Mechanical treatment is necessary to reduce the particle size of biomass which includes milling and grinding. Several other physical treatments like irradiations (γ

rays, microwave and electron beam) and ultrasonication are also used for pretreatment at laboratory-scale but are not much preferable methods for full-scale operations because of high energy inputs and capital investment (Menon and Rao 2012).

4.2 Chemical Methods

Chemical pretreatment is the most widely used process which includes several methods to disrupt lignocellulosic structure.

4.2.1 Acid Hydrolysis

Weak and strong acid hydrolysis is considered as the most effective and preferred methods over other physico-chemical and thermo-chemical methods.

Dilute acid hydrolysis: Dilute acid treatment uses mild concentrations of sulphuric acid (0.5–1.0%) at moderate to high temperatures and performed in two ways:

- Temperature below 160 °C and high biomass loading (10–40%) for batch processes
- High temperature (160 °C or above) with low biomass loading i.e. 5–10 wt% for continuous process.

Dilute acid treatment often dissolves the lignin contents partially and hydrolyses the hemicellulosic fraction which releases the pentose monomeric sugars into hydrolysate. These treatments increase the porosity and give the access to the cellulolytic enzymes for enzymatic hydrolysis of cellulose. This process is advantageous in terms of recovering the hemicelluloses based sugar monomers but the main disadvantage associated with the generation of furfural and hydroxymethyl furfural which act as inhibitors during microbial fermentation process and lower the ethanol yield (Taherzadeh and Karimi 2007; Yu et al. 2010; Brodeur et al. 2011; Menon and Rao 2012).

Concentrated acid hydrolysis: In 1819, Braconnot discovered that cellulose can be hydrolysed by concentrated sulphuric acid or hydrochloric acid into fermentable sugars. Concentrated acid hydrolysis process carries out with high acid concentrations (30–70%) at ambient temperatures (35–40 °C). Unlike dilute acid treatment, this process yields higher amount of fermentable sugars (90% of theoretical glucose yield). Although, this process is the most preferred method till date for lignocellulosic biomass hydrolysis but this method is not considered as an environment friendly. The use of high concentrations of acid is corrosive in nature and needs non-metallic or expensive alloy vessels. Further issue is the production of high amount of gypsum during neutralization process of the hydrolysate which

needs proper dumping. The impact of concentrated acids during process is neither environment friendly nor economic and requires the high maintenance cost to recycle the acids (Taherzadeh and Karimi 2007; Yu et al. 2010; Brodeur et al. 2011; Menon and Rao 2012).

4.2.2 Alkaline Pretreatment

Alkaline pretreatment effectively works in the removal of lignin. This pretreatment can be performed using calcium or sodium hydroxide and ammonia, sodium carbonate at the temperature ranges 25–200 °C depending on the type of feedstock to be hydrolysed. Alkaline pretreatment is effective in delignification, partial decrystallization and solvation of cellulosic and hemicellulosic fractions. This pretreatment deliginifies the biomass and gives access to the hydrolytic enzymes for the further degradation of hemicelluloses and cellulose (Kim et al. 2015; Bali et al. 2014). This method is effective for the pretreatment of corn stover, switchgrass, bagasse, and wheat and rice straws. Although Bali et al. has reported the hydrolysis of *Populus* by using sodium hydroxide, calcium hydroxide and ammonia at different time interval keeping the temperature constant 120 °C.

4.2.3 Organosolv Pretreatment

Organosolv pretreatment is the process, where an organic solvent or mixtures of organic solvents is used with water for the removal of lignin before enzymatic hydrolysis of the cellulose fraction. Besides the removal of lignin, hemicellulose hydrolysis also occurs which facilitates the enzymatic digestibility of the cellulose fraction. Ethanol, methanol, acetone, and ethylene glycol are some common solvents used in this process. The reaction condition varies on the utilization of type of biomass and catalysts, where temperatures can be setup from ambient to 200 °C. Organosolv pretreatment is advantageous in biorefineries, where high quality lignin can be separated and converted into value-added chemicals and the cost of cellulolytic enzymes used for the hydrolysis of cellulosic fraction can be reduced. The only disadvantage of this process is solvent inhibition for further enzymatic hydrolysis and interference in fermentation process too. Therefore, removal and recovery of the solvent requires high maintenance cost and cannot be considered an economic process at commercial-level (Akhtar et al. 2015; Zhang et al. 2016).

4.2.4 Liquid Hot Water

Liquid hot water (LHW) pretreatment, hydrothermolysis, hydrothermal pretreatment, aqueous fractionation, aquasolv, solvolysis or autohydrolysis is a process for biomass pretreatment which is carried out with water at high temperature and pressure. Temperatures can range from 160 to 240 °C which depends on the type of

feedstock used for the hydrolysis e.g. autohydrolysis of corn fibre can be carried out at 140–180 °C but some feedstocks such as hardwood are processed at higher temperatures (190–230 °C). About 40–60% of the total biomass is dissolved in the process, with complete solubilisation of the hemicelluloses (recovered as monomeric sugars), 4–22% of the cellulose and 35–60% of the lignin. Cellulose can be recovered as solid fractions and further subjected to enzymatic hydrolysis. Temperature and reaction time play important role to avoid the inhibitors formation such as furfural and 5-hydroxymethyl-2-furaldehyde (HMF) formed by the degradation of pentose and hexose sugars, respectively. Yu et al. (2010) reported the autohydrolysis of rice straw at 180 °C (low end of temperature range) for 30 min (moderate to high time scale range) with minimal inhibitors generation (Yu et al. 2010; Brodeur et al. 2011; Menon and Rao 2012). The advantage of this process is that there is no requirement of acids, solvents or catalysts for the hydrolysis, hence, neutralization and recovery steps which require high capital investment can be avoided. However, there is necessity of high energy input to maintain high temperature and pressure for the process. Denmark based biomass refinery Inbicon established a demonstration facility based on hot-water pretreatment technology to demonstrate 4 ton/h of continuous operation at industrial-scale (Zhang and Shahbazi 2011).

4.2.5 Oxidative Delignification

Some oxidising agents such as hydrogen peroxide, ozone, oxygen or air have the property of delignification. These oxidizing agents have high reactivity with the aromatic rings of lignin which convert it into carboxylic acids. The acids formed during pretreatment process further interfere into fermentation process and need hydrolysate neutralization which can be done by washing with the stream of water at room temperature (Bensah and Mensah 2013; Akhtar et al. 2015). In addition to oxidation of lignin, oxidative pretreatment partially hydrolyses the hemicellulose fraction of the lignocellulose complex. Some of the preferred oxidation methods are as follows:

Ozonolysis

The cleavage of aromatic rings of lignin complex via ozone pretreatment is termed as oznolysis. Ozone treatment primarily targets aromatic rings structure of lignin, while hemicellulose and cellulose fractions remain intact and hardly decompose. Removal of lignin facilitates the enzymatic hydrolysis of hemicelluloses and cellulose. The process is advantageous for its ambient operational conditions (room temperature and normal atmospheric pressure) which do not require high energy input to maintain temperature and pressure. However, the ozonolysis requires large amount of ozone, thus, makes this process expensive. Ozonolysis has been studied to degrade lignin in various lignocellulosic materials such as wheat straw, bagasse, peanut, pine, cotton straw, and poplar sawdust (Bensah and Mensah 2013; Behera et al. 2014; Akhtar et al. 2015).

Wet Oxidation

Wet oxidation (WO) is an oxidative pretreatment method which utilizes oxygen or air at elevated temperature and pressure. Wet oxidation is considered as an alternative to steam explosion which became the most widely used pretreatment method in recent years. Earlier, wet oxidation processes have been used for the treatment of wastes consisting of high organic matter by oxidation of soluble or suspended materials at high temperatures (150–350 °C) and high pressure (5–20 MPa). Wet oxidation pretreatment oxidizes the phenolic structure of lignin and partially hydrolyses the hemicellulosic fraction into the intermediates such as carboxylic acids, acetaldehydes, and alcohol. The rate of oxidation is high at elevated temperature, pressure and catalysts. Alkaline wet oxidation was found to be effective while considering the formation of inhibitors such as furfural and HMF as compare to acid hydrolysis. Wet oxidation has been successfully studied in several lignocellulosic biomass like wheat straw, rice husk and hardwood. Compared to other pretreatment processes, wet oxidation has been proven to be efficient for the pretreatment of lignocellulosic materials because 90% of the lignin is removed and hemicellulose gets solubilised while the cellulose remains as solid fraction which facilitates the enzymatic hydrolysis (Martin et al. 2008). The reported advantage of the wet oxidation process is the lower production of furfural and HMF, which are potential inhibitors in the fermentation process but the high operating cost associated with the process cannot be ignored (Bensah and Mensah 2013; Behera et al. 2014).

4.2.6 Ionic Liquids (ILs)

Ionic liquids often considered green solvents, are the salts exist in the liquid phase at room temperature and can dissolve cellulose efficiently under mild operating conditions. ILs have low melting points (<100 °C), high polarities and high thermal and chemical stabilities (Zhang and Shahbazi 2011; Behera et al. 2014). This method has been applied to modern fiber making industry, where cellulose is directly dissolved by using ILs. ILs dissolve cellulose by breaking the hydrogen bonds between molecular chains of the cellulose strands. Electron donor-electron acceptor complexes are formed by the ILs by interacting with hydroxyl groups of cellulose. This decreases the crystallinity of cellulose which gives access to the cellulolytic enzymes for further hydrolysis. Recent studies have been reported the application of imidazonium salts (*N*-methylmorpholine-*N*-oxide mono-hydrate (NMMO), 1-*n*-butyl-3-methylimidazolium chloride (BMIMCl), 1-allyl-3-methylimidazolium chloride (AMIMCl), 3-methyl-*N*-bytylpyridinium chloride (MBPCl) and benzyldimethyl (tetradecyl) ammonium chloride (BDTACl)) for the pretreatment of cellulosic biomass (Brandt et al. 2013; Sochaa et al. 2014). Pretreatment of cellulose with ILs is advantageous as they can be used under ambient conditions and the formation of inhibitors is almost negligible. Although

ILs are considered environment friendly chemicals, their high cost impedes their utilization at industrial-level, where tons of biomass needs to be pretreated (Brandt et al. 2013; George et al. 2015).

4.3 Physico-chemical Methods

4.3.1 Steam Explosion

Steam explosion is one of the most preferred pretreatment method amongst chemical and physico-chemical methods owing to its low use of chemicals and less energy consumption. During pretreatment process the biomass is treated with high-pressure saturated steam at high temperature from 160 to 260 °C and pressure 0.69–4.83 MPa (Kumar et al. 2009; Behera et al. 2014). Subsequently, the reduction in pressure creates the explosive decompression of the biomass which results in disruption of lignin matrix and degradation of hemicellulosic fraction. Efficiency of steam-explosion pretreatment depends on the residence time, temperature, particle size of the biomass and moisture content. Studies have been carried out to try to improve the results of steam explosion by the addition of sulfuric acid (Linde et al. 2008). Steam explosion pretreatment is found to be the cost effective and advantageous due to low energy requirement. The conventional mechanical methods require 70% more energy than steam explosion to achieve the same particle size reduction. The only disadvantage associated with steam explosion is the formation of degradation products that may inhibit downstream processes (Behera et al. 2014).

4.3.2 Ammonia Fibre Explosion (AFEX)

In ammonia fibre explosion pretreatment process, biomass is treated with liquid ammonia at high temperature (60–100 °C) and pressure (250–300 psi) and after few seconds, pressure is reduced slowly (Chaturvedi and Verma 2013). The conventional AFEX process is carried out with 1–2 kg ammonia/kg dry biomass at 90 °C for 30 min. AFEX efficacy depends on the operating conditions such as ammonia loading, temperature, water loading, blow down pressure, reaction time and number of treatments. AFEX pretreatment alters the lignocellulosic structure by reducing the lignin content and lowering the crystallinity of cellulose. The advantage of this pretreatmnet is: no inhibitors formation takes place, does not require conditioning for fermenting microbes, gives about 99% recovery of sugars, and does not require addition of nitrogen source for fermentation as residual ammonia serves as a nitrogen source (Zhang and Shahbazi 2011; Brodeur et al. 2011; Menon and Rao 2012; Behera et al. 2014).

4.3.3 CO_2 Explosion

CO_2 explosion is similar to steam and ammonia fibre explosion. CO_2 is used as supercritical fluid at high pressure and then liberated by an explosive decompression which disrupt the lignocellulosic matrix and facilitates the enzymatic hydrolysis. It is postulated that CO_2 reacts to carbonic acid (dissolved carbon dioxide in water), and catalyze the hydrolysis of biomass. Although, carbonic acid catalysis offers the benefits over the use of acids like sulphuric acid, but sugar yields from CO_2 explosion is lower than those obtained with steam or ammonia explosion. However, it's found to be higher than those obtained with enzymatic hydrolysis without pretreatment. This pretreatment may be advantageous for the bioprocess industries produce carbon dioxide and carbonic acid may be utilized as viable reagent for biomass hydrolysis without using mineral acids (Brodeur et al. 2011; Menon and Rao 2012; Behera et al. 2014; Akhtar et al. 2015).

4.3.4 Combined Mechanical/Alkaline Pretreatment

A combined mechanical and alkaline pretreatment includes continuous mechanical pretreatment (e.g. milling, extrusion, refining) of lignocellulosic biomass with the aid of an alkali effectively solubilises the lignin fractions while leaving cellulose remains in the solid fraction. The cellulose fraction can easily be recovered for enzymatic hydrolysis. By performing extrusion in combination with chemical pretreatment in a single step, the accessibility of cellulose for cellulolytic enzymes is improved, which results higher delignification values and improves enzymatic hydrolysis of pretreated biomass. Moreover, the moderate operation temperature in this process prevents the formation of degradation and oxidation products such as furfural and HMF. The combined mechanical and alkaline pretreatment increases the efficiency of the pretreatment compared to alkaline pretreatment alone. However, the expensive chemicals used in the process require the recycling to make the process economically viable (Harmsen et al. 2010; Wang et al. 2014).

4.4 Biological Pretreatment

Biological pretreatment deals with microbial assisted biomass degradation. Microorganisms such as white, brown and soft-rot fungi have been successfully employed to degrade lignocellulosic biomass. Biological pretreatments are environment-friendly and advantageous over other pretreatment methods because it requires low energy, can be operated at ambient conditions. Biological pretreatment is getting wider attention in consolidated bioprocessing, therefore, present research studies are focused on the exploration of new fungi and bacteria which can

efficiently degrade the lignin, hemicelluloses and cellulose fractions with simultaneous saccharification to make the process cost effective (Menon and Rao 2012; Narayanaswamy et al. 2013).

5 Biorefining of Lignocellulose: Biorefinery Concept and Strategies

Biomass-based biorefinery concept emerged to harness every bit of component present in biomass to useful products including biofuels, platform chemicals, heat/power, etc. with an idea of sustainable human development to deal with the trilemma of fuel, food and environmental sustainability issues. Biorefinery encourages zero waste discharge which facilitates not only economical production but also resolves the waste disposal issue. Figure 4 depicts "the sustainable processing of biomass into a spectrum of bio-based products (food, feed, chemicals, materials) and bioenergy (biofuels, power and/or heat)" (Fig. 4) (Jungmeier et al. 2015; IEA Bioenergy 2009). Biorefinery concept follows the crude oil refinery, where crude oil is refined for transportation fuel, electricity and high value-added petroleum products (Fig. 5). Unlike oil refineries, biorefineries use inexpensive renewable feedstocks such as forest biomass, agricultural residues, energy crops, aquatic biomass (macro-algae) and ensure the sustainability of the biomass to avoid the land and water conflicts. The trapped biomolecules in these feedstocks mainly hexose/pentose sugars, triglycerides and other phenolics are then processed for the generation of power, biofuels other value-added chemicals via a series of physical, thermo-chemical and biochemical processes (Fig. 5) (Cherubini 2010; Jong and Jungmeier 2015).

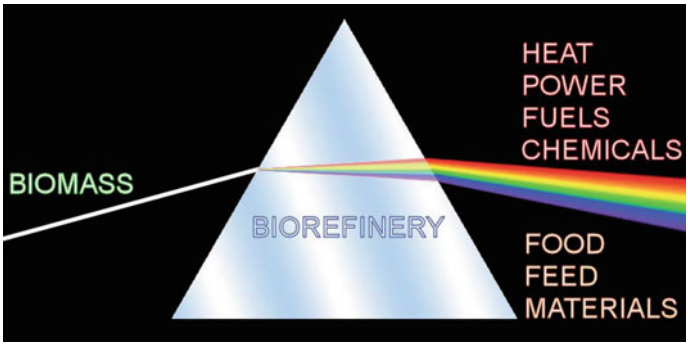

Fig. 4 Biorefinery spectrum (the figure is reproduced with prior permission from the authors of IEA Task 42) (IEA Bioenergy 2009)

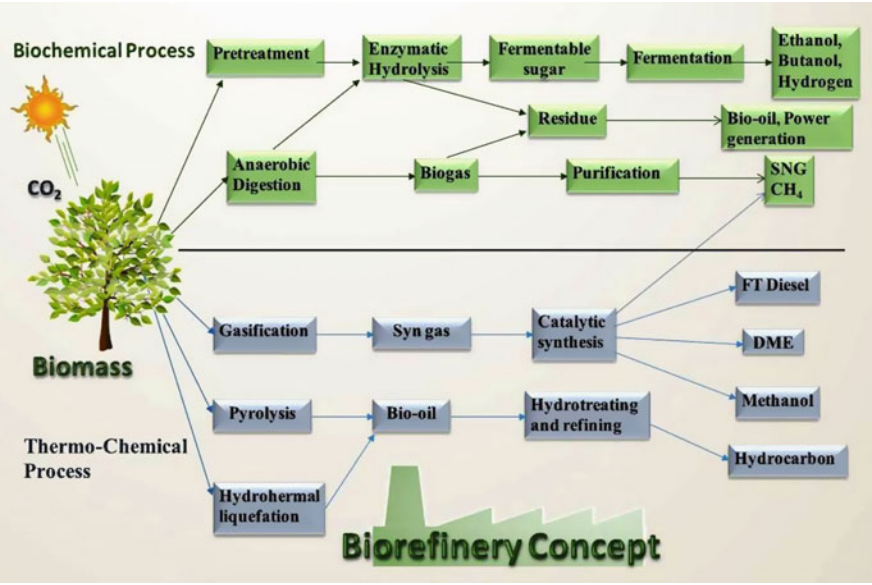

Fig. 5 Illustration of lignocellulose based biorefinery

5.1 Biorefinery Classification and Processes

- The advanced biorefinery is an amalgamation of a series of upstream, mid-stream, and downstream processing of biomass to produce renewable biofuels and chemicals which can utilize a wide range of agro-forestry and marine feedstocks by implementing mechanical, chemical, thermo-chemical and bio-chemical conversion technologies coupled with microbial fermentations (Demirbas 2010; IEA Bioenergy 2009; Jong and Jungmeier 2015; van Hal et al. 2014). Thus, biorefineries are classified on the basis of given criteria:
- Energy and products (Generations): It deals with the generation of biofuels (bioethanol, biodiesel and synthetic biofuel) and value-added products (chemicals, food and feed, and materials) and classified further in first generation, second generation, third generation and fourth generation biorefinery.
- Feedstocks: Utilization of energy crops such as starch crops, short rotation forestry and biomass residues from agriculture, forestry, trade and industry (straw, bark, wood chips from forest residues, used cooking oils, waste streams from biomass processing)
- Biomass conversion processes: Implementation of the conversion processes such as mechanical, thermo-chemical, biochemical and fermentation technology to convert the biomass into energy and bio-based chemicals.

Table 5 Classification of biorefineries (IEA Bioenergy 2009; Demirbas 2010; González et al. 2015; van Hal et al. 2014)

S. no.	Biorefinery	Classification criteria	Feedstocks	Products
1.	Conventional biorefineries	First, second, third and fourth generations (Gen) biorefineries	Starch biomass, vegetable oil, corn stover, wheat straw wood waste, energy crops and algae	Bioethanol, biodiesel, bio-oil, syngas, biogas, Fischer–Tropsch (FT) diesel
2.	Feedstock based biorefineries	Green biorefinery, Whole crop biorefinery, lignocellulose biorefinery (includes forest biorefinery)	Grasses, starch crops, saw dust, wood chips, tree bark, lignocellulosic agriculture and forest waste, paper pulp industry waste	Bioethanol, Biodeisel, platform chemicals, heat and power
3.	Marine biorefineries	Seaweed biorefinery	Green, brown and red seaweeds	Bioethanol, bulk chemicals and feed
4.	Conversion based process biorefineries	Gasification, pyrolysis, hydrolysis, fermentation	Agro-forestry biomass, urban waste	Syngas, dimethyl ether, biodiesel, hydrogen, FT diesel, bioethanol

- Biorefinery Platform: Platform are the key intermediates generates during the bioprocessing of raw materials to the end products such as C5 and C6 sugars, syngas, lignin, and pyrolytic liquid (Table 5).

First generation biofuel production from corn and cereal grains falls into two phases. Phase-I biorefinery deals with limited production of bioethanol and co-products due to process associated technological challenges, whereas, Phase-II biorefinery deals with the production of ethanol and various co-products like gluten feed, high fructose corn syrup (HFCS), starch, dextrose, gluten meal, and corn oil using the corn as feedstock (Jong et al. 2015; Luo et al. 2010).

Phase-III biorefinery is an extension and advanced form of biorefinery concept which can utilize various types of lignocellulosic as well as marine algae as an alternate feedstock to produce biofuels, variety of chemicals and intermediates by implementing different processing methods (Jong et al. 2015; Luo et al. 2010). Phase-III biorefinery combines the whole-crop, green and lignocellulosic feedstock (LCF) biorefineries. A whole-crop biorefinery uses the entire crop to process into useful products. Green biorefinery consists of multi-product system which utilizes natural wet feedstocks such as grass, green plants or green crops whereas lignocellulose biorefinery utilize multiple agriculture (wheat straw, rice straw, corn stover, sugar cane bagasse) and forestry (wood waste) waste hence solely depends on the availability of the surplus biomass. Besides the utilization of terrestrial

biomass, phase III biorefinery envisaged the potential of marine biomass often called as seaweed such as kelp, red macroalgae and green macroalgae for being rich in lipids and carbohydrates (Demirbas 2010; González et al. 2015; van Hal et al. 2014).

IEA Bioenergy Task 42 developed a more pertinent classification system which illustrates the full biomass to end-product chains and divided into two categories (Jungmeier et al. 2015):

- **The Energy-driven Biorefinery**: production of biofuels, energy and value-added co-products.
- **The Product-driven Biorefinery**: production of food/feed/chemicals/materials by biorefinery processes. Side-products are used for the production of secondary energy carriers (power/heat) both for in-house applications as well as for distribution into the market.

LCF-based biorefinery is getting preference over other biorefineries for several reasons: (a) feedstock sustainability throughout the year, (b) renewable and inexpensive, (c) doesn't fall in food, land, and water controversies. Lignocellulose comprises of hexose/pentose sugar rich cellulosic and hemicellulosic components which requires an extensive conversion processing in order to release the trapped carbohydrate. The conversion technologies include mainly series of (a) mechanical, (b) chemical, (c) thermo-chemical and (d) biochemical processes, where lignocellulosic biomass or wastes depolymerise into pentose/hexose sugars which are further utilized to produce useful products such as biofuels and chemicals. On the other hand, lignin utilizes as a fuel for direct combustion to generate steam and electricity (Fig. 6) (Kamm and Kamm 2004; Luo et al. 2010).

5.2 Current Scenario

The history of ethanol production from sugarcane dates back to 6000 BC, whereas, lactic acid production at industrial level was developed by A. Boehringer in Germany in 1895. Thus, biorefinery is not a new concept but is an advanced extension of conventional industries e.g. paper and pulp or sugar industries, where biomass is converted into sugar, molasses, alcohol and papers. The new concept of advanced biorefineries emerged to deal with the trillema of Energy, Environment and Economy is still in its nascent phase working either at R&D or pilot-scale. Despite of the steady growth, and technical challenges in terms of process development, low yield bio-based biorefineries are working on the development of bio-based platform chemicals and biofuels such as: succinic acid, lactic acid, levulinic acid, diphenolic acids, bioethanol, biomethane, etc. Some of the existing commercial biorefinery initiatives summarise below in Table 6:

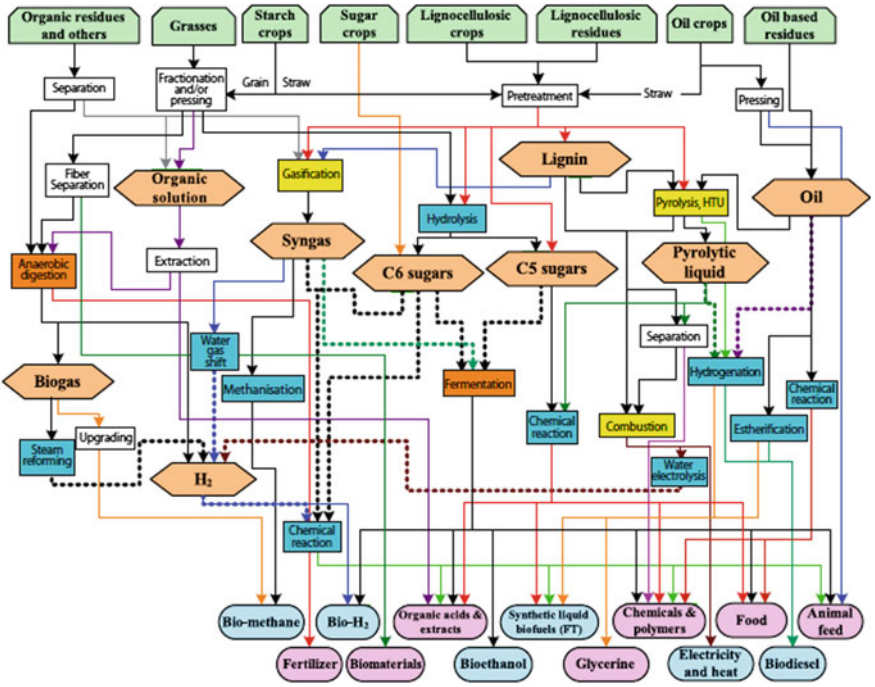

Fig. 6 An overview of the platforms, energy/products, feedstocks and conversion processes based biorefinery classifications (IEA Bioenergy 2009) (the figure is reproduced with prior permission from the authors of IEA Task 42)

Table 6 A summary of existing commercial biorefinery initiatives (IEA Bioenergy 2009; IEA Bioenergy Task 42) (permission has been taken from the authors to reproduce the table)

Country	Feedstock	Product
Austria		
Lenzing AG	Fibre and pulp	Furfural, acetic acid, sodium sulphate, potassium-lignin-sulphate
Danisco	Waste water of pulp and paper industry	Xylose
Canada		
Ensyn	Agricultural and wood residues	Bio-oil, charcoal, food flavours, adhesive resins, green gasoline, diesel and jet fuels
Tembec	Pulp mill biomass	Ethanol, acetic acid, phenol-formaldehyde resins and lignosulfonates
Dynamotive	Waste sawdust/recycled lumber	Bio-oil, char
Nexterra/Tolko	Wood residue	Heat energy

(continued)

Table 6 (continued)

Country	Feedstock	Product
Denmark		
Daka Biodiesel	Fat from slaughterhouses	Biodiesel, glycerol and potassium sulphate
France		
Roquette	Wheat, potato, maize, pea straw	Starch, food, feed, bulk and fine chemicals, succinic acid, ethanol
Tembec, Smurfit	Wood	Cellulose, paper, tall oil, lignosulfonates, electricity, steam
ARD, Cristal Union, Chamtor	Wheat, sugar beet	Food, feed, ethanol, succinic acid, cosmetics, electricity
Germany		
Südzucker	Sugar, grain	Sugar, palatinose, food additives, feed, ethanol, biogas, electricity
Zellstoff Stendal	Wood	Cellulose, paper, tall oil, methanol, turpentine, electricity, steam
Emsland-Stärke GmbH, Wietzendorf	Whole crop biorefinery (potato starch and biogas) demonstration and commercial	Potato starch and biogas
CropEnergies	Sugar, grain	Ethanol, DGGS, electricity

6 Conclusion

Lignocellulose-based biorefineries with integrated conversion processes in analogy with oil refinery have promising future to produce bioenergy and biochemicals. Transportation fuel and related high oil prices are the main driving force behind the development of advanced biorefineries concept. Lignocellulose-based biofuel driven biorefineries are the most promising approach towards production of cost effective biofuels by the adoption of efficient conversion technologies for multiple feedstocks. Presently the lignocellulose biorefineries are in its nascent phase to prove its potential in real commercial world but the continued development will inevitably arise the opportunities for the sustainable human development.

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Biomass, Its Potential and Applications

Rakesh Kumar Maurya, Amit R. Patel, Prabir Sarkar, Harpreet Singh and Himanshu Tyagi

Abstract India is an agrarian country with the abundant availability of biomass. Therefore, it is essential to make the best use of the biomass potential. It is indispensable to effectively use this resource so that the problem of energy could be eased. In order to accomplish this objective, an effective and efficient utilization of the biomass is the key. Biomass is the only source of energy that stores carbon into its structure and releases back into the environment upon the use. Uses of biomass for different application results into various operational, environmental, financial and even logistic issues. The aim of this chapter is to make the reader aware of the liberal spectrum of biomass available in general and India in particular. This chapter also presents the utilization of biomass under different technology pathways for various applications and operational issues are discussed. The data is helpful to arrive at the correct technology alternate for the use of a given biomass type.

Keywords Biomass · Renewable Energy · Gasification · Pyrolysis
Fuel

1 Introduction

Humans have been using biomass as a prime source of energy for a very long period of time, in fact, biomass was the first ever source of energy human had used. The innovation of the steam engine in 1698 by a British engineer 'Thomas Savery', made coal as an important fuel option. The constant pursuit of technology to get quicker transportation alternate lead to the search of cheap fossil fuel sources, which was

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successful by exploring cheap and abundant oil discovery in the twentieth century. This made petroleum, coal, natural gas based refinery a prime activity for economic consumption of fossil fuel. A significant portion of the global energy requirements depends on these choices. Merchandise such as fuel, fine chemicals, pharmaceuticals, synthetic fiber, plastics, fertilizers and so on, to meet the growing demand, are formulated and employed for solving human need. Wild and inappropriate use of fossil fuel gave birth to large-scale production of carbon dioxide and other harmful gases, at the scale that is several times larger than the capacity of the earth to absorb as a natural sink. Slowly the world started facing the bitter reality of global warming. Today we are in the transition towards the warm earth, due to the greenhouse effect caused because of the excessive use of fossil fuel. The earth is on the cross road to two distinct possible future scenarios. The most optimistic scenario is an environmental abatement of greenhouse gases, whereas irreversible degradation of the global environment is the harsh reality we are drifting towards.

2 Biomass as Renewable Energy Alternative

Large emissions of greenhouse gases accumulated in the atmosphere contributes to the problem of global warming. This situation made renewable energy attractive, particularly biomass. Thus, in that respect there is a renewed interest in the production and use of fuels from plants and organic waste (Naik et al. 2010). Biofuel when produce from the plant or organic waste will help cut dependence on already depleting fossil fuel reserves and avoid the production of greenhouse gases, and help mitigate global warming. Comparing the carbon cycle of fossil fuel and biomass, it can be noted that the production of biofuel is sustainable and therefore follows a closed cycle, while production and use of fossil fuel leads to the accumulation of carbon dioxide in the environment, which is a principal cause of global warming (Kavalov and Peteves 2005). This non-sustainable mode of energy production is one of the causes of environmental degradation.

Biomass is the oldest, and as on date, a major supply of renewable energy. It is the only renewable energy alternative that holds the capacity to absorb the carbon from the environment. Biomass has been a major resource of bioenergy that can offset the economic consumption of fossil fuel and mitigate the environment from the global warming crisis. Currently, biomass is the fourth largest source of energy after coal, oil, and natural gas (Ladanai and Vinterbäck 2009). Various conversion techniques such as physical, thermal, chemical and biological schemes have been utilized in order to produce utilization of biomass as energy resources. Amongst all other fossil fuel options, biomass is observed to have the shortest span of growth and formation of fuel, as it can be viewed from the Fig. 1. Petroleum, natural gas and coal take a longer period to get converted into fuel, this makes them nonrenewable in character. Peat is a collection of partially decayed vegetation, available from natural areas called peat lands which is essentially forest areas, the products of which is set aside to be employed as a fuel due to environmental concern.

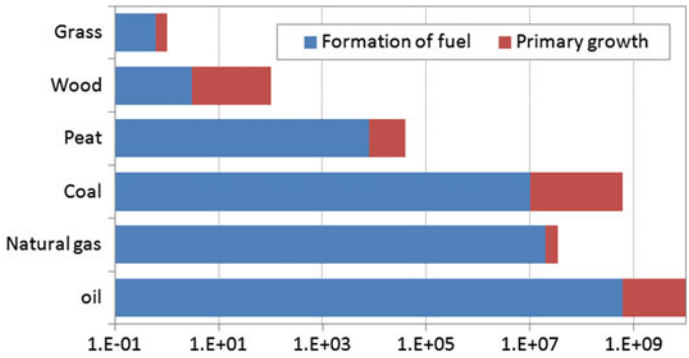

Fig. 1 Stations of Carbon during a growth and formation of fuel (Judex 2010)

Wood and grass are the most frequently used biomass materials for energy. This category of biomass has the capability to replenish in a short span, and thus affected as a renewable energy reserve (Judex 2010).

2.1 Biomass Potential

Biomass is certainly an only renewable energy option, which is having potential to store carbon into its structure with the help of photosynthesis activity. The selection of biomass is even more hopeful given the fact that the biosphere is witnessing large potential of photosynthesis and can play as a large carbon sink. It is possible to achieve a sustainable bioenergy supply of at least 270 EJ and that can satisfy almost 50% of the global primary energy need (Ladanai and Vinterbäck 2009).

As per an estimate, the production of biomass on the earth is around 100 billion tons on land (Naik et al. 2010). Sun is the principle source of exergy on the earth, which makes the biosphere act as a vast biomass generation facility. The energy of the sun is utilized on the earth for a different activity, towards natural decay and growth of vegetation, utilization towards human activity, etc. It is important to mention here that the energy from the sun will not be reduced, but its capacity to do the work or exergy will be destructed. Figure 2 shows the flow of such exergy as it is released from the sun in the process of decay or it may be used by the humans for the purpose of releasing energy. The values quoted in the bracket show the progressive reduction in the exergy values. Out of 86 PW delivered by the sun to the earth is in the form of radiant energy. Out of all this, radiation, 90 TW is utilized by photosynthesis to the plants, this is the net primary production (NPP) and includes land biomass (65 TW) as well as marine biomass (25 TW). This NPP is utilized to add to the reserves of plants on the earth in the form of terrestrial biomass reservoir. The reservoir is assumed to be as large as 30 ZJ. A similar reserve of marine plants may be contributed (0.15 TW) which eventually goes to decay and will be buried in the deep earth to be converted into fossil fuel. Out of the land, biomass reserves

human use are responsible for the exergy destruction equivalent to 15 TW of biomass. Note that any additional use of a portion of the terrestrial biomass reservoir of 30 ZJ may lead to a reduction in the land plant reserves. This is what has been observed in the recent past, the land use change is responsible for a reduction in the natural forest and reserves on the land, and such irreversible degradation of environmental sink is responsible for the decrease in the carbon absorption capacity and increase in global warming phenomenon. Out of this around 5 TW of human use are associated with harvest of wood. This activity resulted into 1.5 TW of exergy destructed through the utilization of biomass for burning. Another 5 TW of exergy destruction is in the form of vast reserves of plant that is grown due to agricultural activity in the form of agro products. Out of this only 0.8 TW is the useful product and the production of transportation fuel, 0.02 TW (e.g. 30 billion liters of ethanol are utilized for producing ethanol) as well as the remaining exergy is locked in the form of agro residues. This indicates the huge potential of biomass is still available for generation of energy through the use of agro residue.

Previous discussion leads to some interesting conclusions. It is interesting to note that out of the total global biomass production, forest contributes largest option of biomass reservoir and has the greatest potential in terms of the return of energy (Table 1). The potential of growth and productivity of forest reservoir is also high amongst different alternatives. This helps one to infer that growing biomass under forest reservoir has the highest capacity not only to store carbon but also to recycle the same from the energy point of view. The example of use of agro residue is an attractive alternate and superior compared to the use of forest biomass reserves. Since using agro residue, the dual objectives can be achieved, i.e. obtaining food as well as energy from residue.

Biomass contributes about 12% of today's world primary energy supply, while in many developing countries, its contribution ranges to even 40–50%. Use of biomass has certain distinct benefits such as, renewable in nature, and in most of the cases a source of indigenous fuel that help improve energy security of a nation.

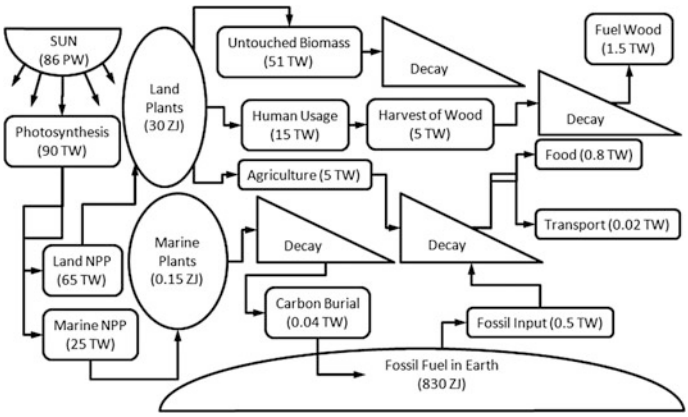

Fig. 2 Exergy flow through the terrestrial biomass system [adapted from (Hermann et al. 2005)]

Table 1 Biomass production and productivity (Adapted from Hermann et al. 2005)

Biomass	Forests	Savanna and grasslands	Swamp and marsh	Other terrestrial
Biomass reservoir (ZJ)	29	1	0.5	1
Biomass per area (MJ/m^2)	600	40	250	10
Net productivity (TW)	42	10	3	9
Surface area (10^6 Km^2)	48.5	24	2	74.5

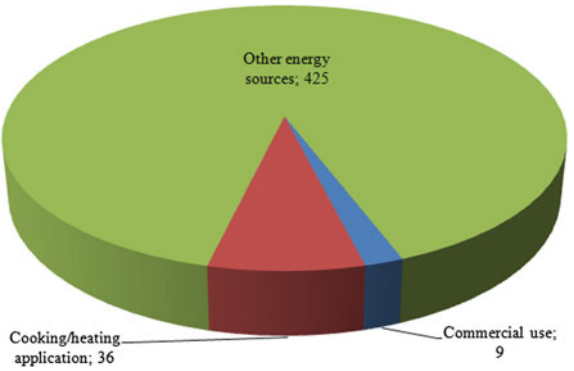

Fig. 3 Contribution of biomass in global primary energy demand of 470 EJ (2007) [Adapted from (Ladanai and Vinterbäck 2009)]

Utilization of biomass as a fuel also offers an opportunity of rural employment, if not utilized as a fuel, its natural decomposition will produce harmful gases such as methane (CH_4), which is causing a global warming potential (GWP) 20 times greater compared to CO_2, this is also one of the causes why it is indispensable to use biomass. Biomass does not contain sulfur so no SO_2 is produced and offers low NO_x when burned. Its use result in reduced economic pressure on the economy, due to lower cost of biomass. However, use of biomass as an energy source has certain limitations also. They are as follows - it differs to the other options in terms of variability of resources, the use of biomass is suitable to low or medium application due to poor perennial availability, poor supply chain management, low energy density, high moisture content, etc.

The above-mentioned advantages and limitations when evaluated, it is found that there is a long way to go for biomass to be used as fuel compared to conventional energy options. The effect is reflected while summarizing global primary energy usage (Fig. 3). It is mentioned that commercial use of biomass is limited to just 2% of the amount. The continuing use of biomass is found limited to the cooking and other thermal applications. The use is essentially limited in rural regions and associated with inefficient use of biomass. Use of biomass is largely limited to underdeveloped and developing economy and less prevalent in developed

countries. However, the issue of global warming has renewed interest in the use of biomass. Use of forest and wood scrape consists of nearly half of current biomass usage, while the bulk of the balance of biomass use is intended for the production of biofuels (Kushwaha 2011).

A large diversity in the supply of biomass is associated due to the different source of origin, drying of wood, different size, and so on. These factors result in large variability in the biomass resource supply, the variation can be divided into the four categories (Fig. 4); biomass generated due to wood waste, agricultural residue, and residue collected from forests (trees, wood and logging residues, bark, plant and leaves). Another category is human induce wood waste, this includes waste generated from industrial activity like, paper and pulp industry waste, waste generated from the textile industry, and waste due to sewage sludge (Lewandowski et al. 2012). Another source from this category is dedicated biomass grown for energy, short rotation crops, herbaceous plant, bagasse, from management of forest and from aquatic source such as algae and water weeds (Ciubota-Rosie et al. 2008). Miscanthus (average yield 15 t/ha/year) and switchgrass (average yield 10 t/ha/year) are the two grass types grown on wasteland especially for the energy purposes (Kavalov and Peteves 2005).

Traditionally, the use of wood logs obtained from trees is the oldest type of fuel source. Due to its low surface area and high moisture content it can be used only for low heating rate applications. Wood logs have other non-energy uses, e.g. for paper and pulp production, furniture industry, etc. This type of use of biomass is not sustainable due to large mismatch in the growth and consumption pattern. To counter this limitation, option of short rotation biomass forestry is adopted, since the cultivation and harvesting consumes much lesser cost (around 4%), the dedicated plants grown for energy purpose are managed for regular dressing and removal. Growing willow and poplar are two most popular selections, under this it is possible to increase the output of biomass to 10–15 t/ha/year, which is normally in the range of 5–10 t/ha/year. Option of planting such dedicated tree at a certain distant location in the existing farm along with wheat and rice crop is another alternate. Wood collected from short rotation forestry many times loses its source, hence this type of

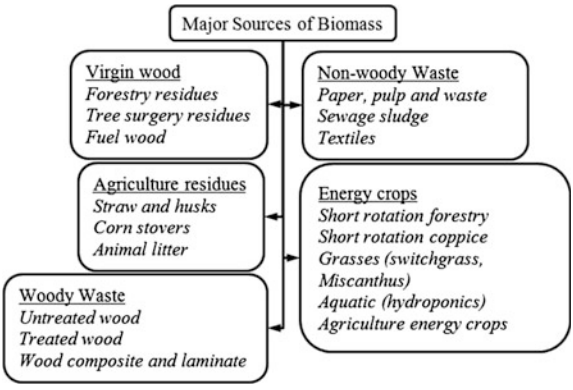

Fig. 4 Sources of biomass for energy production (Ladanai and Vinterbäck 2009)

wood is essentially heterogeneous in nature. The material obtained under this category is high in moisture and requires pre-drying, moisture content in the forest residue account for 20–45% of the harvested wood (Judex 2010).

Drying of wood is essential for effective heat release. Drying can be carried out in three possible ways; by natural drying in which moisture can reduce from 50–55% to 35–45%, by forced drying either by passing waste heat or by making pellets of biomass, both can reduce the moisture to less than 7% and can benefit in terms of calorific value, transportation, storing, etc. However, energy cost may rise by 7–10% of the net available. It is worth noting that more than 15% of moisture content will encourage bio-degradation of material and development of fungi and bacteria (Judex 2010).

The large agricultural residues are another source of biomass. Table 2 gives an idea of vast possibility of the biomass as a source. It is possible that the ample amount of biomass can also be made available from the agricultural activity, this is in terms of residue, husk, straw, and other residues of the crop. Crops such as rice, wheat, sugarcane are prime choice, the residue of these crops can be targeted for use as a waste. Significant potential of these lignocellulosic biomass is available for possible use, given the fact that, biomass under this category accounts for about 50% of the total inventory of biomass in the world, which measures up to around 10–50 billion tons (Claassen et al. 1999). A survey of the global agricultural production suggests that Asia is the hub of large agriculture activity involving these crops, followed by North and Central America. Residue straw from different crops is an example of herbaceous material; which could be made available at relatively low or negative price in most of the instances. Straw has a lower bulk density, lower calorific value and high ash content (Kavalov and Peteves 2005). Given the fact that 23% of rice straw residue produced in India, 48% in Thailand, and around 95% in the Philippines burns in open field (Gadde et al. 2009), possibility of use of residue straw is large. The use of corn residue is well developed in the USA and Brazil. The residue is utilized by way of producing biodiesel to be used as transportation fuel.

Table 2 Estimated global production of major agricultural crop (MMT) § (Reprinted with permission © Springer)

	Asia	Africa	Australia	Europe	N&C America	South America	Oceania	Total
Rice	513	16	~	3	10	18	24	562
Wheat	230	23	~	127	96	21	~	585
Cane	505	80	40	~	156	404	45	1192
Corn	155	44	~	60	264	48	1	576
Soyaben	21	~	~	1	67	39	~	130
Beet	36	4	~	188	25	3	~	255
Potatoes	89	8	1	156	28	12	1	295
Sweet potatoes	124	7	~	~	1	1	~	234
Cassava	46	85	~	~	1	31	~	163

§FAO production year book, 1996 referred in Claassen et al. (1999), ~ negligible quantity

If the biomass potential is assessed from an Indian perspective, one can notice that cotton stalk, sugarcane and rice could also be a principal crop residue that can be targeted for biomass energy (Table 3). Cotton stalk is an attractive fodder for the cattle and hence it has a strong alternate value chain. Sugarcane is largely used as a fuel for cogeneration plant for the sugar industries. However, the use of rice residue is an attractive alternate since rice straw generated in the field is not a preferred fodder alternate. Use of maize cobs can be explored further under fermentation route to extract energy. Crop and wasteland are two alternates that can be explored, but wasteland is highly stressed due to the change of land use and the quantity of this type of biomass obtained tends to reduce with the passage of time. Municipal solid waste is also a good potential to explore, but it requires a well-developed supply chain management. Use of '*Jetropha curcas*' can be another good potential, however, its use is under scanner due to 'fuel-for-food' debate. Cattle dung also needs strong supply chain management and segment is highly unorganized.

One of the biggest limitations in the usage of biomass residues are poor supply chain management. Poor volume density and low calorific value are the main reasons which make them unattractive from the transportation point of view. The limitation to carry over a long distance limits an alternate supply chain. This fact is demonstrated with the help of density statistic for straw under various compacting options as presented in Table 4, as it proceeds from the field under various options of its utilization, the corresponding density change is shown in the table. To begin with, the fresh straw has density of the order of 50 kg/m^3; the filling will increase its density to some extent, chopping and drying can further help to increase its density.

Table 3 Bioenergy potential for India (Ravindranath and Balachandra 2009) (Reprinted with permission © Elsevier)

Feedstock	Area (Mha)	Biomass potential	Pathway	Qty. (MT/year)	Energy potential
Rice	46.1	Straw + husk	Gasification	41	4700 MW
Maize	6.6	Stalk + cobs		6.2	700 MW
Cotton + coconut	16.8	Stalk, coconut shells		240	28,000 MW
Sugarcane	5.5	Bagasse + leaves	Cogeneration	163.5	8900 MW
crop land	14	Woody	Gasification	84	9700 MW
Waste land	28.5	Mixture and woody		171	20,000 MW
MSW		organic matter	Biomethanation	56	6500 MW
Jatropha curcas	65	1.50 MT of oil seeds	Biodiesel for transp.	3.23	34.11 PJ
Jatropha curcas	13.4	Jatropha curcas oil	Biodiesel for transp.	16.08	530.6 PJ
Sugarcane	5.5	Ethanol	Transportation fuel	20.9	562.2 PJ
Cattle		Dung	Biogas for cooking	344	336 PJ

Making pellets are a better option to compact the straw, this will enhance its density due to compaction as well as removal of moisture from the biomass due to heat during the process of pelletization. It is possible to achieve a density of around 1000 kg/m^3 by pelletizing.

Table 5 shows the Indian scenario of the potential of residue for the crops produced in India. It is important to mention that not all biomass available in the form of residue can be practiced for the energy purpose. For example, coconut fronds has strong alternate supply chain system and hence cannot be made available for the energy use, the same is true for banana residue (Singh and Gu 2010). Out of them all, rice and wheat are promising residues that could be utilized for energy use.

Rice-wheat growing pattern is very popular in South Asian countries, which includes India, Pakistan, Bangladesh, Bhutan, and some parts of China. The rice and wheat straw are an important second-generation biomass material. This establishes the potential of agro residue derived from rice and wheat cultivation is a significant one in South Asian countries. More than 85% of the RW (Rice-wheat) system practiced in South Asia are located in the Indo-Gangetic Plains (IGP). Nearly one-third of the total grains of India are produced in this area. In India, the IGP covers about 20% of the total geographical area (329 Mha) and produce about 50% of the total food consumed in the country (Chauhan et al. 2012). It is estimated that approximately 45 and 35% of the harvested over-ground biomass are accounted towards wheat crop, grain and straw respectively (Claassen et al. 1999). Rice–wheat (RW) cropping system are critical for food security of India. As much as 10 million hectares of land are engaging in rice and wheat production in sequence (Table 6), which expands to about 85% of the total cereal production. This crop system engages around 150 million people in South Asia (Chauhan et al. 2012). In the state of Punjab (India) alone rice and wheat cropping is observed in about 60 and 80% of the total land respectively. It is surprising to note that around 90% of rice straw alone is burnt each year in Punjab, India. The statistic suggests possible utilization of rice straw for power generation as a strong biomass alternate.

Table 4 Density of straw and grass in different form (Lewandowski et al. 2012)

State	Density (kg/m^3)
Fresh (green)	50
Standard fill	85–100
Dried and chopped grass pellets	170–380
Bale (grass)	120–150
Compact roll	350
Bale (straw)	800–1200
Dust (crops)	150
Pellets (bulk straw)	540–660
Pellets (single, straw)	1100
Pellets (agricultural)	950–1250
Pellets (grass)	1300
Oil (rape)	920
Pyrolysis oil	1200–1300

Table 5 Production of crops and availability of residue in India (Singh and Gu 2010) (Reprinted with permission © Elsevier)

#	Name of crop	Per year, KMT	Type of residue	Residue as % of crop
1	Arhar	1950	Husks-Stalks	30-250
2	Bajra	7690	Cobs-Husks-Stalks	33-30-200
3	Banana	80,000	Residue	300
4	Barley	1200	Stalks	130
5	Coconut	13,125	Fronds-Husks-Shell	400-53-22
6	Rice	1,45,050	Husks-Stalks-Straw	20-150-150
7	Sugarcane	2,76,250	Bagasse-Top and leaves	33-5
8	Wheat	78,000	Pod-Stalks	30-150
9	Maize	18,500	Cobs-Stalks	30-200
10	Rubber	825	Primary wood-Secondary wood	300-200

Table 6 Area under Rice–Wheat cropping system in various Asian countries (Adapted from Chauhan et al. 2012)

Country	Area (Mha)	Rice (%)	Wheat (%)	Total (%)
China	13	31	35	72
India	10.3	23	40	85
Pakistan	2.3	72	19	92
Bangladesh	0.5	5	85	100

2.2 *Biomass as a Feedstock*

Biomass feedstock can also be classified based on the way it influences the environment. A tentative classification is presented in Fig. 5. The first generation of biofuels is selected based on the premise that biomass releases the same amount of CO_2 when burned which it absorbs and captures during its growth. Various aspects of economic consideration and increased energy security prompted the use. This trend is significantly reinforced with the growth in the price of fossil fuel. Another consideration made in the first-generation biofuel is the substitution of fossil fuel in existing infrastructure. Biofuels like biodiesel, bio-ethanol and biogas are examples under this category. Its ability to blend with the existing fossil fuel type and possible use of the existing engine facility make biofuel of the category a relatively easy task. Today world is witnessing the commercial success of the first generation of biofuel with approximately 50 billion liters of biofuels produced annually.

The popularity of biofuel is questioned from the environmental considerations. The release of greenhouse gases from the use of such biofuels are a concern for the environment and in turn for human beings. This issue puts environmental impacts and carbon balance of products and technology pathways under scanner. The case is sufficient to highlight the disadvantage of first-generation fuel and is proving that

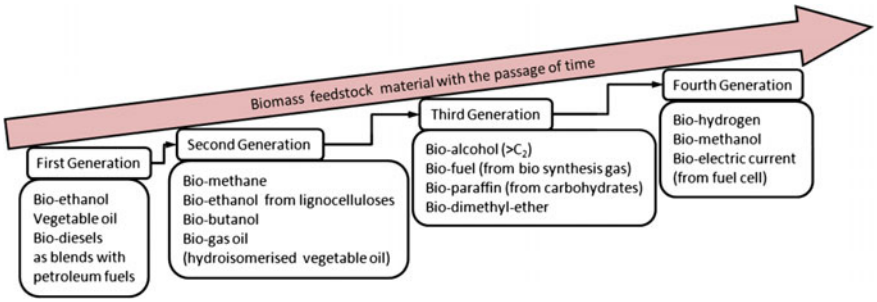

Fig. 5 Classification of biomass based on its chronological use of energy and chemicals [Adapted from (Lako et al. 2008)]

these types of fuel are not sustainable, and it puts stress on existing use of land. There is a debate over whether the race of scarifying food-for-fuel is justified, especially when a large sections of humankind is malnourish and has a scarcity of food. This involves people from Africa that includes large numbers of malnourished children (Naik et al. 2010).

Second-generation biomass are a biomass option that arises out as an alternate to the limitation of first-generation biomass (Naik et al. 2010). The want of a sustainable biomass fuel source gave birth to the second-generation biofuels. Second-generation biofuels is produced from Lignocellulosic biomass, primarily derived from agro residues or 'plant biomass'. The line of reasoning in favor of this feedstock is; cheap and abundant availability and its lower price sensitivity to the existing food cycle and not competing right away with food. Few of the feedstocks worth mentioning are agriculture and forestry residue, weed, aquatic biomass, and water hyacinth, etc. These biomass materials are burned conventionally in an inefficient manner and in some cases leads to release of harmful aerosols posing threats not only to the environment but also to human lives. Likewise, if not utilized and left unattainable in the fields, these feedstocks may lead to the decay and be responsible for the release of methane (which is having even greater environmental threat). Therefore, the use of second-generation biomass may result in lower cost and greater capability to tackle the environmental problem and hence it is the most effective route to renewable, low carbon energy for road transport. The products in this category are, hydro-treated oil, bio-oil, Fischer-Tropsch (FT) oil, lignocellulosic ethanol, butanol, mixed alcohols. The technology for the second-generation biomass are in progress, a number of technology barrier that need to be overcome and therefore large-scale production of second-generation biofuels are a distant reality. Conversion process of second-generation biomass involves thermochemical as well as biochemical routes. Under thermochemical processing biomass are subjected to thermal decay and chemical reformation by heating biomass in an environment of different concentrations of oxygen (Naik et al. 2010).

Third and fourth generation biomass feedstock take care of not only the economic and environmental concerns, but its use results in societal benefit as well and

thus addressing all aspects of sustainability (economic-environmental-societal). One of the examples of third and fourth generation biomass feedstock is ethanol-gel, which is a clean burning fuel made-up of gelatinized ethanol bound, thickened by cellulose and water. The feedstock can be readily used with an existing cook stove with slight modification in design. The fuel gives clean smokeless combustion, which presents a drastic decrease in CO_2 emission and hence leads to a decrease in indoor air pollution. The improved combustion results in higher combustion efficiency of around 40%. In a separate study an LPG-assisted conventional cook stove is compared with gasifier based cook stove using rice husk as fuel. The testing results along with an economic analysis for the gasifier based model after a successful field operation of 2 years are reported (Suvarnakuta and Suwannakuta 2006).

3 Assessment of Biomass

It is indispensable to evaluate suitability of a fuel option for the purpose of employment to obtain energy. Prof. Van Krevelen suggested a simplified diagrammatic method to identify the suitability of fuel option based on its molar ratio. He defined the zone for different fuel options (Fig. 6). From the figure, it is observed that a fuel with the lower value of (H/C) and (O/C) gives a high quality of fuel. Under this mode of classification it is possible to allocate definite zone for a particular type of fuel or its constituents, anthracite and coal will occupy lower value of H/C and O/C, followed by lignite and peat. Biomass is the next fuel option and occupies a wider range of the variation. Due to the higher oxygen content, biomass has a high score on O/C ratio.

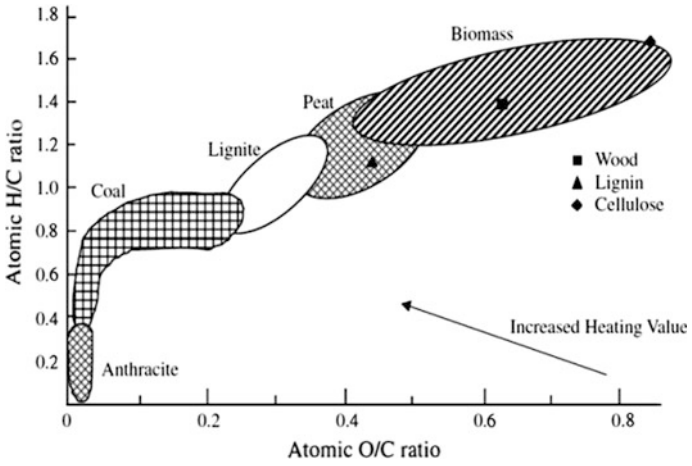

Fig. 6 Van Krevelen diagram. Plot of fuel for H/C and O/C ratio (Judex 2010; Prins et al. 2007)

Some other popular method of mapping the fuel structure is by the use of C–H–O diagram. The advantage of using this three-axis diagram is that on this diagram not only the biomass feedstock can be precisely figured, but this can also depict the effect of biomass change, when followed by a particular conversion process. It is also possible to identify clearly core zones, solid zone as well as gaseous fuel zone. One such example is as shown in Fig. 7. The state of biomass is shown on the diagram and the occurrence of different processes on the path are shown on this. For instance, H shows the process of biomass treatment by addition of hydrogen, S shows the use of steam for processing of biomass, O shows the addition of oxygen, P shows slow pyrolysis and F shows fast pyrolysis treatment of the biomass material.

Suitability of biomass feedstock is ensured predicting different constituents of biomass. This is obtained by carrying out different types of analysis, which is useful to predict the various constituents of biomass. Lignocellulosic biomass is made of three basic structural units - cellulose, hemicelluloses and lignin. Cellulose is a simple and crystalline glucose polymer, while hemicelluloses is an amorphous polymers of xylose, arabinose. Lignin a large poly-aromatic compound consists of 3D structure and gives the biomass much needed rigidity and support (Naik et al. 2010). Heating value of dry biomass is around 17 MJ/kg. Out of the basic component of the biomass, cellulose (17 MJ/kg) and lignin (25 MJ/kg) contribute to the list and maximum respectively to the calorific value of biomass. Out of two fundamental building blocks (C and H), C has a mass fraction of 0.48 kg/kg and a molar fraction of 40.36 Mol/kg, while energy from the combustion as −15.88 MJ/kg. H has a mass fraction of 0.0298 kg/kg and a molar fraction of 27.56 Mol/kg, while energy from the combustion as −6.67 MJ/kg (Judex 2010). One such comparative analysis of herbaceous, woody and waste biomass is as shown in Table 7. It is important to note that the woody biomass contains a high percentage

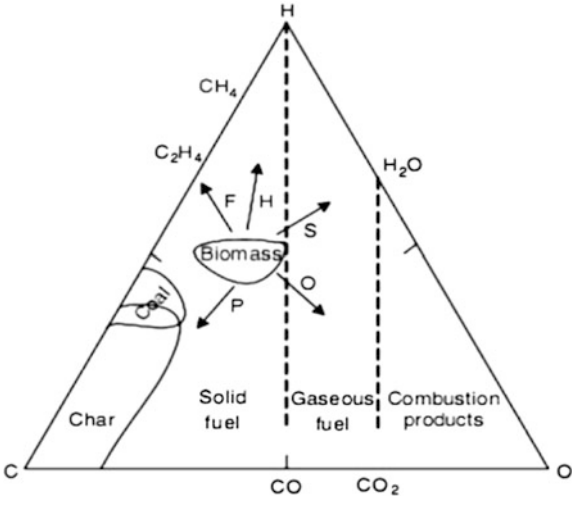

Fig. 7 Location of Biofuels in ternary C–H–O diagram (Basu 2010)

of lignin, which is having high carbon content and effective use of this carbon would give high calorific value. Herbaceous type of fuel has high quantity of cellulosic and hemi-cellulosic parts. Since the cellulose and hemicellulose contain relatively simple construction and therefore possess lower quantity of carbon content compared to lignin, which is essentially a 3 dimensional structure. Lower carbon content in turn gives lower calorific value as a fuel.

The detailed characterization of biomass as a fuel material is as shown in Table 8. Compared to coal it is observed that although the ash content of coal is high, however, very low moisture content and high amount of carbon content makes it dense and energy efficient and results in high net calorific value. High H content and low O content make coal an ideal choice for higher calorific value fuel. However, large S content goes against a selection of coal as a good fuel option. Comparing different biomass material it is noticed that poor density can have certain operational issues. This can be overcome by making pellets to improve the operation. Another important issue in the case of the use of biomass is low melting temperature of ash due to high minerals in biomass. Such melting can form clinker and agglomeration and in extreme case can chock the combustion chamber. This type of phenomenon can occur frequently in the case of rice straw (due to the high silica content) and use of poultry litter (high mineral content) as fuel under gasification route.

Elemental analysis along with specific exergy is another way of determining suitability of biomass as a fuel option. Table 9 indicates these data for the different biomass materials. It is easy to observe that large carbon content gives higher specific exergy for the biomass material. However, while in operation different proportion of moisture content may affect the results to a large extent. It is essential to estimate the calorific value for the biomass in order to access the suitability of the fuel. An empirical rule for finding the energy capacity of biomass (MJ/kg) are given by Eq. (1) (Judex 2010) and Eq. (2) (Channiwala and Parikh 2002) given as under:

$$\mathrm{LHV} = 34.8\,\mathrm{C} + 93.9\,\mathrm{H} - 10.8\,\mathrm{O} \tag{1}$$

$$\mathrm{HHV} = 34.91\,\mathrm{C} + 117.83\,\mathrm{H} - 10.34\,\mathrm{O} - 1.51\,\mathrm{N} + 10.05\,\mathrm{S} - 2.11\,\mathrm{Ash} \tag{2}$$

Table 7 Biomass composition and properties [Adapted from (Hermann et al. 2005)]

Biomass component	Bermuda grass (herbaceous)	Poplar (woody)	Pine (woody)	Refuse fuel (waste)	Carbon content	HHV (MJ/kg)
Cellulose	32	41	40	66	40–44	17
Hemicellulose	40	33	25	25	40–44	17
Lignin	4	26	35	3	63	25
Protein	12	2	1	4	53	24
Ash	5	1	1	17	0	0

Table 8 Properties of biomass considered as feedstock (Adapted from Kavalov and Peteves 2005)

	Coal	Wood	Forest residue	Wood chips	Wood Pellets	Cereal straw	Energy crops
Ash (d%)	8.5–10.9	0.4–0.5	1.0–3.0	0.8–1.4	0.4–1.5	3.0–10	6.2–7.5
Moisture (w%)	5.0–10	5.0–60	50–60	20–25	7–12.0	14–25	15–20
NCV (MJ/kg)	26–28.3	18.5–20	18.5–20	19.2–19.4	16.2–19	16.5–17.4	17.1–17.5
Density (kg/m^3)	1100–1500	390–640	–	250–350	500–780	100–170	200
Volatile (w%)	25–40	>70	>70	76–86	>70	70–81	>70
Ash melting (°C)	1100–1400	1400–1700	–	1000–1400	>1200	700–1000	700–1200
C (d%)	76–87	48–52	48–52	47–52	48–52	45–48	45.5–46.1
H (w%)	35–5	6.2–6.4	6.0–6.2	6.1–6.3	6.0–6.4	5.0–6.0	5.7–5.8
N (d%)	0.8–1.5	0.1–0.5	0.3–0.5	<0.3	0.27–0.9	0.4–0.6	0.50–1.0
O (d%)	2.8–11.3	38–42	40–44	38–45	40	36–48	41–44
S (d%)	0.5–3.1	<0.05	<0.05	<0.05	0.04–0.08	0.05–0.2	0.08–0.13
Cl (d%)	<0.1	0.01–0.03	0.01–0.04	0.02	0.02–0.04	0.14–0.97	0.09
K (d%)	0.003	0.02–0.05	0.1–0.4	0.02	–	0.69–1.3	0.3–0.5
Ca (d%)	4–12	0.1–1.5	0.2–0.9	0.04	–	0.1–0.6	9

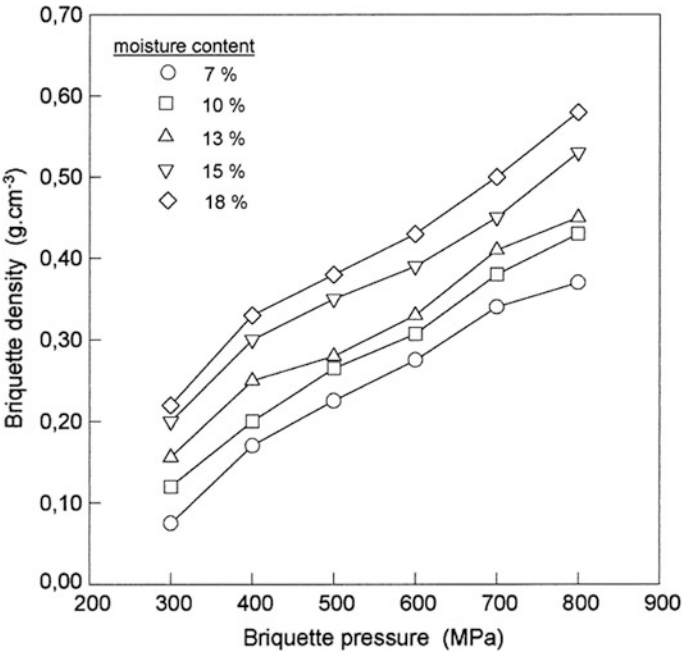

Fig. 8 Effect of pressure on the wheat straw briquette density (Demirbaş and Şahin, 1998)

Table 9 Specific Exergy on dry basis of certain biomass materials (Adapted from Hermann et al. 2005)

Biomass	Elemental mass fraction (%)						Sp. exergy (MJ/kg)
	C	H	O	N	S	Ash	
Poplar	49	6	43	0	0	1	19.2
Corn stover	44	6	43	1	0	6	18.2
Bagasse	45	5	40	0	0	10	17.8

4 Use of Biomass as Fuel

Conventional use of biomass for energy generation, that includes heat as well as electricity, depends on several factors. Few of them are—type of biomass, available quantity, the application for which energy is intended, capital investment and operational issues, availability of resources for operations, existing supply chain for biomass. Biomass utilization in the existing thermal and power generation application is prompted by economic considerations. Few successful applications are listed as below:

- Substituting part of fossil fuel by means of co-firing of biomass in existing coal-fired boiler application.

- Use of biomass for cooking and drying application (fish drying, onion drying), small gasifiers for cooking using rice husk, pellets of rice straw, wheat straw or bagasse, etc.
- Use of biomass for cooking, that save the use of LPG by
  - use of gasifier or
  - use of anaerobic digestion.
- Replacing fossil fuel completely with biomass in steam generator or hot water boilers using logs of wood.
- Use of biodiesel or bio-ethanol fully or in part with fossil fuel in the engine.
- Use of bio-oil obtained via pyrolysis to produce chemical compounds.
- Use of gasification technology to produce producer gas from biomass, and to produce H_2 or use of reforming reaction to produce NH_3.
- Producing high quality carbon dioxide from combustion of producer gas via gasification route.
- Producing charcoal through slow pyrolysis.

Biomass suffer several limitations. Tables 10 and 11 give a direct comparison of the biomass with the other fuel options. Clearly, it is found that the biomass has only 1/3rd available energy at 50% moisture. The dry wood has a calorific value between 19 and 20 MJ/kg. It consists of roughly 70% volatile matter 28% fixed carbon and 2% ash (de Miranda et al. 2013). On thermal efficiency count the biomass has lower thermal efficiency. This is due to slow rate of combustion, high moisture content, large surface area and higher excess air for combustion.

Table 12 shows the comparison of thermal efficiency of different fuel options. It is noted that the use of wood releases more carbon dioxide than fossil fuel, besides this it has a poor combustion efficiency compared to fossil fuel. Wood ends-up consuming more energy per kJ of energy released and also releases higher green-house gases. Due to this fuel switching option from fossil fuel to biomass would not be advisable in the short run (Ingerson 2009). Besides this, the use of some of biomass materials is technically difficult to process thermally, e.g. excessive tar production when using rice husk in the small cooking gasifier stove is a problem for rice husk gas technology (Belonio 2005). On the emissions front, compared to conventional fuel options, biomass use is found to emit large amounts of particulate matter, this leads to the release of fly ash in the form of black carbon and harmful aerosols. Use of pellets is a safe alternate for this problem. Due to the large volume-to-surface area and slow release rate, biomass use (woody as well as pellets of biomass) will contribute to the discharge of unburned carbon dioxide gases. The consequences of carbon monoxide are more serious in the urban area due to

Table 10 Comparison of biomass and fossil fuel energy (Adapted from McKendry 2002)

Material	Energy Density
Mineral Oil	42 GJ/t
Coal	28 GJ/t
Biomass (wood, 50% moisture)	8 GJ/t

Table 11 Combustion characteristics of coal and biomass (Adapted from Tumuluru et al. 2011)

	Coal	Wheat straw
HHV	20.42	17.99
LHV	19.65	16.73
Yield (t/ha)	–	2.2
Moisture (d%)	5.5	10
H_2 (w%)	3.49	5.7
Ash (w%)	34.2	7.9

Table 12 Average efficiency of various fuel options (Adapted from Ingerson 2009)

Fuel	Power plant (%)	Other use (%)
Coal	35	45–60
Gas	45	80–90
Oil	38	80
Wood	22–25	65–80

Table 13 Emission from wood and fossil fuels in kg/kJ (Adapted from Ingerson 2009)

Pollutant	PM10	CO	NO_x	SO_2
Oil boiler	0.0060	0.0151	0.0615	0.2150
Natural gas boiler	0.0030	0.0344	0.0387	0.0002
Coal boiler	0.0176	0.1208	0.3909	0.4408
Woodchip boiler	0.0430	0.3139	0.0710	0.0035
Wood pellet boiler	Low	0.2193	0.1170	Low

stagnant air conditions. Table 13 gives emission comparison of different fuel options. It is likewise observed that biomass when used as a fuel releases a similar amount of NO_x compared to petroleum, and higher amount of particulate matter. Small participles are more harmful, for two reasons, they remain in the air for longer duration of time and another is it contained toxic substance which can reach to the lungs because of inhalation (Ingerson 2009). Home wood stove releases a considerably large amount of pollutants compared to a commercial arrangement. Outdoor boilers limit combustion air and create fire smoldering, produce more particulate release and with its low elevation of the chimney the effect is even more serious (Ingerson 2009).

4.1 Use of Biomass as a Boiler Fuel

The most feasible low-cost option for the use of biomass is co-firing with coal in existing boilers. Co-firing technology is demonstrated in almost all types of boilers, include pulverized coal boilers (wall-fired and tangentially fired designs), coal-fired

cyclone boilers, fluidized bed boilers, and spreader stokers. It is seen that about 15% of the total energy input can be substituted with biomass under co-fired option requiring little or no modification. Under such switchover there is little or no loss in overall efficiency. Conversion efficiency from Biomass to electricity is observed at 33–37%. Since biomass have very less sulfur than coal, significant decrease in SO_2 is observed. A further NO_x reduction of 30% is observed (Bain et al. 2002). Developing local biomass supply option is a great challenge to the use of biomass as fuel.

4.2 Use of Biomass to Generate Power

Power generation from coal can be a disadvantage from the environmental point of view. Using only biomass for power generation can get important advantages; even so, there are a number of limitations to overcome. Few limitations of use of 100% biomass applications are; large storage space, need of supply side management, intensive biomass feeding system, biomass availability due to seasonal variation and adaptability to different types of fuel, capital investment required for modification in existing system, lower heating value, poor bulk density, high moisture and ash content, stability during storage and grind-ability, flame stability, formation of smoke and flue gases, fouling of boiler tube due to higher particulate release, etc. (Tumuluru et al. 2011). The issue of ash after the combustion of biomass also has serious consequences, for instance a 50 MW plant has to handle about 16–20 tons of ash per day (Ingerson 2009). For such plant approximately 70–80 truck-loads are required per day. The issue of a sustainable provision of wood is also in doubt. The particles generated from the usage of biomass can contribute to fouling and corrosion of hot gas parts as well as erosion of the nozzle and valves (Ingerson 2009). It is likewise observed that the wood fuel feed rate needed for an equivalent heat input is nearly double that of coal on a weight basis, and more than four times that of coal on a volume basis (Ingerson 2009). Wood combustion required more excess air compared to coal, this requires large induced draft fan compared to coal. CO in wood fired flue gases is more compared to coal burning, this make option of preheat combustion air is essential.

4.3 Option of Biomass Co-firing

Residues, energy crops, herbaceous crops and woody biomass can be utilized as fuel for co-firing, by using the cyclone type combustion chamber. Co-firing results in little derating of boiler thermal efficiency and boiler capacity. Due to such derating it is not preferred to go for co-firing greater than 20%. The capacity derating can be overcome by increasing the thermal loading or by increasing fuel combustion rate. Reduction in terms of NO_x, SO_2 and mercury emission can take

place. Co-firing option is also feasible in a fluidized bed boiler, where saw dust is supplied along with the coal powder. At higher proportion of coal replacement by the biomass, problem of slagging and fouling become serious because of interaction between alkaline in wood and sulfur in coal (Wiltsee 2000).

Compared to coal, biomass has a higher composition of hydrogen and oxygen and lower amount of carbon, this gives biomass a very low calorific value compared to coal. Moreover biomass has a higher fraction of volatile matter, this will result into the combustion with flame, while in the case of coal the combustion will be followed by glow almost without flame. These issues need to be considered when fuel switch over is considered with respect to heat load and sizing. The issue of high moisture content is also critical for the biomass combustion. At the time of cutting, the moisture in the biomass is in the range of 40–45%, this high moisture is not acceptable from the combustion point of view. Especially when mixed with coal having moisture levels as low as around 5%. Sun drying is the most sought option to drive out high moisture content, however this option is limited to the space available to store the biomass and the capacity of the plant. High moisture content reduces the combustion temperature and increase the time to release the same amount of energy by burning in the combustion chamber. As far as the ash is content, biomass has low ash and this reduces the heat carried away from the combustion chamber along with ash. Some biomass has high sand, salt or clay (rice straw $\sim$ 15% silica). High alkaline matter in the ash can cause fouling of heat transfer surface. Chlorine and sulfur content in the coal can cause corrosion problem, use of biomass as fuel help resolve this issue (Tumuluru et al. 2011).

The quantity of moisture content can reflect in terms of low calorific value, durability, storage and self-ignition chances. Poor bulk density may result into problems in storage, transport and handling of fuel. High ash content can result in the formation of dust on heat transfer surfaces, emission of particles in the environment as well as additional cost of ash handling. Potassium and sodium may result in corrosion, lower ash melting temperature and aerosol formation. Another limitation of biomass use is a mixture of the biomass with different origin, these cannot be burned together in most of the furnaces. However, fluidized bed combustion is more flexible from that angle.

Besides this, there is technical complexity when biomass is used along with the coal; for instance, pyrolysis temperature for the biomass is much lower compared to coal. Since biomass have high volatile matter the heat supplied from it is about 70% in the case of biomass while the same in the case of coal is only 30–40%. Although specific heat released due to volatile matter is lower for biomass compared to coal. Due to the high oxygen content in the biomass the char made from biomass contains more oxygen and is highly porous compared to char produced from coal. Highly porous char is a good candidate for use in the applications as biochar in the field. Biomass ash is more alkaline in nature, and leads to high fouling of heat transfer surfaces.

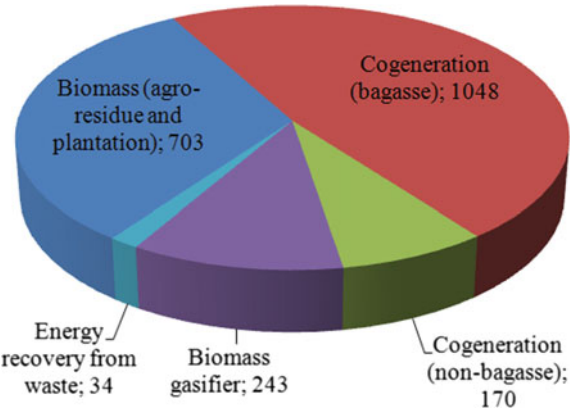

Fig. 9 Actual power generation (MW) from biomass in India 08–09 (MNES report) (Singh and Gu 2010)

4.4 Use of Biomass by Making Briquettes/Pellets

Densification of biomass by pellets and briquette making is an essential biomass-preprocessing requisite. This is due to the fact that certain agro residue and waste biomass materials are often difficult to directly utilize as biofuel material. This is due to its poor bulk density thin size and at times high and uneven moisture content. Studies suggest that densification improves homogeneity, reduction in particulate matter and lower moisture content, and also ease in storing and transportation (Naik et al. 2010). Figure 8 shows the effect of briquette density with briquette pressure for different levels of moisture content.

4.5 Use of Biomass by Grinding and Torrefaction

Option of grinding of biomass (to a size <5 mm) can be chosen to overcome the slow combustion and reduction in moisture content of biomass. Another viable option is to pelletize the biomass (6–8 mm diameter), this brings homogeneity in the fuel structure, reduction in moisture content as well as, reduction in ash generation. Use of pellets or briquettes can help increase the rate of combustion to the coal, and also improve the combustion characteristic.

The option of torrefaction can also be considered. This is achieved by thermochemical heating of biomass in the absence of oxygen for 30–60 min. This process will drive out moisture and volatile matter from the biomass. This will give increased heating value, improves grinding and binding properties, because now more lignin is available in biomass. It makes biomass hydrophobic in nature and can be stored for longer duration, as now biomass will not absorb moisture from the surroundings. Table 14 presents a comparison between three solid wood fuel options - wood chips, pellets and torrefied wood pellets. It is observed that making wood chips, pellets and torrefied wood pellets will reduce moisture content,

Table 14 Physical properties of wood chips, wood pellets and pellets made out of terrified wood pellets (Adapted from Tumuluru et al. 2011)

Physical property	Wood chips	Wood pellets	Torrefied wood pellets
Moisture (%)	35	6–10	1–5
Density (kg/m^3)	300–500	600–650	750–800
Calorific Value (MJ/kg)	10.5	16	21
Energy bulk density	5.8	9	16.7

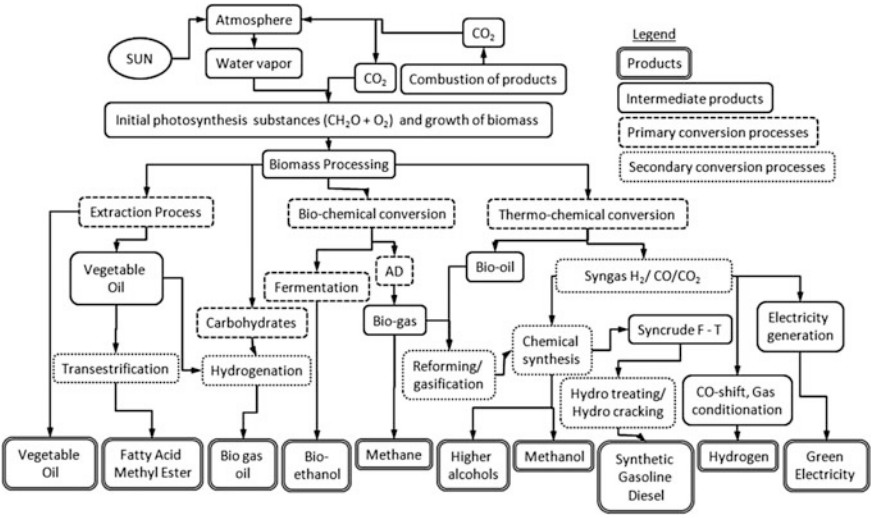

Fig. 10 Technology options for biomass adapted from (Lako et al. 2008; Adams 2011; Ciubota-Rosie et al. 2008)

increase density and improve the calorific value of fuel, in the given order. The option of torrefied wood pellets gives the extra-long storage capability. This is because the pellets are hydrophobic in nature and do not absorb moisture from the environment and increase the shelf life of pellets, without collapsing shape or disintegrating.

5 Technology Options

Utilization of biomass is traditionally dominated in rural India, this includes primary utilization of biomass for cooking and heating applications. Besides this, biomass is also utilized largely for power generation. Figure 9 gives the statistics of actual power generation pattern (in MW) from different routes of biomass used in

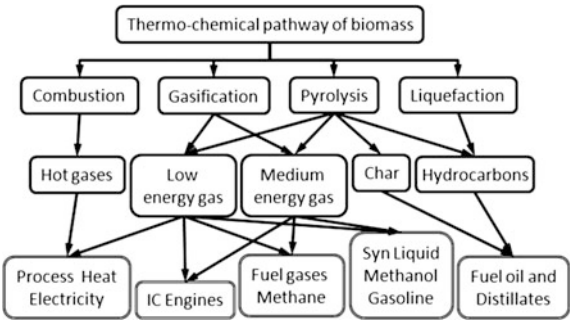

Fig. 11 Biomass utilization pathways under thermo-chemical conversion [Adapted from (McKendry 2002)]

India. Cogeneration using bagasse is the largest biomass power generation option, practiced at a large sugar belt in India (1048 MW). This is followed by power generation by use of biomass by direct combustion, this includes agro residue and yield from energy plantation (703 MW). Biomass gasification route is demonstrated at several installations (243 MW), this includes gasification of woody biomass as well as gasification of rice straw and a small number of briquettes/palletized fuel using agro residues.

Figure 10 shows the broader picture of biomass production to its usage. The production of biomass takes place by consuming CO_2 and water vapor in the presence of energy from the sun. This initial photosynthesis substance grows over a period to produce different biomass products. These biomass products when subjected to different processes based on the physical and chemical composition of biomass result in different pathways for the generation of energy. Direct combustion is the oldest method known to humankind for heating and power generation. It is a chemical reaction between heated biomass and oxygen that result in the release of heat and gaseous products. Theoretically complete combustion releases gases and vapor (CO_2 and H_2O) (Naik et al. 2010). A common application of combustion is found in domestic stoves and water heaters and small boilers. Direct combustion does not require any pretreatment of biomass and therefore is excluded from the present discussion.

5.1 Direct Extraction Process

First and oldest type of use of biomass is direct extraction. Under extraction process, biomass is used to obtain crude vegetable oil from oil seeds using a screw press. The residues are many times used for producing other biofuels. The residue oil cakes can also be used as a binder for making pellets and briquettes. Production of vegetable oil was once encouraged by the Planning Commission of India by launching massive plantation of Jatropha (*Jatropha curcas*) and Karanj (*Pungamia pinnata*) under biodiesel project, which include 200 districts and 18 states. Although, there are several problems to be resolved if vegetable oil is to be used in

place of diesel in existing engines. Major limitation is the high viscosity of oil, which limits proper atomization, poor mixing of oil with the combustion air leads to incomplete combustion and high smoke and carbon deposition, sticking of piston ring and scuffing of the engine liner, and failure of injection nozzle (Singh and Gu 2010). Also, high cloud level and pour point, compared to diesel, limits its use in cold climate. Research suggests that the large molecule of triglycerides of heavy molecular weight are responsible for this. Chemical treatment of this molecule via transesterification to produce biodiesel can be an option to resolve this issue. Biodiesel is mono alkyl esters of long chain fatty acids, which can be readily used in engine for power generation. Production of biodiesel is not attractive from an Indian perspective due to factors such as—low productivity of oil seed cultivation (1 t/ha), and large gaps in production and import of vegetable oil, which may find it difficult to see the feasibility of biodiesel in India. Due to this non-editable oil produced from Jatropha oilseed as biodiesel feedstock which has high yield (3.75 t/ha) is preferred (Singh and Gu 2010).

5.2 Biochemical Conversion

Biochemical conversion includes two primary processes anaerobic digestion (AD) and fermentation. It is the process that includes the conversion of organic material directly into a gas, popularly known as biogas. The AD route results in the formation of methane and carbon dioxide gas. While, fermentation process can generate bio-ethanol. The conventional AD process may yield methane of the order of 60%, however, with the use of advance bi-phasic system yield can be increased to as high as 80%. Organic, wet and non-lignocellulosic material is ideal for this type of reaction process. The feedstock is converted into gas in the absence of oxygen. This natural breakdown of the biomass in the absence of air can be a useful process to use for even energy grass and animal manure. The residue of the process is a slurry which is a stable, commercially useful compound. The slurry can be considered as a soil conditioner.

Fermentation process can produce large-scale bio-ethanol from sugar crops like sugar cane and sugar beet and starch crops like maize, wheat. The solid residue produced from the fermentation process can be used to feed the cattle. Production of large-scale ethanol from sugar cane can be a total substitute for the fossil fuel and is found popular in Brazil. Likewise, the use of maize for the ethanol production is also widely practiced in the US. Although, uses of wheat and sugar beets have been restricted since both these feedstock violate the condition of fuel-for-feed, still production of ethanol from wheat and sugar beets is picking up in the UK.

5.3 Thermochemical Conversion

Under thermochemical conversion process the conversion of biomass using thermal energy is considered. Under this, four main processes are available combustion, gasification, pyrolysis and liquefaction (Fig. 11). Combustion is burning of biomass in the air. The combustion of biomass can take place in different places and for different applications. This includes combustion of biomass over the grate to generate steam in the boiler, combustion at fireplaces for thermal heating, combustion at stoves for cooking application, in the combustor for the gas turbine power generation application. Combustion results in the generation of hot flue gases at around 800–1000 °C. There is a large variation observed in the temperature of the flue gases. It mainly depends on the factors like type and size of biomass and moisture content. Due to poor energy density of the biomass and variability in the quality and other logistic issues the plant size of biomass is generally low and ranges from 5 to 15 kW.

Generation of bio-oil using pyrolysis technology is a recent development. Pyrolysis technology can use agro residue and it produces viscous dark liquid called bio-oil (Bridgwater 2012a) which can be used to partially replace diesel to generate off-grid electricity (Sagi et al. 2014). Pyrolysis is a thermochemical conversion process in which feedstock is heated at less than 400 °C in a reactor in the absence of oxygen to obtain vapor and char (Evans and Milne 1987). Pyrolytic thermal biomass decomposition is a complex process which consists of simultaneous reactions of dehydration, isomerization, aromatization, carbonization, oxidation. While secondary reactions like thermal water decomposition into synthesis gas, cracking, condensation also take place (Lewandowski et al. 2012).

Vapor fractions from pyrolysis process are condensed in a condenser to obtain bio-oil (Bridgwater et al. 1999), while char can be utilized as a fuel in the boiler and for other thermal applications or as a slow-release fertilizer in the field (Woolf et al. 2010; Major 2009; Lehmann and Joseph 2009; Novak et al. 2009). The advantage of pyrolysis is that it requires lower temperature compared to gasification. Product yield can be controlled by varying different residence time for the vapor in the reactor. High temperature and longer residence time can increase the conversion of biomass to gas, whereas the moderate temperature and short residence time is ideal for producing large quantities of liquid in the form of bio-oil.

Pyrolysis can be of three types. Fast pyrolysis, in which biomass are subjected to high heat transfer rates and low residence time to maximize bio-oil generation (Bridgwater 2012b). Slow pyrolysis, in which biomass are subjected to slow heating rates and longer residence time to maximize char production (Bridgwater 2012b). It is a typical process by which charcoal is produced. The third variant of this process is Intermediate pyrolysis. It is characterized by low heat transfer rates, short residence time for vapors and longer residence time for solids. The intermediate pyrolysis technology, developed by EBRI at Aston University, Birmingham, U.K. (Hornung 2011) is a process which reduces the formation of high molecular tars and produces dry and brittle char. Intermediate pyrolysis works

with a reactor system called "Pyroformer" which is a specially designed reactor to process straw pellets and to separate ash rich residues from fuel on a continuing basis.

Liquefaction is the conversion of biomass into a stable liquid hydrocarbon using low temperature and high hydrogen pressures. Interest in the liquefaction process is low due to the requirement of high pressure parts, especially reactor and fuel feed system, and complexity of the process. High cost and complexity deter the growth of the liquefaction process.

Franz Fisher and Hans Tropsch proposed syngas production from biomass. Under this process, biomass is first converted into gases by way of gasification process, and using these gases CO and H_2 gases are converted into liquid fuels, with the help of a metal catalyst. The technology suffers from disadvantage of production of large products due to polymerization of fuel. This wax-like material is required to undergo complex hydro-cracking reaction (McKendry 2002). Under hydro treating or hydro thermal upgrading, liquefaction is the process that converts biomass into partly oxygenated hydrocarbons under wet environment and at high pressure (McKendry 2002).

6 Summary

It is very important for an agrarian economy like India to appreciate its waste biomass resources. It is essential that an extensive use of these resources be made so that the huge availability of biomass can be utilized to address the issues of energy scarcity in the country. Biomass is the only renewable energy source capable of storing carbon in its structure. With the advent of efficient technology and the use of second-generation biomass fuels, it is possible to control the emission arising from the use of agro residue. India has traditionally been an agrarian economy and has the potential of large biomass waste. However, currently these biomasses are not efficiently utilized, remain unattended and are allowed to decay. This may result in the generation of greenhouse gases like CH_4, which are 21 times more severe compared to carbon dioxide to cause global warming phenomena. Any efficient use of biomass would not only provide much wanted electricity, but will also provide an efficient alternate to the use of agro residue. The biggest limitation of the use of biomass under thermal application is its moisture content and release of soot particles. Biomass can also be treated under biochemical and thermochemical conversion processes. It is observed that anaerobic digestion and pyrolysis are promising technology alternates available for treatment of biomass under these options. It is concluded that biomass when used in the industrial unit as a fuel, several factors need to be addressed before such switch-over could take place. Treatment of biomass using biochemical and thermochemical conversion option is fast developing and may lead to an economic and efficient alternative.

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Biomass Gasification and Sustainability Assessment of Biomass Utilization

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Abstract India, being a predominately agrarian country, large amount of biomass is abundantly available throughout the country. This biomass, if effectively utilized, could generate off grid electricity, reduce environmental burden from fossil fuels, and can help to achieve sustainable living. In order to achieve these requirements, effective and efficient use of the biomass is the key. The purpose of this chapter is to discuss biomass gasification technologies that are suitable for sustainable energy generation. Additionally, sustainability in general and sustainability aspects of gasification technology (to review how much these technologies are sustainable in the long run) are also discussed. A technology is said to be sustainable if its implementation results in improvement in the execution of all the three pillars of sustainability simultaneously, namely environmental, economic, and societal. This chapter discusses the implication of gasification technology for all three pillars of sustainability. Section 1 discusses the gasification in brief and its types. Section 2 covers discussion on sustainability and its three pillars: environment sustainability, social sustainability, and economic sustainability, with the help of relevant studies. Section 3 summarizes the discussions in this chapter while Sect. 4 concludes the chapter.

1 Gasification

Gasification is a process in which biomass is converted into combustible gases. This is a thermo-chemical process, which requires high heating of biomass. Thermo-chemical degradation of biomass results into vapor and other gaseous matter. The gasification process can be either direct (using oxidant) or indirect (transforming heat

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to biomass by heating the reactor). The reaction goes to make synthesis gas or producer gas. The gas created by gasification of biomass is of low thermal energy and can be utilized to produce residential heat or to run the gas engines or gas turbines. Gasification is a thermal degradation generally carried out in the presence of an externally supplied oxidizing agent, e.g., air, steam, or oxygen. The reactor, which facilitates these reactions are known as gasifiers. Gasifiers are simple in design and easy to construct, however, its operation is not so simple. Its operation is governed by a complex combination of temperature, air supply, and other operating variables (Reed et al. 1988). Based on the principle of operation, the gasifiers are updraft or downdraft type. Typically, updraft gasifiers generate up to 20% of tar-oil, on the other hand, downdraft gasifier possesses less than 1% tar-oil. Therefore, former is suitable only for thermal applications, while latter can also be considered for power generation applications. Typical composition of producer gas from biomass is CO_2–9.7%, CO–21%, H_2–14.5%, H_2O(v)–4.8%, CH_4–1.6%, N_2–48.4%, higher heating value on wet basis as 5,506 kJ/Nm^3, and air ratio required for gasification is around 2.38 kg of wood/kg of air, while the air ratio required for the combustion of these gases is around 1.15 kg of wood/kg of air (Reed et al. 1988).

Biomass gasification is a technology, which is useful to process, not only woody biomass but also biowaste (moisture <20%), which is having low or negative disposal value, to generate heat and power. These waste materials otherwise would be burned inefficiently and on combustion, generate harmful gases and invite environmental problems. If not allowed to burn, it can undergo the processes of natural decomposition or decay that would generate greenhouse gases like, methane which has 21 times higher global warming potential compared to carbon dioxide (Adams 2011). On storing, biomass would attract transportation cost and corresponding indirect emissions due to the use of fossil fuel. On using as landfill, it would incur landfill tax in certain advanced countries. Therefore, increasing knowledge and use of biomass gasification technology to process biomass would be advantageous to the society (Adams 2011). High gas generation rate at a low gestation period is a major advantage in favor of biomass gasification vis-à-vis other technology options, that includes, methanization by anaerobic digestion or production of biodiesel through transesterification. Biomass gasification can offset the use of fossil fuel when employed to generate power and offers an option of clean burning of biomass. Provided biomass is obtained from a sustainable source and technology used for combustion or conversion is appropriate.

Replacement of fossil fuel by producer gas for several thermal applications is technologically an easy option for an agrarian country like India and at the same time it can conserve precious foreign exchange and improves energy reliability. Biomass gasification can also employ for power generation. Due to lower bulk density, transportation of biomass is not a cost-effective option. Under such circumstances, power generation by biomass gasification at a remote location not only solves waste disposal issues but also offers much needed off grid electricity for the rural area, in an environment-friendly manner.

Figure 1 shows the gasification of a small particle, its state at different temperature levels, and the corresponding gas, steam, and vapor generation.

The beginning stage of biomass heating involves drying, where the moisture from the particles and also the structural moisture get discharged in the form of water vapor. It shrinks the biomass to some extent and also changes its color from brown to dark brownish. Further increase in temperature of biomass results into preheating of biomass (before actual thermo-chemical conversion) and pyrolysis. Under this, decomposition of biomass takes place and this contributes to the liberation or release of volatiles. Heating of biomass vaporizes the volatile portion of the feedstock (devolatization), the volatile matter consists of gases (H_2, CO, CO_2, CH_4, and hydrocarbon gases), tar and water vapor and the phase is also known as pyrolysis. Some of the tar and hydrocarbons in the vapors convert into smaller molecules at this stage by means of thermal cracking. Steam follows reaction along with carbon dioxide to form carbon monoxide and hydrogen (also called as gasification reaction), this associated with the reduction in the biomass size. A further increase in the temperature leads to partial combustion of biomass (under the limited supply of air or oxygen) as well as the way in which biomass reacts at a surface due to the reaction of delayed volatile particles, releasing and liberating from the inner core of the biomass. At this stage, initiation of thermal cracking of the volatiles as well as gases starts. Prolonged exposure of these liberating volatile particles and gases in the environment of higher temperature leads to the secondary cracking reaction of the constituents, which results into gases and tar. At higher temperature, the tar is subjected to further complex cracking reactions and releases gases and cock-like substance. Tar is basically a long chain molecule which in most of the cases leads to irreversible thermal degradation in the form of solid structure on the surface at which the reaction takes place. The delay in the release of volatile particle is due to the low value of thermal conductivity of the biomass, which leads to the large temperature gradient in the biomass. These volatiles react to the surface of biomass as well as in the gas–gas type of reaction and release gases. These reacting gases and additional heat further convert volatiles into the gases and contribute to the formation of producer gas. The requirement of heat for these reactions is supplied by the partial combustion of biomass that is taking place in the reactor and is made possible by supplying a limited amount of oxygen (pure or along with the air) into the reactor. There is char-to-gas and gas-to-gas reaction that changes the final composition of gas, prominent among all is water–gas shift reaction. The result of these reactions produces H_2, CO, CO_2, and CH_4 (the exact concentration depends on several other factors) and the mixture of these gases is called producer gas.

Biomass gasification takes place in a two-step process, pyrolysis followed by gasification. Following reactions takes place in a process of gasification (Rajvanshi 1986; McKendry 2002; Lubwama 2009):

Combustion occurring in the oxidation zone

$$\text{Partial oxidation } (C + 1/2\,O_2 \rightarrow CO + 123\,\text{MJ/kg mole}) \quad (1)$$

$$\text{Complete oxidation } (C + O_2 \rightarrow CO_2 + 393.8\,\text{MJ/kg mole}) \quad (2)$$

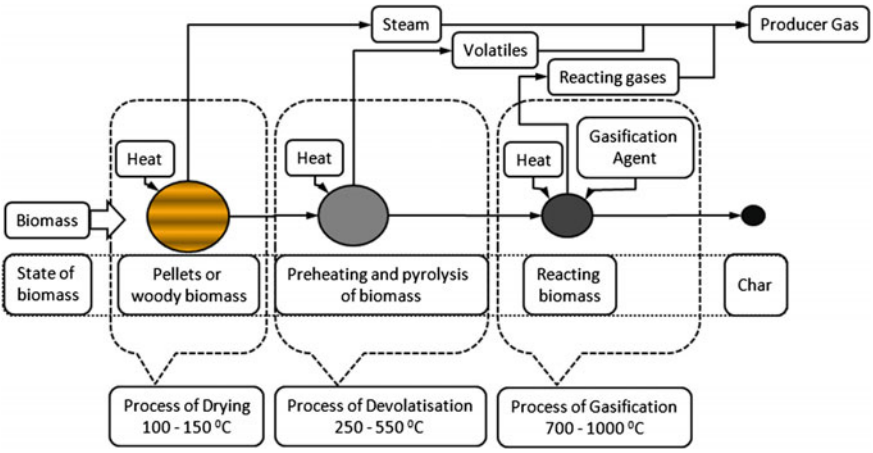

Fig. 1 Gasification of a small particle (Adapted from Adams 2011)

$$\text{Methane formation reaction}(C + 2H_2 \rightarrow CH_4) \tag{3}$$

Equations (1–3) are exothermic and provide autothermal gasification and provide feasibility for endothermic reactions for drying, pyrolysis, and reduction zones. Water vapor resulted from drying and due to presence in air reacts with the hot carbon results in a heterogeneous reversible water gas reaction, due to this H_2 generated at this stage is considered as unstable

$$\text{Water gas reaction}(C + H_2O + 118\,\text{MJ/kg mole} \leftrightarrow CO + H_2) \tag{4}$$

Water gas reaction is followed by boudouard reaction

$$\text{Reduction reaction}(C + CO_2 + 159\,\text{MJ/kg mole} \rightarrow 2CO) \tag{5}$$

Due to above endothermic heterogeneous reactions increase in the CO and H_2 at high temperatures and at low pressures is observed in the reactor. Under these conditions, several reductions take place, out of which water gas shift reaction and methanization reaction are important. Both of these reactions will tend to consume major portion of unstable hydrogen, generated during water gas reaction, as per Eq. (4).

$$\text{Water gas shift reaction}(CO_2 + H_2 + 40.9\,\text{MJ/kg mole} \leftrightarrow CO + H_2O) \tag{6}$$

$$\text{Methane formation reaction}(C + 2H_2 \rightarrow CH_4 + 84\,\text{MJ/kg mole}) \tag{7}$$

The partial combustion of biomass releases around 65% of the energy compared to complete combustion Eqs. (1) and (2). Advantage of partial combustion is the production of steam, hydrogen, and carbon monoxide, which can react with the

volatiles and produce useful gases. These gases have combustion value and can be used for obtaining energy. Out of all water gas reaction, Eq. (5) and water gas shift reaction Eq. (6) are important, which results in the release of useful by- products in the form of carbon monoxide. The larger recovery of carbon monoxide from carbon dioxide increases the thermal value of the producer gas. Such recovery is enhanced by providing charcoal bed in the reactor, which acts as a catalyst. Producer gas consists of a mixture of CO, H_2, CH_4, and water vapor. Producer gas has a considerable amount of nitrogen (around 50%) due to the use of air as gasifying agent. This large component of nitrogen results in the reduction in the calorific value of the gases. The use of pure oxygen as a gasifying agent may overcome this issue. However, high cost of oxygen is a limiting factor, owing to low calorific value of producer gas. However, the same is justified if the end product is energy-intensive fuel like methanol (Reed et al. 1982).

Producer gas composition can be further controlled by obtaining more complete gasification of biomass, this reduces undesirable components at the gasification step. It is found that increase in gasification temperature has a positive effect on the reduction of various hydrocarbons. Reducing the residence time for the feedstock is another factor to improve gas quality. This is achieved by reducing the particle size of the feedstock. Small particle size and lower residence time for the feedstock can improve the gas quality. Figure 2 shows the variation in the composition of the producer gas with an increase in moisture content in the wood. As the moisture content in biomass increases, CO recovery from the producer gas decreases and therefore CO_2 in the producer gas increases. In spite of, higher moisture in biomass increase the hydrogen generation rate, the overall net calorific value of the gas decreases due to the presence of large quantity of CO_2.

The mass flow rate of air is very crucial for determining temperature of producer gas and quality of gas generation. For atmospheric air gasification, increase in temperature in producer gas is observed from a very low equivalence ratio (Ø) of 0.4 up to 0.9. However, beyond this range, increase in equivalence ratio decreases the temperature of producer gas and reaction will switch more to combustion rather than gasification. Heating value (per volume of gas) will continuously decrease with increase in equivalence ratio, irrespective of any value of equivalence ratio.

1.1 Fixed Bed Gasifier Designs

Fixed bed gasifiers are the oldest in design, relatively simple, and less complicated to operate, under this the movement of biomass relative to the gas flow is very slow, and biomass almost remains stationary over a reacting bed. Consequently, these types of design are known as fixed bed design. Three variations of this design are in use: they are updraft, downdraft and crossdraft design. Figure 3 shows a brief classification of different gasifiers. Figure 4 shows the path of air, gas, and biomass in these three fixed bed gasifiers.

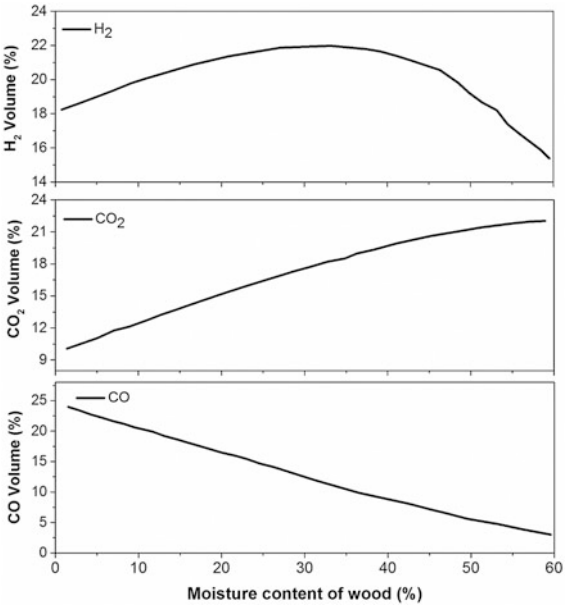

Fig. 2 Producer gas composition as a function of moisture content in wood (Adapted from Cala et. al 1986)

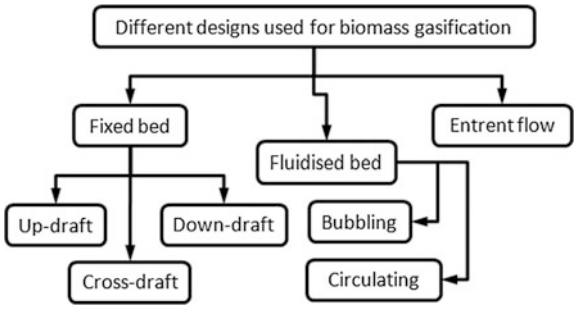

Fig. 3 Classification of different designs of gasifiers

Figure 5, shows a typical biomass gasifier of updraft and downdraft design, showing clearly the reacting zones and temperature levels for these zones. The bed, i.e., reacting front in the biomass, in updraft gasifier moves from bottom to top. The different zones of biomass gasifiers are as shown here. The biomass feed dried by using heat from the gas leaving the gasifier. The pyrolysis zone is the next, it is

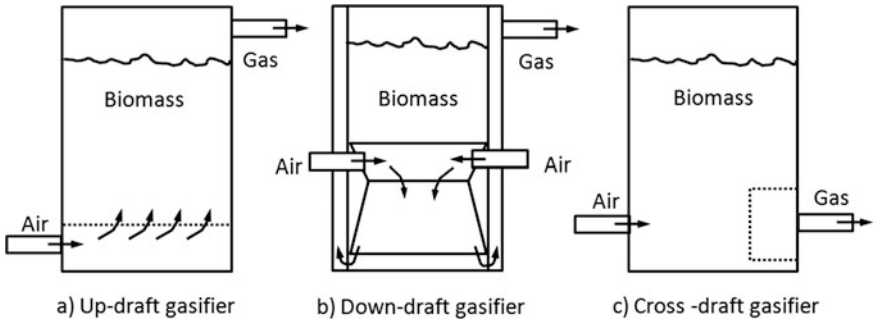

Fig. 4 Different designs of fixed bed gasifier

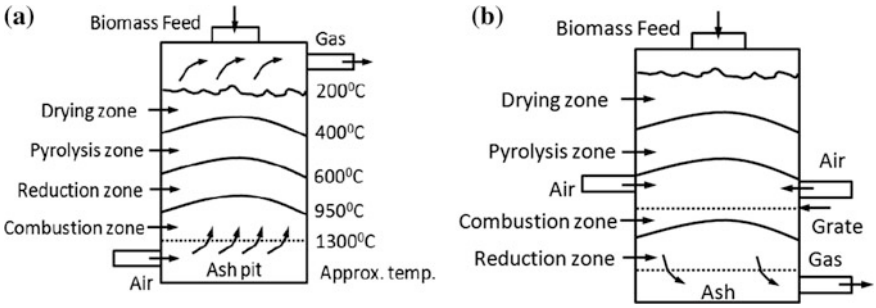

Fig. 5 Biomass gasifier **a** updraft design and **b** downdraft design

under this zone that the thermo-chemical degradation of biomass takes place. Therefore, drying zone is always at the top and pyrolysis zone is next, however, position of reduction and combustion zone is reversed for updraft and downdraft design. Reduction zone is the zone in which gases come across the hot char bed, which acts as a catalyst and encourage reduction reaction in which CO_2 from the flue gases gets converted into CO in the presence of hot char. Combustion zone is located where the air is entering and interacting with the biomass first. In case of updraft design, significant amount of tar presents in the gases leaving the pyrolysis zone, as this is the zone where significant amount of thermal degradation of biomass occurs. In case of downdraft design, these gases pass through the high-temperature combustion zone followed by a reduction zone (unlike updraft design). This ensures high recovery of CO in the final gases and significantly low tar content. Advantages of updraft gasifiers are its simple design, high charcoal burnout, lower gas exit temperature, high thermal efficiency, and wide range of flexibility as far as feedstock is a concern. However, this type of designs is prone to vapor locking and in extreme cases can lead to explosive situations. The efficient design of grate plays an important role in sound operation. Requirement of disposal of the tar as well as condensate that produces at the gas cleaning device are also

important for this type of design. Therefore, this type of design is preferred only for thermal applications (FAO 1986).

Downdraft gasifier was widely used during Second World War as an onboard fuel gas generator to offset lack of gasoline (Overend 2004). Compared to updraft gasifiers, downdraft designs face constrain in scaling and can handle a particular type of fuel for which it is designed. While updraft design has fewer restrictions in scaling, the gas generated carry lots of tars and methane. Due to this, updraft designs are not suitable for the gases used for the power generation application. Another advantage in favor of downdraft gasifier is that it has good turndown ratio compared to updraft design, and can handle large variations in the load. Yet, the higher capital cost limits its use for thermal applications. Problem of tar formation is more severe in the case of updraft design and calls for frequent cleaning and maintenance. In case of downdraft design, the air is supplied beneath the pyrolysis zone, hence combustion of the biomass takes place below the pyrolysis zone. Since all the pyrolyzed vapor and gases passes through the combustion zone, the gases have the opportunity to burn the tar carried along with it. It is also observed that pyrolysis of biomass release large amounts of particles along with the gas, passing of gas from combustion zone and reduction zone release most of the particles and the gas obtain have low tar and low particles. However, at higher turndown ratio, higher tar is expected. A tar-free turndown ratio of 1:3 is ideal, while a value of 1:5–6 can be considered as very common. Major limitation of the downdraft design is that it cannot operate with the different number of unprocessed fuels. Fluffy, low density, and thin material like straw etc., may cause flow problem and excessive pressure drop in these types of gasifier designs. Making pellets or briquettes from a mixture of feedstock is a good strategy under this circumstance (FAO 1986). It is important to note that financial burden of preparing pallets are very low, if pellets are made in bulk (at 1 t/h capacity). Pellets making increases bulk density of biomass, that gives higher capacity to store pellets and at the same time reduces the moisture to a low level (<7%). Downdraft type of design required handling gas with lower tar content and consequently average quality of charcoal at the reaction bed is suffice. Figure 5 shows the different zones in case of a downdraft gasifier.

For updraft design, if mass flow rate of air fed to gasifier (when fed at the interface between biomass and char) is high, it results in large amount of char, under these conditions, the air consumes excess char rather than biomass. Since char consumption rate is high, more biomass can be consumed. Therefore, operation of updraft gasifier is assumed as self-regulating (Reed et al. 1988). While for downdraft design if air quantity is high, this can reach to char bed along with volatiles and tar, this can consume char bed at a faster rate. If this rate is faster than the char production, it is possible that char bed may soon replace by partially burning biomass pieces (not fully burned biomass in form of hot char). This in effect consumes char bed and prevents conversion of CO from CO_2, and resulting in poor gas quality. Therefore, maintaining char bed is crucial in the operation of downdraft gasifiers. Since during operation, chocking of bed occurs more frequently in downdraft design, it is equipped with a pressure drop measurement

device many times. The pressure drop across the bed is continuously monitored during operation. Additionally, the removal of hot char (from bottom) and feeding of fresh charge (from top) should synchronize with the gas generation rate, many manufacturers use an on–off timer to control the char discharge rate. In order to ensure continuous movement of biomass in the reactor, once it enters from top, many downdraft manufacturer employ external vibration generation system usually appended on the walls of the reactor.

Crossdraft gasifier is in the characteristics that lie in between updraft and downdraft gasifies. They are generally of a small-scale device, and associated with the simple gas cleaning device (a cyclone and hot filter) and can be used to serve small engines. One of the disadvantages with the crossdraft gasifier design is gas generation with large tar content. High-quality charcoal is required to crack large tar production.

1.2 Fluidized Bed Gasifier

Fluidized bed gasifier (FBG) came into existence in 1920 (Overend 2004). Under this, the biomass of small size are crushed and circulated in the gasifier. This type of design does not face limitation of scaling, and are flexible to the size of the particle. The risk of slagging and fouling is higher in case of fluidizing bed—this is controlled by observing temperature of the bed in the close range. This may result in incomplete decomposition of feedstock. Another problem in the case of fluidized bed gasifiers is the problem of dust in the gas (Kavalov and Peteves 2005). Under fluidized bed design, the grate is removed and biomass and air is allowed to mix in a highly turbulent mixing zone. Higher heat transfer rates are achieved by reducing the size of biomass and higher turbulence intensity of air, which is gasifying media, due to this high throughput of the order of 1500 kg/m^2 h can be achieved. The advantage of fluidized bed gasifier is a larger residence time of the gas and smaller biomass particle size. This allows for better cracking of the pyrolyzed products and subsequently lower tar particles in the final product. However, secondary tar particles be still there in the gas. Additionally, this type of design is extremely sensitive to the moisture content of the biomass. The technology is complex since heavy blowers are required to inject air at the bottom of the bed observing a higher pressure drop. The volume of the gas as it leaves the bed and gets circulated till the cyclone increases, due to rise in temperature and also due to release of gas from biomass due to decomposition (Overend 2004). The advantage of the FBG is their flexibility to handle feedstock, easy control of temperature, can allow feedstock having low ash melting point (rice husk). However, it has high tar content (≤ 500 mg/Nm^3). These designs are associated with incomplete burnout, also FBG design operation is sensitive to velocity of gas and particles, any load change can cause a detrimental effect on the tar formation and quality of gas (FAO 1986).

Two popular variants under this type are: one is bubbling fluidized bed (BFB) gasifier and another is circulating fluidized bed gasifier, as shown in Fig. 6.

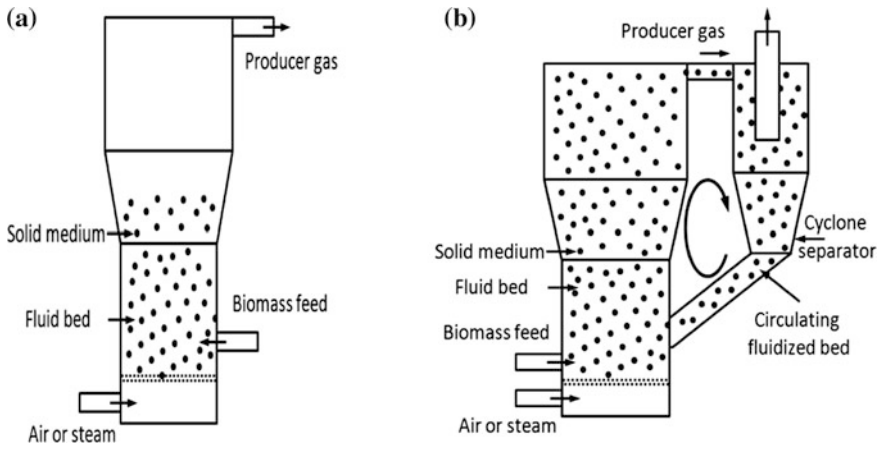

Fig. 6 Fluidized bed gasifier **a** bubbling and **b** circulating fluidized bed gasifier

In BFB, air is allowed to pass from the bottom in an inert bed of silica sand, dolomite, alumina, and olivine. The bed is expanded and remains in dynamic motion due to the flow of fluidizing gas medium entering into the bed from the bottom. This gives the higher terminal velocity of particles and particle floats over the body. If the terminal velocity of the gas flow is increased to such a level that the particles in the bed is transferred out and flow along with the gas, then this design may be termed as circulating fluidized bed. The flowing particles may even reach to the cyclone separator where it is released and allowed to be recirculated (Overend 2004).

The entrant flow gasifier, as shown in Fig. 7, operates at a pressure of around 50 bar and temperature of the reactor is 1300 °C. The residence time of the biomass is also shorter, of the order of only a few seconds. At such a short residence time, complete transformation of biomass is possible if the feedstock is having much smaller size, typically less than 0.1 mm in diameter. At such an extreme condition, thermal treatment of tar is complete and the obtained gas is almost free of the tar. The complete cracking would also result in high yield of CO and H_2. The high degree of conversion is due to the high reactivity of hot biomass. Its large surface area provides sites for the secondary cracking of pyrolysis vapors. Due to extreme conditions of pressure and temperature, the entrant flow gasifier is flexible with the type of the biomass material, and for a broad range of feedstock acceptability. It is possible to simultaneously gasify even biomass, coal, petroleum residue, and waste. At high operating temperature, the ash from the feedstock may turn into molten slag. Such molten slag can be removed from the bottom of the gasifier. To improve slag properties, addition of the fluxing material like silica sand or limestone is preferred.

Large amount of nitrogen in producer gas when used for power generation de-rate the capacity of the engine. It is very difficult to remove nitrogen from the producer gas once gasification is taken place. This is because of liquefaction route to remove nitrogen from producer gas is a costly option and is not economically

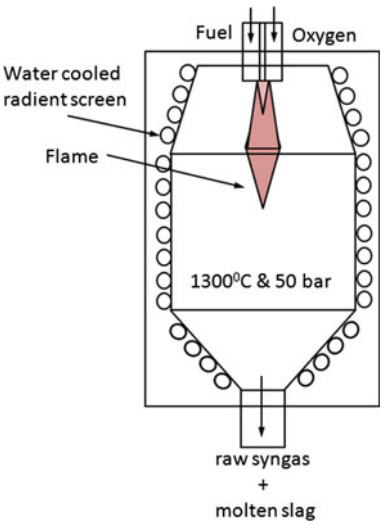

Fig. 7 Entrant flow gasifier design

Table 1 Main component and properties of gas from different gasification processes (Adapted from Kavalov and Peteves 2005)

Composition (vol.% dry)	A	B	C	D	E	F
CO	19.3	26.9	16.1	16.1	42.5	46.1
H_2	15.6	33.1	18.3	18.3	23.1	26.6
CO_2	15	29.9	35.4	46.9	12.3	26.9
CH_4	4.2	7	13.5	13.5	16.6	0
N_2	44.5	0.7	12.3	0.8	0	0.4
C_2H_6	1.4	2.4	4.4	4.4	5.5	0
NCV (MJ/m^3)	5.76	8.85	8.44	8.05	13.64	7.43
H_2/CO ratio	0.81	1.23	1.14	1.14	0.54	0.58

feasible (Kavalov and Peteves 2005). As a solution to this, the use of pure oxygen for the gasification is proposed, from the calorific value enhancement. Amount of other components (e.g., CO_2) can be minimized by more complete decomposition of biomass, which makes the gasification step more energy efficient and cost effective. Table 1 shows the produced gas composition when a different method of biomass gasification processes is adapted. They are A, which is atmospheric air blown direct circulating fluidizing bed gasifier. In this, the gas generated has a calorific value very low (5.76 MJ/m^3). Option B, C, and D are: gasification using pure oxygen under atmospheric pressure at direct circulating fluidized bed gasifier, second pressurized with nitrogen and oxygen at direct circulating fluidized bed gasifier, and third pressurized with carbon dioxide oxygen at direct circulating fluidized bed gasifier, respectively. Since all process used pure oxygen, the calorific value of each option has been in a narrow range (8–9 MJ/m^3). Option E is atmospheric steam blown indirect gasification, which gives a very high calorific value of

the gas (13.64 MJ/m^3). Finally, option F, is pressurized oxygen used at the direct entrained flow gasifier, although it has moderately high calorific value (7.43 MJ/m^3) its advantage is that it has a high fraction of CO and H_2 and a very low value of N_2 gas (Kavalov and Peteves 2005).

2 Sustainability Assessment

It is essential for any country to make the best use of the available biomass in such a way that the technology should make economic return, leading to emission reduction, and achieve social benefits. It is essential to assess the sustainability aspect of gasification technology too. The following sections provide an assessment of biomass gasification across all the three pillars of sustainability viz., economic, environment, and social aspects of sustainability.

2.1 Introduction

The first official use of the word sustainability (German translation: Nachaltigkeit) is found in 1713 in the book *Sylvicultura Oeconomica* written by Hans Carl von Carlowitz. The term has become popular after the Brundtland report, published by the United Nations, defined *sustainable development* as development that "meets the needs of the present generation without compromising the ability of the future generations to meet their own needs" (Brundtland 1987). The report caused a significant impact on the workplace and future direction of the nations, governing bodies and people.

In the 1980s, Swedish oncologist Dr. Karl-Henrik Robèrt played an instrumental role in building a consensus on the need for a sustainable society. In 1989, Robèrt (Heinberg 2010) proposed four fundamental requirements for the condition of sustainability, which in turn became the base for sustainability for any establishment. He termed these requirements as the "Natural Step". The term became popular and many commercial enterprises and municipalities around the world appreciated and adopted the "Natural Step" conditions. The four conditions required to fulfill are: In a sustainable order, nature is not subject to systematically increasing; (1) concentrations of substances extracted from the earth's crust, (2) concentrations of substances produced by society, (3) degradation by physical means and, in the society, (4) people are not subject to conditions that systematically undermine their capability to fulfill their demands. It is agreed that if the above four conditions are followed, the sustainability can be achieved.

The progress in the sustainability proceeded further with the concept of triple bottom line approach. "Triple Bottom Line" (TBL) was introduced by environmentalist and economist John Elkington in 1997. The term is now widely used in discussions on sustainability. Elkington identified three pillars of sustainability

(social, environmental, and economic) and stated that the overall sustainability can be accomplished by attaining sustainability for these three segments simultaneously. Elkington's expression of sustainability helps build increasingly widespread view that, it is essential to achieve at least a basic degree of all three from of sustainability to achieve a desired degree of ecological, or social or economic sustainability, separately (McKenzie 2004). Figure 8, shows the interrelationship of environmental, societal, and economic sustainability. From the figure, it is easy to note that the trinity views of sustainability are interdependent of each other. For example, economic production is possible depending on the availability of natural resources as well as human capital, which is a component of social sustainability. Also, the social growth is possible only from the level of income and the level of income is dependent on a healthy environment.

Considering that environmental issues are the prime drivers for the sustainability, the environmental sustainability and its effective inclusion in the present Life Cycle Analysis (LCA) is pertinent for sustainable technology assessment (Janeiro 2011). The methodology given under general guide for life cycle assessment as proposed by ILCD handbook is widely practiced for the environment sustainability (ILCD Handbook 2010). The prime focus for such LCA is on the environmental pillar, with due consideration to the economic and, to a lesser extent, the social dimension of sustainability (Heijungs et al. 2009). Cradle-to-cradle approach of LCA is a specific kind of cradle-to-grave assessment, where the end-of-life disposal step for the product is a recycling process. It is a method used to minimize the environmental impact of products by employing sustainable production, operation, and disposal practices. In the cradle-to-grave approach of LCA, environmental impacts occur throughout the lifetime of a product from raw material extraction, manufacturing and processing, distribution, use, repair, and maintenance, disposal, and recycling (Life Cycle Thinking 2014).

Social sustainability occurs when the formal and informal, processes, systems, structures, and relationships actively support the capacity of current and future generations to create healthy and livable communities (McKenzie 2004). This implies that the objective of social sustainability is to try and ensure that future

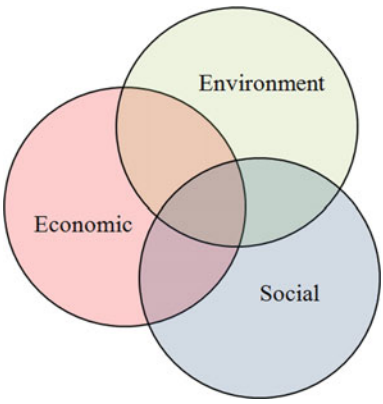

Fig. 8 Conceptualising sustainability (Adapted from Hodeson 2002)

generation should have same or greater access to resources as the current generation by implementing required processes, adopting suitable systems, organizing necessary structures, and building healthy relationships. This means that measurements of social sustainability are healthy and livable communities. Although a social dimension to sustainability is widely accepted, exactly what this means has not been very clearly defined and agreed (Bramley et al. 2006). However, recent authors tried and streamlined the procedure of conducting social sustainability (Benoît et al. 2009).

The economic sustainability, also known as cost–benefit analysis, deals with the assessment of risk and return from a project or investment. Primarily, it deals with the financial aspect, the lack of provision to consider the risk into such analysis reduces its strength. Ignoring risk into the analysis is invalid, yet at the same time, it is also true that some of the risks cannot be measured, e.g., the problem of determining the optimal rate of emissions reduction and the cost associated with it (Diesendorf 2007). Life cycle costing (LCC) is a widely accepted methodology to assess the costs of production under economic aspect of sustainability. LCC allows detailed viability assessment of the projects considering all the costs right from acquisition to operate and to disposal and allows optimizing the economic performances of enterprises. International Organization for Standardization (ISO) defines LCC as a methodology for the systematic economic appraisal of products/processes (ISO 2008) by classifying costs into construction, management, maintenance, and end-of-life cost categories (Biondini et al. 2008). LCC is a useful technique while comparing competing alternatives for projects with different costs and benefits and having different spans of time (Kaan Ozbay et al. 2003). The advantage of LCC is that it can determine the economic success or failure of a product in the market, yet it does not entirely represent the complete economic dimension. LCC fails to handle: uncertainty, irreversible decisions, neglects environment concern, future environmental calamity, availability and reliability of input inventory, and influence of decision maker's personal values (Real 2010). LCC analysis also fails to consider cost added due to the intangible aspects of product, e.g., user's comfort or cost of going for greener building options, selecting recyclable or natural material (Fuller and Petersen 1996). In spite of its widespread use, it is noted that a comprehensive methodology and detailed guidelines are yet to be published (Benoît et al. 2009).

2.2 *Environmental Sustainability for Biomass Gasification*

LCA is considered to be the most comprehensive approach to assessing the environmental impact. LCA started in the early 1970s, initially to investigate the energy requirements for different operations. It is a method in which the energy and raw material consumption, different types of emissions, and other important factors related to a specific product are being measured, analyzed, and summoned over the product's entire life cycle from an environmental point of view. Emissions were added afterward to the orbit of the LCA.

LCA is the method of choice in recent years for new technology alternatives from Bioenergy (Pant et al. 2011). To estimate energy and emissions resulting from a given bioenergy alternative against a conventional means of generation of energy, a thorough evaluation of the LCA must be carefully carried out (Singh et al. 2010). LCA is a systematic, analytical, and a comprehensive method to identify, evaluate, and minimize the environmental impacts of a specific process (Morrison and Sinclair 1998). It considers all the stages of a product's life cycle, such as, raw material, processing, manufacturing, use, and end-of-life scenario (Williams 2009). According to ISO standard 14040 and 14044, consists of four main activities (Fig. 9). First one is the Goal definition (ISO 14040), in which the basis and scope of the evaluation are defined. Next is Inventory Analysis (ISO 14041), which creates a process tree in which all operations from raw material extraction through wastewater treatment are mapped out and connected and mass and energy balances are obtained (all emissions and consumptions are accounted for). This is accompanied by an Impact Assessment (ISO 14042), under which emissions and consumptions translate into environmental effects. Their environmental effects are grouped and weighted. Finally, Improvement Assessment/Interpretation (ISO 14043), under which areas for improvement are identified. Actively involved in the definition and scope consists of determine need, data specificity, decide collection method, and data presentation. Under life cycle, inventory phase involves carrying out process diagrams, data aggregation, and evaluation of the information. While life cycle impact assessment deals with defining impact categories and their weights, and subsequent results. The final report presentation includes significant data, data evaluation and interpretation, final conclusions, and recommendations (Williams 2009). LCA is conducted by consistent, robust, and quality-assured life cycle studies, following ISO compliant procedure, and observing the principle of best practice, reliability, efficiency, flexibility, fairness and acceptance, transparency and reproducibility, and quality (ILCD Handbook 2010).

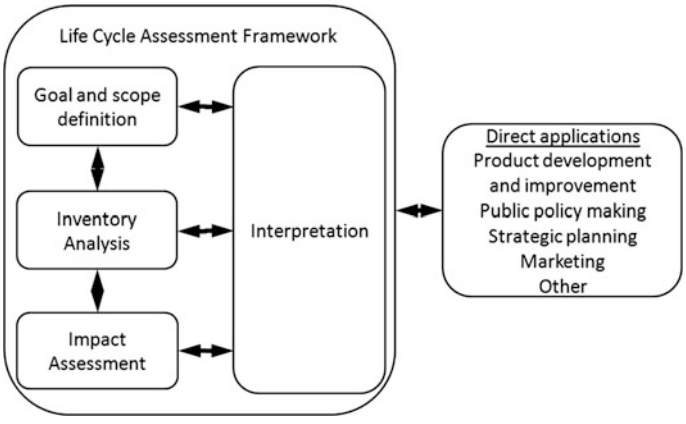

Fig. 9 Life cycle assessment framework as per ISO 14040 and 14044, 2006 (Adapted from O'Connell 2005)

The outcome of LCA study is a quantitative determination of environmental impact and opportunities for improvement by quantitatively justifying a change in the process, and to compare and analyze several processes for their environmental impacts conveyed through the instruments such as eco-labeling, eco-design, carbon footprinting, and green public procurement (ILCD Handbook 2010). In other words, LCA can help in determining environmental hot spot in the process chain so that alternatives may be evaluated to reduce overall environmental impact (Hassan, Jaramillo, and Griffin 2011).

2.2.1 Some Relevant Studies on LCA

Focusing on Indian emission inventory for the year 2000, which is shown in Fig. 10, it is observed that the emissions from power production activity is largest, followed by emissions from industry and from transport activity, both 14%. However, non-energy emissions, i.e., agriculture category also contributes 14% of emission. This implies that the effective use of agricultural products for power generation by means of efficient technology leads to the dual benefit of emission reduction. The role of gasification can fulfill this gap, it can provide power from the use of agricultural waste and on the other hand, it can avoid emission that would have caused due to inefficient use of agriculture waste.

In general, bioenergy has potential to substitute the use of fossil fuel and reduce the effect of CO_2 released into the environment. LCA is widely regarded as one of the best methodologies for the evaluation of environmental burdens associated with Bioenergy production. Use of life cycle study provided vital information regarding the way land used for the production of fuel, embodied energy used in the construction phase, particulate emissions during gas generation, ecotoxicity due to the emissions, the effect of climate change on the health of human, etc. (Adams 2011).

Emission is a cause of concern the way agro-residues are currently burning in the open fields of Punjab, India. To remove the straw and prepare the field ready for the

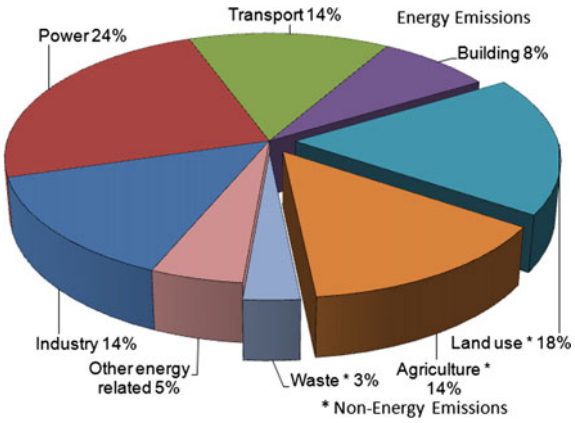

Fig. 10 GHG emission by source for the year 2000 (adapted from Singh and Gu 2010)

next harvest, farmers follow widespread burning of the residue. Emission due to open field burning of agricultural residue is a significant component, comprised of approximately 61% in Asia and 39% for the rest of the world (Garg 2008). Statistics suggest that burning of agro-residue has become a common practice in certain parts of the world, given that currently less than a quarter of rice straw in India, around half in Thailand, and almost all in the Philippines are being burned in open field (Adam 2013). The burning of straw results in emissions of gases such as CO_2, CO, CH_4, N_2O, NO_x, aerosols, and non-methane hydrocarbon (Sharma et al. 2010). The burning of 1000 kg of straw generates approximately 3 kg of particulate matter (0.17%), 60 kg of CO (3.5%), 1460 kg of CO_2 (85%), 199 kg of ash (12%), and 2 kg of SO_2 (0.12%) (Gupta et al. 2004). Burning of straw also leads to adverse health effects (Kumar and Kumar 2010). A study on the nutrient replacement need (Hermann et al. 2005) due to the growth of biomass revealed that uptake of deciduous plants in terms of different elements such as carbon, nitrogen, potassium, phosphorous, calcium, and magnesium, are present in the ratio of 2000–20,000:100:64:15:7:10. Straw is the only organic material available in significant quantities to most rice farmers. About 40% of the nitrogen (N), 30–35% of the phosphorus (P), 80–85% of the potassium (K), and 40–50% of the sulfur (S) taken up by rice remains in vegetative plant parts at the time of the maturity of the crop, refer statistics in Table 2. Open field burning causes almost complete loss of *N*, about 25% loss of *P*, about 20% loss of *K*, and 5–60% loss of *S* (Dobermann and Fairhurst 2002). The study (Lee and Atkins 1994) also revealed that the straw and stubble burning in open field are another source of ammonia, which is till now not accounted in conventional greenhouse gas inventory.

A study carried out by Adam (2011) which is based on producing heat and power using biomass gasification technology compared to options like natural gas or diesel operated power generation system. The aim of the study was also to determine important factors that affect the environment in case of power generation using biomass gasification technology. Findings revealed that upstream emission (i.e., emission incurred in building plant and facility) are responsible for metal resource depletion, since biomass gasification requires high plant liability because of technological complexity. Regarding the emission effect using biomass, during the operation of the plant is concerned, in case of use of biomass, the impact categories like fossil fuel depletion, climate change, and particulate matter formation are relevant. The factors responsible for this are: requirement of wood pre-processing, electricity consumes during operation, and use of natural gas for the start-up. The study suggests that particulate matter released from the combustion of producer gas is relatively high. However, the overall operation of the plant shows

Table 2 Micronutrient requirements (Adapted from Dobermann and Fairhurst 2002)

	Nutrient removal (kg nutrient/t)				
	N	P_2O_3	K_2O	Mg	Ca
Rice grain	10.5	4.6	3.0	1.5	0.5
Rice straw	7.0	2.3	17.5	2.0	3.5
Rice grain + straw	17.5	6.9	20.5	3.5	4.0

that total emissions are low compared to conventional natural gas or diesel operated power generation system. Overall, it can be suggested that biomass gasifier-assisted power generation plant has a low environmental footprint compared to natural gas or diesel operated power generation system.

It is debated that the issue of environmental sustainability is highly sensitive to the emission from the automotive sector, due to the fact that the options of substituting replacement of automotive emission are few. To estimate emission resulting from transportation, Bartolozzi et al. (2013) undertook a study under the project "*Filiera Idrogeno*" (Hydrogen Chain) to determine a life cycle investigation of the possible use of hydrogen as an automotive fuel obtained from different pathways in the Tuscany Region of Italy. The three hydrogen generation options are considered under this study. First, from electrolysis using wind electricity, second and the third, by direct separation of hydrogen in the biomass gasification process. The study shows that option of using of hydrogen obtained from renewable energy source of wind (first option) is the best among all, followed by electrolysis using electricity produced by biomass gasifier and direct separation of hydrogen in the biomass gasification process option, respectively. However, it is noted that inclusion of storage and distribution phase of hydrogen in the case of first and second options may change the order. The study also revealed that 3.28 MJ of energy is generated for every 1 MJ of primary energy inputs (Bartolozzi et al. 2013). The study proposed a promising opportunity for gasification technology considering that a hydrogen economy is tomorrow's reality.

In a study carried out by Mann and Spath (1997), life cycle assessment for the use of biomass are carried out for the production of electricity using a combined gasification cycle in a cradle-to-grave concept of LCA. The scope of the study considers the production of biomass as a dedicated feedstock, its transportation, and finally, the electricity generation. Upstream emission is considered in the analysis. The result in terms of CO_2 emission and energy production is shown in Fig. 11. Statistics revealed that against 1.9 kg of CO_2 production, around 15 kWh of electricity is obtained. The study concludes that life cycle efficiency and carbon closure, which is defined as proportion of carbon that is present in the biomass to the carbon released from the power plant, are very high for such energy pathways.

A study carried out by François et al. (2013) considers the most detailed approach of carrying out LCA for the biomass-assisted gasification plant. Under this, the sustainability of biomass energy chain considered right from the soil up to biomass vaporization process as shown in Fig. 12. The objective of this work was to consider most comprehensive LCA for the study. Different stages considered in the scope are: forest growth of biomass, its management and the wood valorization chain (including pulp, timber, etc., and energy), effect of fertilizer (involving carbon and mineral—N, S, Cl, P, K balances), transportation of biomass, and energy extracted under the biomass gasification unit. The study cautioned that mineral,

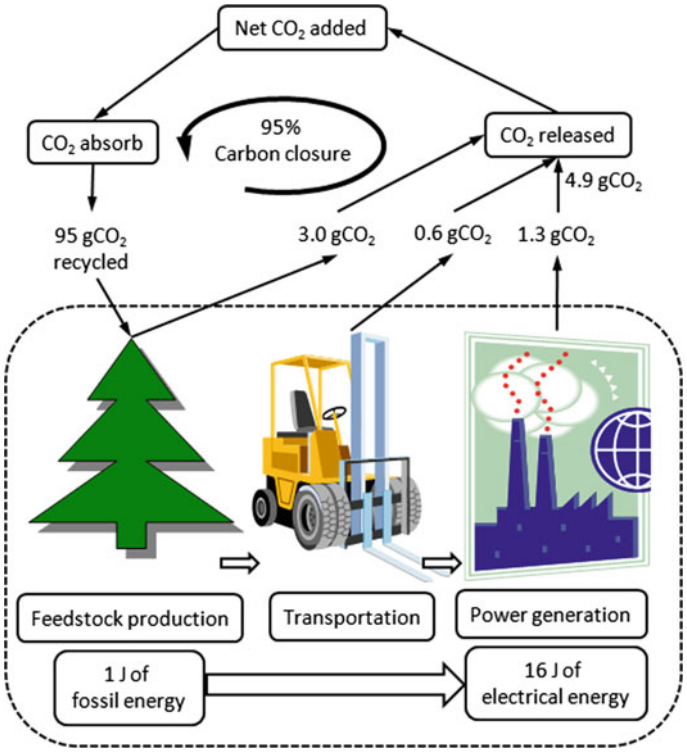

Fig. 11 Life cycle assessment result, CO_2 and energy (adapted from Mann and Spath 1997)

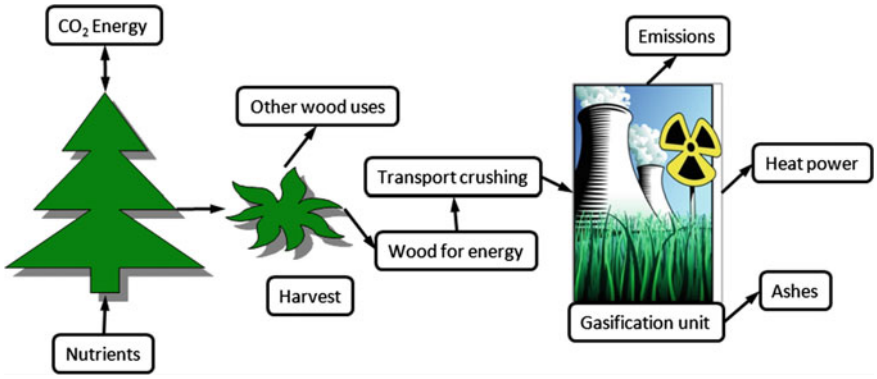

Fig. 12 System boundary under detailed model of biomass power from soil to electricity (François et al. 2013)

exported from the soil during biomass harvest, could be a major issue of the sustainability for the use of biomass. Review of open literature revealed that this fact is not considered by any study till date.

A European study carried out by Oeko-Institut gave exhaustive data required for LCA study. The data are with respect to the life cycle emission of renewable and conventional electricity generation option for European countries. The work was based on the Global Emissions Model for Integrated Systems (GEMIS) software developed in-house by the institute. The outcome of the study also presents upstream life cycle data for fossil fuel (coal, natural gas, and fuel oil), nuclear energy, Bioenergy (straw, wood chips, wood pellets, maize, cereals, use of manure), and certain dedicated crops (rape seeds, Miscanthus, short-rotation forestry). Similar data are also developed for certain nonrenewable electrical energy option (woods as well as nonwoody hydropower, wind power, solar thermal as well as solar PV). The study also presents life cycle emission data for the average energy generation mix of electricity for the year 2005 (based on the historical data) and same for the year 2030 (calculated using PRIME software). The study also presented life cycle emission of some of the transport fuels (Fritsche and Rausch 2009).

In a study carried out by Gilbert et al. (2014), option of the use of biomass gasification as a replacement of natural gas for reforming reaction at ammonia production is evaluated considering life cycle assessment as well as from techno-economic angle. The functional unit for the study is considered as production of 1 kg of NH_3. The result shows significant reduction in the greenhouse gas savings of around 65% for the biomass gasification-assisted NH_3 reforming system. The changes in the system due to the use of biomass gasification lead to a scenario having an Internal Rate of Return (IRR) as 9.8%. It is possible to achieve the scenario of IRR to as high as 20%, that is achieved when biomass is used for the electricity, at a carbon credit price of £ 32/*t* CO_2. However, the study noted the biggest limitation in achieving such fuel switchover is the availability of the large scale of biomass required to feed the present system.

As discussed in the literature above, the biggest limitation of biomass feedstock is its sensitivity to the transportation emission, due to its poor energy density. Even if biomass feedstock transported in the form of pellets, it attracts a high emission cost. A study conducted in British Colombia (Pa 2010) compares the LCA of gasification using exported pellets versus homemade pellets, both replaces natural gas for a district heating application. The study showed that marine transportation of pellets contributes to 40% of life cycle energy consumption and contributes to more than 50% toward environment footprint. GHG saving is about 81%, if woody biomass is utilized, instead of pellets. Wood waste gasification gives a lower footprint compared to the pellets due to extra processing in making pellets. However, analyzing stack emissions pellet gasification found beneficial, since the rise in health impact observed in case of pellets use was only 12%, whereas the same in the case of wood waste gasification was 133%.

It is understood that the transport is the most significant component of the energy as well as emission. Figure 13 shows that European energy consumption (31%) and

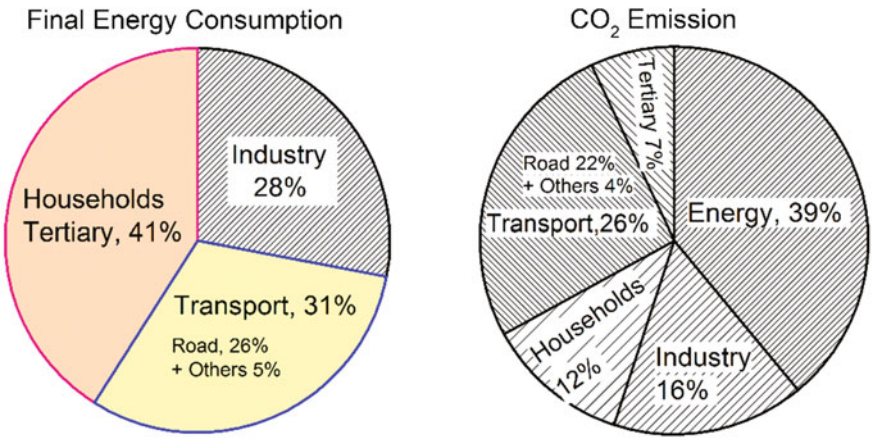

Fig. 13 Breakdown of final energy consumption and CO_2 emissions by sectors in EU-25, 2002, (%) (adapted from Kavalov and Peteves 2005)

a CO_2 emission (26%), which indicates that the emission from road, is the major contributor. As far as biomass is concerned, production of liquid biofuel is the only alternate to overcome the emission under this category. Technology as far as other thermo-chemical conversion is concerned, is not yet ready to bring it to the field, therefore gasification is the only hope to address this issue. Gasification can bring down the ratio of emission to energy in the transportation sector to a low value.

A study carried out by Prins (2005) observed that the high O/C ratio of the biomass and high moisture content reduces the optimum gasification temperature (below 700 °C) for the most of lignocelluloses material. This leads to over oxidization of biomass during the gasification process leading to high thermodynamic losses. Biomass if subjected to a pretreatment process called torrefaction, which is heating up to 250–300 °C, may prove beneficial. Torrefaction drives out moisture and carbon dioxide from the biomass and make biomass more suitable for gasification at higher temperature, this gives gas formation at the much reduced tar level. Torrefaction improves the energy density of the fuel. Improvement in calorific value of torrefied biomass is between wood and coal. Torrefied pellets can be advantageously used in the coal co-gasification (Prins 2005). Since torrefaction improves energy density, gasification with increase gas and reduced tar, the process if applied to gasification has potential to reduce the environmental footprint.

An LCA study is undertaken by Sreejith et al. (2013) to evaluate gasification of coconut shell in comparison with the gasification of coal. The study indicates that the use of coconut shell gasification to generate electricity can reduce emission of global warming gases by 18.3% and increase use of nonrenewable energy by 72%. The study raised a few issues concerned with the use of biomass gasification route, high life cycle emission due to the existing use of fertilizer, tailpipe emission from the gasifier, and use of nonrenewable energy from the grid (having high indirect emission due to high grid emission factor) are the key concerns for this technology. It is proposed that if a coconut shell produced by organic farming and use of green

electricity along with the use of catalytic converters for emission reduction, it may give further greenhouse gas emission reduction of 44%.

Gasification of mushroom-growing substrate is considered by Tippayawong et al. (2011). The results showed that gasification of mushroom substrate leads to a reduction in the smoke emission. The gasification also improves the thermal efficiency to 20% compared to only 5% in the case of burning in open. The arrangement gives attractive financial returns with a payback period of only a year.

A study envisage hydrocarbon biorefinery via gasification pathways, study also showed how operation of such a refinery can be optimized for two opposing criteria, one is fulfilling environmental criteria (GWP) and another is maximization of economic benefit (NPV). The study revealed that high-temperature gasification, direct cooling, internal hydrogen production, and use of catalysis are the key factors affecting the overall performance. At the breakeven point, study yields the cost of operation as US$4.43 and a GWP of 20.92 kg of CO_2 per gasoline equivalent gallon (Wang et al. 2013).

A study carried out by Holmgren et al. (2011) conducted to evaluate how much emission reduction takes place by the use of a biomass gasifier system and which is the best application that gives maximum environmental benefit for such a system. It is found that the use of biomass under co-combustion at the coal power plant has the highest potential for reducing GHG, followed by the use of biomass in transportation application, followed by the use of biomass at the biorefinery.

2.3 *Social Sustainability for Biomass Gasification*

Appraisal of social sustainability is a highly subjective matter. Figure 14 shows various important indicators of social sustainability assessment. Gasification technology can be evaluated on these indicators. The evaluation leads to the success or failure of a technology using these indexes. Since the human needs are highly subjective (Janeiro 2011), usage of integration or aggregation of results obtained by social sustainability study by allocating appropriate weighing factors is left to the reader. This is due to different persons has different answers to the need and their perception toward the social sustainability and issues related to the same (Lako et al. 2008).

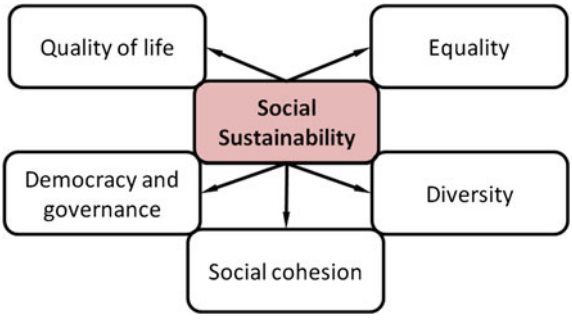

Fig. 14 Aspects of social sustainability (adapted from Wong and Reynolds 2009)

2.3.1 Social Sustainability Using Gasification Technology

The gasification process has a strong social sustainability implication. Biomass gasifier offers a way of achieving social sustainability. It is essential to note that the use of biomass is not limited only to woody biomass, but it also includes the other lignocelluloses material, e.g., agro-residues and forest residues that are considered as waste, this includes rice husk, rice straw, wheat straw, and sugarcane bagasse. The use of gasification technology can be benefitted to rural community in two possible ways (a) it offers clean and efficient cooking solution for rural households and (b) it can generate off grid electricity by offsetting the use of fossil fuels. Figure 15 shows a schematic arrangement for both the applications. Biomass waste is regarded here for producing producer gas with the assistance of a biomass gasifier. Producer gas can be directly be used for the cooking application. For the power generation application, producer gas is routed via further cleaning and cooling, since the engine requires very high quality of ultraclean gases. This level of cleaning is achieved by scrubbing of gases using water jet to remove tar and other dust particles, followed by passing of gas in a bag filter which can remove fine dust from the gas. The clean and cooled gas is then sent to the engine in the power generation application. Both the applications of the producer gas differ in terms of the capacity of the operation. Cooking is a small-scale application and generally accomplished by employing updraft gasifier. However, the power generation applications are of medium- to large-scale application and are sensitive to the quality of the gas and require a downdraft gasifier model.

Quality of Life

The choice of a higher capacity gasification process offers a centralized gasification cum power generation facility (or a community gasification center), having biomass collection centers in several peripheral locations. This helps farmers avail gasification facility at an affordable basis and improves reliability of energy. Use of small gasification facility for cooking may result in not only a reduction in the use of fuel due to the efficient use of biomass but also help improve the health of the other family members, especially female and children member. Indian National Biomass Cookstoves initiative suggested that efficient cookstoves alone could avoid 570,000

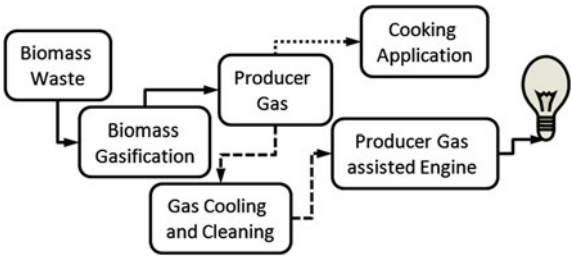

Fig. 15 Rural applications of biomass gasifier

premature deaths and a reduction in 4% of India's greenhouse gas emissions (Bhattacharyya 2012). Since many times timber is treated with toxic substance for insect and rot resistance, this may create harmful gases besides the release of carbon monoxide and soot during combustion (Christa 2011). The healthy environment in the house improves the hours of stay in the house for the children. This improves their involvement in creative activity and improves parent–children interaction, and improves the social-cultural interaction between them. This kind of use of waste biomass, for instance, straw residue, by cooking activity reduce indirect emission that takes place due to inefficient burning and the decay of biomass. For instance, open field burning of farmers leads to greater environmental emission and release of aerosols in the air (causing cancer-like diseases). Finally, building a community-based gasification facility can increase social returns to the stakeholders. Generating electricity using biomass gasifier increase training and skill level for the rural aspirant and can generate employment opportunity for the rural workforce and improves reliability of energy (especially in off grid electricity in rural areas) and independence from the government supply of electricity.

Equity

The income generated from the electricity (or electricity, credit) from a gasification plant can directly reach to the farmers without any intermediaries. Direct return, thus can provide quick and effective recovery of rural economy. Further, managing cooperatives by the stakeholders themselves ensures higher returns, similar to operations of milk cooperatives. The small cooking application of biomass gasifier can help disadvantaged segment of the society, e.g., women empowerment. These people can now involve in any venture based on their cooking skill. This promotes entrepreneurship for the rural masses. Therefore, disadvantaged and marginal people can sustain their family by giving financial stability. The opportunity for women to manage such plants can be increased, since she is familiar with the cooking and can take central role not only in the energy management but also in the commercialization of the cooking skill through the use of gasification. This tends to improve equality and gender balance in the society.

Diversity

Among the target audience (or target group), two financially diverse groups may emerge that take advantage of gasifier technology. One that is not very rich and their scale of operations are small (cooking application) and requires lower skill levels for the operation. Another group that belongs to the strong financial background, which may choose to go for a large capacity gasifier system. That can generate power for a village or for community type of activity, or simply sale the electricity to the grid. Further, there is possibility of conflicts of interest, e.g., a waste management group that already has well-developed supply chain for the

straw to; cardboard industry, with small wood-fired boilers, to cattle feed pellets manufacturers, and so on, may resists not only the very idea of the use of straw but intentionally doubt the technology itself. On the other hand, pellet-making machine manufacturers welcome the move and discover this concept with optimism, and view the technology as a new niche for their well-established machine market.

It is the role of a government agency (and nongovernment organization) to spread the awareness and a systematic campaign for the popularization of gasification technology and to discourage open field burning. The prime idea of the campaign may be to convince farmers to divert the residue straw for gasification technology instead of open field burning. Biochar that they can produce by gasification process is far advantageous compared to ash that is now being made in the area due to open field burning. Besides the financial benefit, there are health, ecological, and environmental benefits as well. It is in the interest of government agencies to advertise and support this technology. However, it requires a systematic campaigning in order to convince and educate farmers. In the present instance, the concept of technology itself fulfills this need and the outcome of the technology implementation can be incorporated into a plan that may change existing practice of open field burning or the propagation of greenhouse gases or by disintegration of waste biomass in the rural region. This serves to shift the entire viewpoint of farmers toward technology option, from a profit-making cooperative unit to the social welfare center. Development of improved understanding toward the environment not only increases the adoption of the technology but may alter cultural belief and strengthen the traditional value systems.

Social Cohesion

Processing biomass through gasification route may help develop awareness among stakeholders to protect the environment and society. This can produce a sense of belongingness among community toward society and boost them to participate aggressively. Development of the community-based pyrolysis facility that can give an opportunity for people to regularly meet and discuss their issues and viewpoint. Their knowledge about the facility and support programs offered by government improves. Because of this, their commitment toward such program increases, which increase effectiveness of environmental mitigation target, and stronger possibility of achieving the same. Such technology can help build a strong link between them and government agency, in person as well as by their exposure to media. This reduces the government burden of achieving social welfare, to some extent, since now the stakeholders become canvasser of the benefit and can bring vital change in the system. The effort of the diffusion of such technology leads to vital knowledge imparted to the stakeholders, which may serve as a tool for long-term environmental mitigation awareness from broader sections of the society.

Democracy and Governance

The implementation of gasification system may increase investment toward rural areas, generating new business opportunity for small- and medium-size entrepreneur and generate income for the inhabitants. A community-based approach improves the possibility of allocating budgets, planning, and quantitative outcome and ensures effective mitigation of target problems along with a pool of well-trained resource persons, and stakeholders, which can be banked for long-term structural changes concerned with the environmental issues. Democratic governance provides flexibility and better adaptability to achieve stated objectives for the projects. This is quite likely that the target audience differs in terms of linguistic communications, education, and having significant cultural variations, particularly for a country like India.

To summarize all, gasification technology is useful in the human development of the society, by improving the environment by way of reducing emission and conserving natural resources. This is accomplished by means of: better waste management, utilizing straw to offset use of diesel, and use of woody biomass for cooking application in rural fields. These factors are studied under social sustainability assessment as a means of achieving improved standards of living. Improved life standard is analogue to an income, which represents a means of increasing social expenditure and, in the end, result in healthy and livable society. This can help to conclude that gasification technology under biochar route provides very high social benefits for stakeholders and should be implemented to achieve social sustainability.

2.4 Economic Sustainability for Biomass Gasification

Figure 16 presents the general methodology adopted for the assessment of economic sustainability under gasification technology. The input and output operating cost component are shown in the figure. The input cost of the process is comprised of the cost due to biomass, labor, and electricity while the financial return expected from the process is in terms of revenue from electricity generation. The difference between input cost and returns from output is the savings from the operation. The time required for obtaining such saving equated against the fixed monetary value (capital cost) is the payback period. When considered together, savings and payback period represent benefit and risk from the operations, respectively. Savings and payback period is most widely considered as an indicator of the process. Note that the labor cost, biomass cost, electricity cost, and revenue from the sale of electricity and biochar are all life cycle cost. Therefore, they can be subjected to a rate of discounting and escalation rate. Also, their net effect can be influenced by the inflation rate.

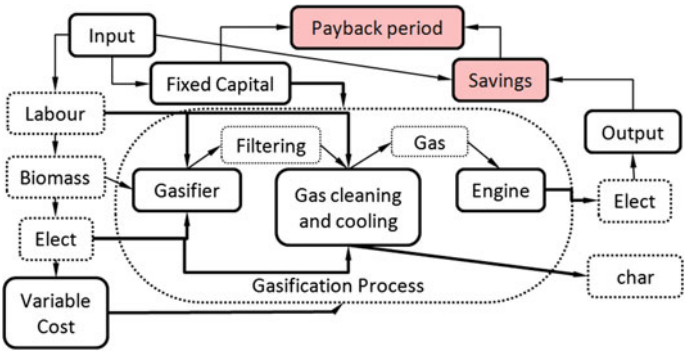

Fig. 16 Illustration of economic sustainability

2.4.1 Some Relevant Studies on Economic Sustainability

As discussed earlier, the sustainability criteria mandates to look financial gain not in the isolation, but instead considered along with environmental and social criteria. When different renewable energy options are proposed as a replacement of the conventional fossil fuel, choice is not always straightforward. Due to its characteristic they results into different results with respect to economic and environmental outcome. Certain renewable option has high capital expenditure and has an almost no cost of electricity generation; wind energy system-WES and solar PV are a good example to cite. Certain renewable energy options have moderate to small investment liability, but they are part of the price of electricity produced is a significant one, biomass energy via gasification system (BES) is one such example. On the environment front, WES and PV has high fixed or embedded emission, their energy production is emission free. BES, on the other hand, has less embedded emission and also has near zero electricity emission.

A study carried out by Afzal (2011) determines the best combination of renewable for a load of 1.5 MW. These combinations are WES-PV system. PV system and PV-BES; and BES-WES. Under PV-BES, energy generated by use of biomass gasification of rice husk near Chennai, Tamil Nadu is considered for the study, while for BSE-WES, rice husk-based biomass gasification near the Veraval, Gujarat is considered. The result in terms of electricity generation, electricity export income, GHG emission reduction, income from carbon trading and equity payback period is interpreted. Option of PV-BES combination generated maximum energy (8672 MWh), while only 50% energy obtained under WES-PV combination, mainly due to poor plant load factor for both options. Maximum net surplus energy is obtained under BSE-WES option. However, PV-BSE combination attracts highest total annual cost due to the cost of rice husk. On emission front, BSE-WES combination gives the highest annual GHG reduction of emission, and hence high income from carbon trading. The same combination gives a shortest equity payback

period of 2.7 years. This makes the option of BSE-WES as most profitable one from the environment as well as economic considerations.

Biomass gasifier can be successfully employed in several low-temperature thermal applications, where at present fossil fuel or biomass is being used as a fuel. One such application is a paper mill industry. Paper mill generally employs boiler to recover energy from waste wood, bark, and sludge. Due to high moisture content of the biomass, biomass is pretreated by drying of fuels to reduce moisture content and requirement of excess air. This helps reduce NO_X and improve boiler thermal efficiency. A study is considered to determine techno-economic feasibility of using a biomass gasifier-assisted power generation system, with an objective of utilizing waste wood by replacing fossil fuel use and reducing NO_X and CO_2 emissions. The implementation of the gasifier leads to the improvement in the boiler efficiency to 80% and an increase in power generation up to 332%. Added benefit of NO_X reduction of the order of 30–50% is obtained, while net CO_2 reduction of 33% is observed (Bryan et al. 2003).

An effort to support rural electrification by the use of a small-scale wood gasification (10 kW gasifier) is carried out in Uganda. Economic operation of such system for daily use of 6 h is demonstrated and the payback period worked out as less than 3 years. However, the study noted that supply chain management for ensuring wood supply and higher capital cost is key to successful implementation of such project in a rural area (Buchholz et al. 2007).

The health damage due to indoor air pollution has been estimated to result in about lakhs of deaths in India, and a study is carried out to obtain a solution for replacing conventional "*chullas*" by an efficient biomass gasifier-assisted compact firing system cum water heater. The application can substitute both poor efficiency and high indoor air pollution. The design proposed in the study gave a lower smoke generation, higher thermal efficiency (20%), and an attractive payback period of 1.45 years (Chendake et al. 2014).

A field testing study carried out by Dingra et al. of TERI (Dhingra and Kishore 2001) evolved a biomass gasifier-based silk reeling oven specifically for the silk industry. The paper presents a detailed explanation of the use of gasifier-assisted cocoon cooking and also worked out its economic for operation of such a system. The proposed system gives silk yield improvement. The payback period for such system is only 126 days. The paper noted one very important criteria for the success of biomass-assisted switchover applications that considerable user training and maintenance support and user acceptance can come only after painstaking and sustained interaction with users.

Currently, the majority of hydrogen is produced by steam methane reforming of natural gas. A study is carried out by Lau et al. (2003) to find out the best choice out of three biomass materials for the production of hydrogen via gasification route. The feedstock considered are: sugarcane bagasse, nutshell, and switchgrass. The results were analyzed for availability, cost of production, and scale of operation. The effective thermal efficiencies observed at 58% for bagasse, 74% for switchgrass, and 76% of the nutshell mix. This study has shown that gasification of biomass can compete positively with steam methane reforming. However, the study

cautioned challenges that gasification technology that need to overcome, viz., in need of improvement in cleaning hot gases, design of improved membrane for syngas reforming inside the gasifier reactor and improved feeding system.

Design of gasifier-based stove to use rice husk is well documented in the literature and sufficiently explored experimentally. A study was carried out by Suvarnakuta and Suwannakuta (2006) over the period of 2 years to develop a biomass gasifier-based efficient cooking stove. The stove was designed to use different feedstock like rice husk and small chopped wood pieces. The thermal efficiency obtained for the stove was 21.77%, with a clean burn, flame-like LPG, having starting time as low as 2 min, and having attractive payback periods of 5.12 months only (Suvarnakuta and Suwannakuta 2006).

3 Summary

By the advent of efficient technology like gasification and the use of second-generation biomass fuel, it is possible to control the emission arising from the use of agro residue. Any efficient use of biomass by use of gasification technology would not only provide much wanted electricity but also provide an efficient alternate to the use of agro-residue. Out of all other technology options discussed, biomass gasification technique leads to efficient use of biomass by generating combustible gases and generation of electricity from the biomass waste. Use of biomass is preferred from economic, environmental, and social consideration. This study showed how the use of biomass for the two biomass gasification-assisted applications; power generation and small cooking applications are sustainable in the long run by citing several studies and the results of experimental outcomes.

4 Conclusion

It is very important for an agrarian economy like India to appreciate sustainable technology like gasification, to efficiently utilize large biomass resources. Implementation of gasification technology can provide benefit to all the three pillars of sustainability: economic, environmental, and social. The technology provides a means to effectively utilize large potential of biomass economically. Further, the utilization of existing biomass can be undertaken by a clean technology or environment friendly option that reduces emission. Last but not the least, it results in a healthy and lively society that promotes: equality, improving quality of life, offering required diversity, introducing democracy, and social cohesion.

On environmental front, gasifier technology is assessed by several authors using LCA method. It is observed that large emission is reported due to burning of agro-residues. Gasification of these agro-residues can reduce lots of emission. Release of particulate matter is high when biomass is used under gasification

option; besides, this overall emission from use of biomass gasification under power generation is low compared to other options like natural gas of diesel for power generation. Use of gasification technology for the production of hydrogen by direct separation is also a superior option. However, when gasification option considered for a greater distance, transportation emission is large due to low bulk density of biomass. Similarly, excess use of fertilizer consumed for biomass production can also be a source of high emission under this route. Gasification route when employed for production of ammonia also found reducing large emission liability for the process.

On the social front, it is noted that gasification technology has a strong influence on our life. Thermal use of biomass gasification for domestic or community cooking and power generation application both can improve lives of the rural community, increase in capability to pay for social benefits, a chance to coexist diverse groups, can improve social cohesion and can offer democracy and a chance to govern community project based on gasification. On economic front, there is a clear advantage of use of this technology due to large availability and low cost of biomass as a fuel compared to other options like natural gas and diesel. However, large capital cost goes against this technology option. The payback period for the thermal application of biomass gasifier is of the order of 5–6 months only when evaluated against LPG for domestic applications. The same under power generation, application ranges in 2–3 years, owing to greater technological complexity.

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Advances in Transformation of Lignocellulosic Biomass to Carbohydrate-Derived Fuel Precursors

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Abstract Cellulosebased, second-generation biofuels have been considered as an alternative fuel source to compensate for depleting fossil fuel reserves. Considering compounds that may be obtained from lignocellulose during biorefining, furfural, 5-hydroxymethylfurfural, and levulinic acid are among the most promising building blocks for energy fuels preparation via chemical or biological synthesis reactions and are thus described as platform chemicals. In this chapter, we present a review on advances made over the traditional strategies for the preparation of these fuel precursors from biomass. The recalcitrant nature of biomass, caused primarily by cellulose crystallinity and nonreactive lignin, hampers the successful commercialization of these valuable by-product chemicals. To date, different processes and production schemes have been adapted to improve product yields and lower production costs and include examples such as supermolecular structure modification of cellulose for improved saccharification using solvents or selective removal or displacement of biomass constituents such as lignin to improve enzyme mobility. Additionally, schemes have included direct conversion of biomass including forestry and secondary agricultural residues to platform chemicals using novel catalysts and reaction systems such biphasic or extractive distillation. Unfortunately, the details of most biomass chemical and biochemical reactions are still unclear, due to their complex nature, hindering improvements to the process. Thus, continued research and development is needed to further understand the biomass component characteristics, the overall cell wall, interrelationships between fractional components, transformation of component during reaction, and competing degradation

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reactions. This research is critical to enable natural lignocellulosic materials utilization to value-added chemicals during the production of fuels.

Keywords Lignocellulose · Glucose · Furfural · 5-hydroxymethylfurfural Levulinic acid

1 Introduction

Transformation of lignocellulosic biomass from energy crops, forestry resources, and agricultural residues to energy fuels and chemicals has garnered considerable research and commercialization interest over the past few decades. The increased awareness has been driven by the desire to solve nonrenewable energy economic and political or environmental issues, primarily being greenhouse gas emissions (Hahn-Hägerdal et al. 2006; Goldemberg 2007; Yuan et al. 2008). The U.S. Energy Information Administration (EIA) projects that world energy consumption will grow with population and country development, and is projected to increase by 56% between 2010 and 2040, moving from 553 EJ (524×10^{15} Btu) to 865 EJ (820×10^{15} Btu) with the most growth in developing countries (EIA 2011). Although other renewable energy resources such as solar, wind, and hydrothermal have the potential to contribute to the growing energy demand, biomass-derived energy is the only renewable option that can viably replace petroleum-based liquid transportation fuels and chemicals (Yuan et al. 2008). Fortunately, biomass has an abundant availability, with the world annual production of biomass is estimated around $1.7–2.0 \times 10^{11}$ tons per year. Additionally, much of the chemical energy content stored inside the plant cell wall in lignocellulosic biomass is an ideal material for renewable energy applications, as it is composed of up to 75% carbohydrates from the cellulose and hemicelluloses, with a mix of hexose and pentose sugar polymers (Sun and Cheng 2002). However, release of fermentable sugars from lignocellulosic materials either using chemical or biological processing methods is a challenging task due to its structure and the presence of the amorphous lignin polymer.

Biomassderived glucose is the primary feed material for cellulosic bioethanol production via microbial fermentation (Sarkar et al. 2012). Cellulosic ethanol has the potential to replace petroleum-based transportation fuels mainly in the light-duty automotive sector (Galbe et al. 2013; Binod et al. 2010). Blending of ethanol with traditional gasoline at levels up to 10% level is common in most countries, with the proportion of ethanol to gasoline varying according to individual country regulatory mandates and ethanol production capacity. Global bioethanol production has significantly increased in recent years from ~70–85 billion liters during 2009–2013 (Sawin et al. 2014). Unfortunately, greater than 60% of the world bioethanol is obtained from food materials such as corn and sugarcane, with the majority of the fuels being produced from these two feedstocks by the USA and Brazil, respectively (Balat and Balat 2009). Although enormous availability of

biomass could serve as potential alternative resource for the cellulosic biofuel production, its recalcitrance nature hinders the viability for industrial commercialization (Himmel et al. 2007; Zhao et al. 2012). Although numerous researches are being carried out worldwide investigating physical and chemical modification methods so far, conversion of carbohydrate polymers to monomers and further to transportation fuel production remains an economic challenge (Wyman et al. 2005b; Mosier et al. 2005). Still with persisting challenges, DuPont, Abengoa, Alberta, and INEOS are the some US-based industries that have recently started commercial small-scale cellulosic ethanol plants with annual production capacity of maximum 30 million gallons bioethanol per year.

Recently, a substantial amount of attention has also been given to the preparation of sugar-based platform chemicals such as furfural, a pentose (also less commonly hexose) sugar derivative (Rogers 2008) and 5-hydroxymethylfurfural and levulinic acid, a hexose sugar derivatives, from lignocellulosic biomass (Sheldon 2014). These furanic chemicals have potential to find application in energy fuel production as precursor for hydrocarbon fuel preparation to go beyond their current industrial use as either solvents or feedstocks for specialty chemicals. Chemical catalysis method is generally employed for the synthesis of these chemicals from lignocellulose via hydrolysis and subsequent dehydration reaction (Tong et al. 2010). However, formation of undesirable humin substances from degradation products and polymerization reactions lower product yield and profitability of the production process (Chheda et al. 2007). For the past few decades, numerous studies have been conducted to improve product yield, which we present as a review in this chapter, with the focus of this review is only on carbohydrate transformation to monomers and the monomers to furanic chemical fuel precursors.

1.1 Lignocellulose and Its Chemistry

Lignocellulose is a generic term for describing the material that constitutes the major polymeric substances such as cellulose and hemicellulose (composed of pentose and hexose sugar monomers), and lignin (composed of polyphenol aromatics). The biogenic polymers are closely associated with each other via covalent linkages, resulting in provides mechanical strength and offers resistance to microbial degradation (Betts et al. 1991; Rubin 2008). These polymers are at the highest concentrations in the primary and secondary cell walls of the plant, as shown in Fig. 1. Considering component functionality, cellulose forms the framework of the cell walls, hemicelluloses cross-link the noncellulosic and cellulosic polymers by a covalent linkage, and lignin covers these polysaccharide polymers providing mechanical strength, especially under wet conditions, and as a hydrophobic barrier to allow transport of water and other essential nutrients through the plant cell wall. The fractional composition of biomass differs considerably depending on species, genetic, and environmental factors (Sun and Cheng 2002).

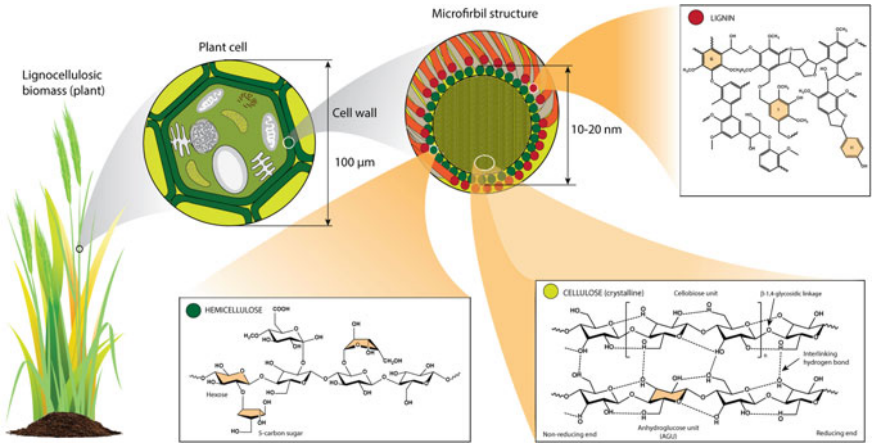


Fig. 1 Schematic representation of plant cell wall micromolecular structure containing all three major polymeric components (Agarwal et al. 2017)

The lignocellulosic biomass includes a variety of materials such as energy crops (i.e., switchgrass and short rotation woody crops), forestry residues (i.e., branches and unused trees), agricultural residues (i.e., straws and stover), and industrial residues (i.e., sawdust and brewers spent grains). Even with these wide range of sources, most lignocellulosic biomass sample has components in between the range viz. cellulose 30–50%, hemicellulose 20–30%, and lignin 20–30%, (shown in Table 1) with herbaceous plants having more hemicellulose and less lignin than woody materials. These biomass substrates also contain minor amounts of low molecular weight components, mostly in the form of organic extractives and inorganic minerals referred to as ash (Elumalai and Pan 2011). The chemical structures of the above polymeric components and their important properties are discussed in the later sections.

1.1.1 Cellulose

Cellulose is a linear, unbranched homopolysaccharide consisting of D-glucose (D-anhydroglucopyranose) subunits linked via β-1,4-glycosidic linkages. Individual cellulose molecular weights vary widely depending on the polymer chain length, with some cellulose polymers containing as few as 3000 DP (degree of polymerization), whereas others may contain as many as 20,000 DP, with most cellulose polymers in the 8000–12,000 DP range. Structurally, each glucose molecule unit present in cellulose chain is rotated at an angle of 180° with respect to its immediately adjacent unit. The fundamental molecular structure drives upon cellulose properties that are different to those of starch derived amylose, which also consists exclusively of glucose residues linked by α-1,4-glycosidic linkages, creating a helical structure. β-1,4-glycosidic linkages create a linear structure which allow the

Table 1 Cellulose, hemicellulose, and lignin contents (% by weight) in common agricultural residues (Sun and Cheng 2002)

Lignocellulosic materials	Cellulose	Hemicellulose	Lignin
Softwood stems	45–50	25–35	25–35
Hardwood stems	40–55	24–40	18–25
Wheat straw	30	50	15
Switch grass	45	31.4	12.0
Sorghum straw	36	26	8
Cassava stem	39	7	12
Sugarcane bagasse	55	11	26
Rice straw	36	18	29
Grasses	25–40	35–50	10–30
Leaves	15–20	80–85	0
Corn cobs	45	35	15
Cottonseed hairs	80–95	5–20	0
Coastal bermudagrass	25	35.7	6.4
Corn stover	40	25	17

cellulose chains to be tightly packed into bundled fibrils structures consisting of several parallel cellulose molecules. Besides β-1,4-glycosidic linkages, individual glucose molecule are also engaged in having intra- and intermolecular hydrogen bonding within the cellulose chain and to the adjacent cellulose chain in glucose units, which also contributes to the overall stiffness of the molecule. In detail, there are two types of intramolecular hydrogen bonding present, as follows: (i) hydrogen bonding between the C3 hydroxyl (OH) groups and the pyranose ring oxygen of an adjacent glucose residue (O3–H…O5′), and (ii) hydrogen bonding between the C2 OH and the C6 oxygen of a neighboring glucose residue (O2–H…O6′), as shown in Fig. 2. Furthermore, the major intermolecular hydrogen bonding occurs between the C6 OH and C3 oxygen (O6…O3′) along the b axis. Because of these hydrogen bonding and weak van der Waals forces, cellulose fibrils are aligned together in a highly ordered fashion forming a crystalline region, which is water insoluble and very resistant to chemical or enzymatic attack. The less ordered region of cellulose molecules being noncrystalline are usually termed amorphous, which can be easily hydrolyzed under mild acidic or basic conditions. So far, at least six different cellulose structures has been elucidated, which are as follows: Cellulose I, is the native form of cellulose having parallel glucan chains and strong hydrogen bonds; Cellulose II, is obtained after alkali treatment of native cellulose resulting in mercerized cellulose with antiparallel arrangement; Cellulose III_I and III_{II} which are produced by the aqueous ammonia treatment of Cellulose I and II at −80 °C, respectively; whereas, Cellulose IV_I and IV_{II} are obtained after glycerol treatment of Cellulose III_I and III_{II} at 260 °C, respectively (Pérez and Mazeau 2004; Elumalai and Pan 2011).

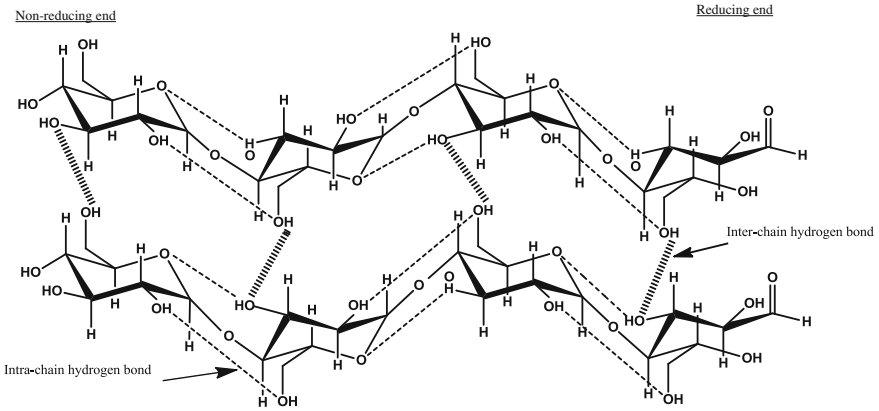


Fig. 2 Cellulose structure with intra-and interlinked hydrogen bonding (Elumalai and Pan 2011)

1.1.2 Hemicellulose

Hemicelluloses are short-branched heteropolymers composed of primarily 5-carbon (pentose) and 6-carbon (hexose) sugars or sugar acids, and are typically located in primary and secondary cell walls of biomass. Unlike cellulose, hemicellulose polymers have a low degree of polymerization (only 50–300) and thus have a significantly lower molecular weight than that of cellulose. This characteristic polymer forms a complex network to the other neighboring components by different linkages such as hydrogen bonds to cellulose, covalent bonds with lignin (mainly α-ether linkages), and ester linkages with acetyl units and hydroxycinnamic acids. The chemical structure of hemicelluloses in wood and herbaceous plant materials is well established and contains a monomeric sugar component in combination with side groups such as uronic acids, acetyl- and methyl-substituted groups (Cengiz et al. 2010). The common polysaccharides present in hemicelluloses are xylans (arabinoxylans and 4-*O*-methyl-glucuronoxylans), galactomannans, glucomannans, β-D-glucans (3- and 4-linked), β-D-glucan-callose (3-linked), and xyloglucans (4-linked β-D-glucans with attached side chains), some of which are depicted in Fig. 3. These polysaccharide backbones usually consist of one repeating sugar unit linked through β-(1 → 4) with branch points (1 → 2), (1 → 3), and/or (1 → 6). The predominant polysaccharide in hemicellulose is the glucuronoxylans, which contains a xylan backbone of β-D-xylopyranose units linked through (1 → 4) with acetyl groups at C-2 or C-3 of the xylose units (Elumalai and Pan 2011; Wyman et al. 2004). Therefore, xylan is the simplest general representation of a typical hemicellulose.

Another group of polysaccharides that may account for a portion of carbohydrates in some plants is pectins. Pectins contain polycarboxylic acid and function together with hemicelluloses as matrix substances, which provides structural support to the cell walls. Galacturonic acid is the major constituent of all natural

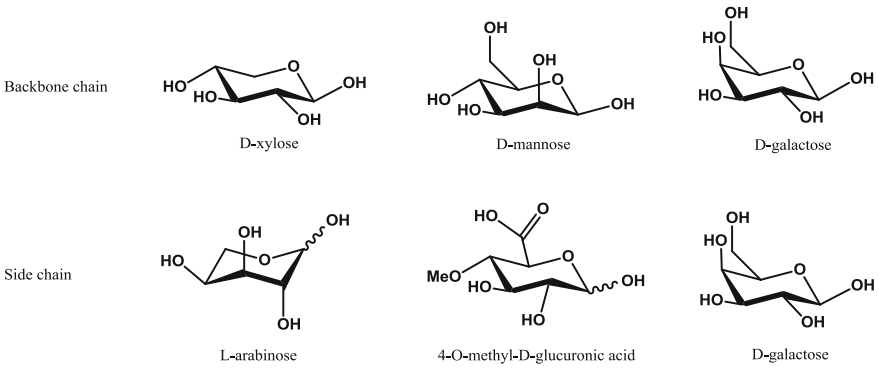


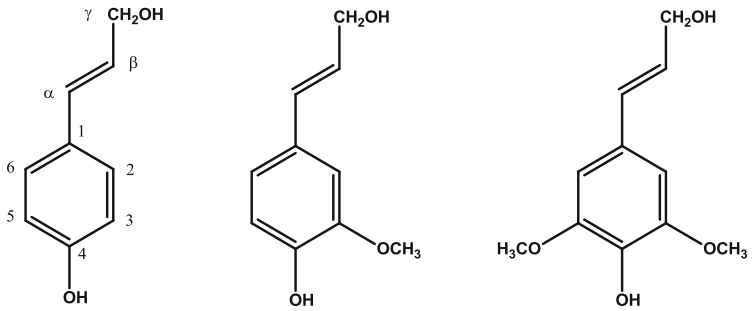
Fig. 3 Hemicellulose sugars including C5- and C6-sugars as backbone and side chain (Bordy 1999)

pectins. Various other pectic polysaccharides has also been detected in the cell wall like homogalacturonan (HG), xylogalacturonan (XGA), apiogalacturonan, rhamnogalacturonana I (RGI), and rhamnogalacturonan II (RGII). These substances are not separate molecules but covalently linked domains (Bordy 1999).

1.1.3 Lignin

Lignin is a three-dimensional, highly cross-linked macromolecule composed of three types of substituted aromatic phenols which include: *trans*-*p*-coumaryl, *trans*-coniferyl, and *trans*-sinapyl alcohols formed by enzymatic polymerization yielding a massive number of functional groups and linkages. These precursors form main kinds of lignin units in plants, i.e., *p*-hydroxyphenyl-propane (H), syringylpropane (S), and guaiacylpropane (G) units (Fig. 4), and these units vary depending on the species. For example, softwood lignin is composed almost entirely of guaiacyl-propane units, whereas hardwood lignin contains varying ratios of syringylpropane (S) and guaiacylpropane (G) units (Table 2). Furthermore, grass lignin is even more complicated than woody species because it contains substantial amounts of *p*-hydroxyphenylpropane (H) unit along with S and G units (Obst and Laaducci 1986).

The cross-linked, lyophilic nature of lignin makes it insoluble in water, stable in nature, and acts as the "glue" that connects the polysaccharides such as cellulose and hemicellulose together. Within the lignin polymer, the phenylpropane units (S, G, and H units) are bonded together by a set of linkages, as summarized in Table 3 and depicted in Fig. 5. By far, the most common linkage between the lignin monomer units is reported to be the *β*-*O*-4 linkage, which accounts for 50–60% of total linkages. This linkage is typically targeted during delignification or modification processes as this bond is both common and although not easily cleaved, is one of the most reactive linkages in lignin. Other lignin monomer linkages,



trans-p-coumaryl alcohol *trans*-coniferyl alcohol *trans*-sinapyl alcohol

Fig. 4 Three important precursors involved in lignin biosynthesis (Chen 2014)

Table 2 Abundance of lignin units in different biomass materials (Li et al. 2015)

Units	Abundance of monomer units (%)		
	Softwood	Hardwood	Grasses
p-hydroxyphenylpropane (H)	<5	0–8	5–35
Guaiacylpropane (G)	>95	25–50	35–80
Syringylpropane (S)	Trace amount	45–75	20–55

Table 3 Common linkages in softwood and hardwood lignin structure (Elumalai and Pan 2011)

Linkage type	% of total linkages		
	Softwood (spruce)	Hardwood (beech)	Grasses
Arylglycerol-β-aryl ether (β-*O*-4)	50	60	93
Noncyclic benzyl aryl ether (α-*O*-4)	2–8	7	–
Phenylcoumaran (β-5)	9–12	6	37
Biphenyl (5-5)	10–11	5	–
Diaryl ether (5-*O*-4)	4	7	–
1,2-diaryl propane (β-1)	7	7	0.5
Linked through side chains (β-β)	2	3	4
In glyceraldehydes-2-aryl ether	2	2	–
In structures condensed in 2- or 6 positions	3	2.5	–
In biphenyl	11	4.5	–
In diphenyl ether	4	6.5	–
In quinine ketal structures	Traces	–	–

including 5-5, β-5, β-1, β-β, and 5-*O*-4 are stable under most conditions. Lignin also has additional functional groups, such as hydroxyl, methoxyl, and carbonyl, which plays an important role in delignification and lignin modification reactions (Li et al. 2015).

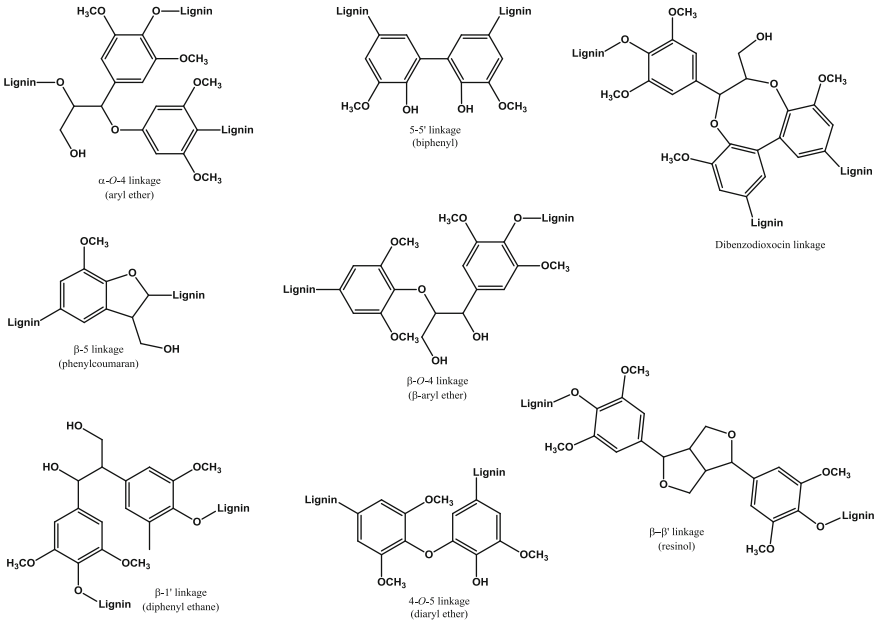


Fig. 5 Structures of chemical linkages found in lignin polymer (Brunow and Lundquist 2010; Takanori et al. 2014)

2 Hydrolysis of Lignocellulose

Cellulosic biofuels, including bioethanol, have been considered as critical alternative energy sources for the near future to replace nonrenewable petroleum fuels. Glucose, the main component, is primary feedstock required for bioethanol production through microbial fermentation. Inherently, lignocellulosic biomass consists of glucose residues as cellulose units (glucose subunits linked via β-1,4-glycosidic linkages). Individual cellulose molecules vary widely with respect to polymer length. As described previously, cellulose molecules consist of 8000–12,000 glucose monomers. Breakdown of this cellulose polymer (glucan) into monomer units (glucose) can be accomplished via hydrolysis either using chemical catalysts, typically mineral acids, or biocatalyst, typically with cellulose enzymes. The hydrolysis reactions are discussed briefly in the following sections.

2.1 Acidic Hydrolysis of Cellulose to Glucose

Chemical hydrolysis of cellulose for the glucose ($C_6H_{12}O_6$) release has been comprehensively researched over the past century. The most investigated approach for the depolymerization of cellulose is using strong acids including sulfuric,

hydrochloric, and phosphoric acids. Among acids, sulfuric acid hydrolysis has been extensively studied because of promising results achieved and the relatively lower cost of sulfuric acid compared to other acids (Binder and Raines 2010). Conventionally, sulfuric acid hydrolysis method can be divided into two categories: (i) dilute acid hydrolysis and (ii) concentrated acid hydrolysis. The operating conditions maintained in the former method is typically less than 1% by wt. H_2SO_4 conc., 200–220 °C for few minutes, whereas later employs 30–70% by wt. H_2SO_4 conc., 35–40 °C for few hours. In this approach, the most typical degradation reactions of glycosides, di-, oligo-, and polysaccharides of lignocellulose occur. A typical reaction condition, β-1,4-glycosidic linkage in cellulose is mainly susceptible to acid-catalyzed hydrolytic attack leading to molecular degradation. However, the extent of hydrolysis depends on the following interaction conditions, such as nature of the acid, its concentration, reaction temperature, and duration. For instance, at increased acid concentrations of more than 63% wt., the molecules are capable to cleave the hydrogen bonding and penetrate into crystalline and amorphous regions of the cellulose polymer with intermediate complexes formation, as depicted in Fig. 6. Several mechanistic studies have also been shown that apparent swelling of cellulose at acid concentrations above 50% leading to the dissolution of cellulose (Saeman 1945; Camacho et al. 1996).

In addition, partial esterification of hydroxyl groups of cellulose and their substitution by sulfonic groups has also been observed. Breaking of hydrogen bonds as a result of the intermediate complexes formation, sulfate ester formation, and the depolymerization of macromolecular chains/fibrils are the main factors allowing for cellulose dissolution at the higher concentrations of sulfuric acid. Chemical hydrolysis, necessary for cellulose depolymerization to glucose is governed primarily by the crystallinity of cellulose and its limited solubility under typical reaction conditions. Preferably, glucose is produced after sufficient hydrolysis of cellulose; however, due to its reactive nature in the acidic medium, the reaction is susceptible to further dehydration to produce derivative chemicals such as 5-hydroxymethylfurfural (HMF), levulinic acid (LA), which may polymerize with unreacted carbohydrates forming humins, an undesired by-product.

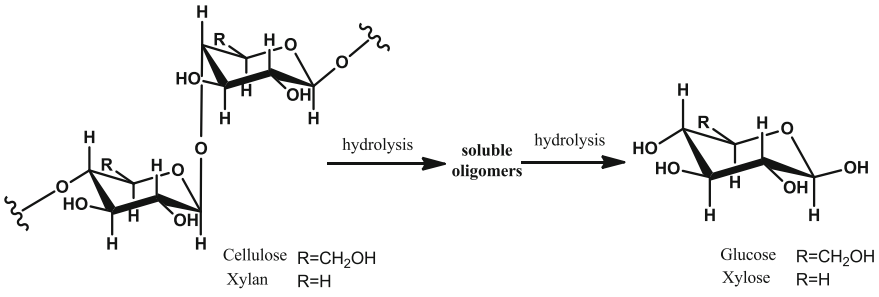


Fig. 6 Proposed reaction scheme of cellulose and hemicellulose decomposition during acidic hydrolysis (Binder and Raines 2010)

The reaction pattern of acidic hydrolysis of cellulose by either dilute or concentrated acid method is represented in Eq. (1) as a sequential first-order reaction, according to Saeman's kinetic model (Saeman 1945):

$$\text{Cellulose} \xrightarrow{k_1} \text{Glucose} \xrightarrow{k_2} \text{Decomposed products} \tag{1}$$

where k_1 and k_2 are the rate constants of the corresponding subsequent reaction steps. The rate constants of the inorganic acid hydrolysis reactions are represented by an Arrhenius Eq. (2) in which the pre-exponential factors (k_{i0}) includes the acid concentration term to account for the effect of acid as well as the temperature:

$$k_i = k_{i0} \times [A]^{ni} \times \exp(-E_i/\mathrm{RT}) \tag{2}$$

where i = 1 (hydrolysis reaction), i = 2 (decomposition reaction), n_i = acid exponent, [A] = concentration of sulfuric acid, and E_i = activation energy. Wyman et al. (2004) have proposed that at elevated temperatures, the kinetic pattern of hemicellulose hydrolysis can be expressed in a manner similar to that of cellulose hydrolysis (i.e., sequential first-order reactions in series).

One of the advantages of the acid over enzymatic catalyzed hydrolysis method is faster reaction, however, though this method offers low potential sugar yields, lower selectivity, and higher energy requirements. Alternative acidic hydrolysis strategies using homogeneous or heterogeneous catalysts either separate or in combination for the conversion of biomass have been proposed so far, in order to improve the final product yield of glucose. During the 1980s, Ragg et al. (1987) have demonstrated aqueous phase conversion of cellulose to glucose in HCl (6–7 mol/lit) containing LiCl or $CaCl_2$ salts, as additives and achieved approximately 85% glucose yield. In an advanced study, Luterbacher et al. (2014b) have recently demonstrated the homogenous conversion process for glucose production from biomass substrates in a biphasic reaction system containing organic (γ-valerolactone) and aqueous (dilute acid) phase and reported >70% glucose yield. In perception, several studies have recommended for the pretreatment of lignocellulose to make the cellulose more susceptible for hydrolysis due to its complexity nature. Accordingly, Cao et al. have employed homogeneous cellulose pretreatment using ZnCl followed by hydrolysis with dilute HCl and achieved improved glucose yield (91.5%) (Cao et al. 1994).

In an alternative approach to homogeneous catalysis of cellulose to glucose, ionic liquids (ILs) has become more popular in recent years, because of their unique solvent properties including low volatility, nonflammable nature, and good recycle performance (nearly complete recovery is possible) (Rinaldi et al. 2008). ILs are a new class of salts that exists as liquids at temperatures <100 °C and have unique solvent properties. A series of studies have demonstrated for the dissolution of lignocellulosic materials for glucose production using hydrophilic imidazolium-based ionic liquids (combination of different cations and chloride anion) such as 1-butyl-3-methylimidazolium chloride, 1-allyl-3-methylimidazolium

chloride, 1-benzyl-3-methylimidazolium chloride, and 1-ethyl-3-methylimidazolium acetate with improved glucose yields (>80%) (da Costa Lopes et al. 2013; Li and Zhao 2007; Rinaldi et al. 2008). After the treatment with ILs, the fermentable sugars can be recovered by the addition of anti-solvents such as water and ethanol. Recently, Binder and Raines have reported maximum glucose yield nearly ~90% from cellulose using chloride-containing ionic liquid (1-ethyl-3-methylimidazolium chloride, [EMIM]Cl) by gradually adding water to the reaction mixture (Binder and Raines 2009). Although ILs provide an excellent approach for the rapid conversion of biomass cellulose to glucose, several factors including viscosity, toxicity, and high cost are associated with their use which have to be seriously considered for the commercial process development. However, ionic liquids with anions such as acetate, formate, and phosphate possess lower viscosities that facilitate their use for various applications (Lucas et al. 2012).

In contrary to homogeneous catalysis method, heterogeneously acid catalyzed cellulose hydrolysis to glucose eliminates the product separation difficulty and contributes to the economic process development by facilitating repeated reuse of the catalyst. Generally, the reactions are initiated by acid–base interactions (surface reaction) in which the solid catalysts act as acids and the reactants (liquid) act as a base (Onda et al. 2008). Heterogeneous catalysis methods offer many advantages over liquid catalysts, including continuous production operation, easy recovery, noncorrosive nature, and the possibility for multiple reactions using a single catalyst bed, or multifunctional solid catalyst. The proposed general mechanistic pathway of cellulose hydrolysis by a solid acid catalysts consists of the following steps: (i) dissolution of crystalline cellulose using some chemical agents or solvents, (ii) a water molecule adsorbs onto the acid site of an solid acid via an intermolecular hydrogen bonding, (iii) the soluble polysaccharide diffuses into the internal pores of the solid acid catalysts; (iv) the diffused polysaccharide undergoes hydrolysis over the acid sites with the adsorbed water, and (v) the hydrolysis products, mainly glucose, diffuse into the reaction medium (homogenous). However, the properties of solid acid catalysts, such as acid site density, acid strength, acid site distribution, structure of supports and tolerance to water, have significant influence on their activities and selectivities (Van de Vyver et al. 2011). Table 4 lists some examples of the classified solid acid catalysts that could possibly be used for cellulose hydrolysis. The details of preparation and structural information of the synthesis materials have been described elsewhere. Among notable studies, Onda and co-authors were the first to demonstrate cellulose hydrolysis using solid acid catalyst and achieved more 90% glucose selectivity. The solid catalysts tested were H-form zeolite, sulfated and sulfonated-activated carbons (Onda et al. 2008). Among them, sulfonated solid acid catalyst showed a remarkably high yield of glucose. In another study, Shimizu et al. (2009) have evaluated different heteropoly acids (a type of solid acid consisting of early transition metal-oxygen anion clusters) for cellulose hydrolysis to glucose and compared with the traditional homogenous mineral acids. The sugar release from the reaction were observed to decrease in the following order: $H_3PW_{12}O_{40} > HSiW_{12}O_{40} > HClO_4 > H_2SO_4 > H_3PO_4$ (Rout et al. 2014). To date, a variety of solid acids differing in terms of their structure and

Table 4 Classified heterogeneous solid acid catalysts

Class	Example solids	References
Zeolite and zeolite-like	*X*-, *Y*-zeolites (faujasite), chabasite, ferriertie, beta-zeolite, mordenite, erionite, 5-ZSM, 5-HZSM, MCM-22, metalloaluminophosphate (e.g., silicoaluminophosphate, gallosilicate, beryllosilicat, titanosilicate, stanosilicate	Hattori and Ono (2015), Szostak (1991)
Clay	Montmorillnite, saponite	Hattori and Ono (2015)
Metal oxide and mixed metal oxide	Al_2O_3, TiO_2, SiO_2, Nb_2O_5, WO_3, SiO_2–Al_2O_3, SiO_2–ZrO_2, SiO_2–MgO, TiO_2–SiO_2, WO_3–ZrO_2, WO_3–Al_2O_3, WO_3–SnO_2, Nb_2O_5–Al_2O_3, B_2O_3–Al_2O_3	Fierro (2005)
Acid supported	H_3PO_4/SiO_2, $HClO_4/SiO_2$, SO_3H/SiO_2, SO_3H/C, $AlCl_3/SiO_2$, BF_3/SiO_2, SbF_5/SiO_2–Al_2O_3, SbF_5/TiO_2, CF_3SO_3H/SiO_2, heteropoly acids/SiO_2	Hattori and Ono (2015)
Sulfated oxide	SO_4/ZrO_2, SO_4/TiO_2, SO_4/SnO_2	Fierro (2005)
Layered transition metal oxide	$HNBMoO_6$, $HTaWO_6$, $HNbWO_6$	Fierro (2005)
Metal salt	$AlPO_4$, $Nb_3(PO_4)_5$, $FePO_4$, $NiSO_4$	Izumi et al. (1992)
Heteropoly compound	$H_3PW_{12}O_{40}$, $H_4SiW_{12}O_{40}$, $H_3PMo_{12}O_{40}$, $H_4SiMo_{12}O_{40}$, and their salts (e.g., $H_{0.5}Cs_{2.5}PW_{12}O_{40}$)	Izumi et al. (1992)
Ion exchange resin	Amberlyst-15, Nafion, Nafion-silica composite/nanocomposite	Hattori and Ono (2015)

functionality have been investigated for cellulose hydrolysis reactivity for glucose production (Huang and Fu 2013).

2.2 *Enzymatic Hydrolysis of Cellulose to Glucose*

Extensive research has been conducted on conversion of lignocellulosic materials to glucose via a biocatalytic route using cellulase complex in the last three decades (Sun and Cheng 2002; Ståhlberg et al. 1991). Studies have suggested that enzymatic saccharification is a promising method for effective cellulose hydrolysis though the current cost of cellulose enzymes hinders wide-scale commercialization. The synergistic action of three distinct classes of enzymes that accomplishes the depolymerization of cellulose molecule are: (i) the "endo-1,4-β-glucanases" or, 1,4-β-D-glucan 4-glucanohydrolases, which act randomly on soluble and insoluble 1,4-β-glucan substrates, (ii) the "exo-1,4-β-D-glucanases", including both the 1,4-β-D-glucan glucohydrolases, which liberate D-glucose from 1,4-β-D-glucans and hydrolyze D-cellobiose slowly, and 1,4-β-D-glucan cellobiohydrolase, which liberates D-cellobiose from 1,4-β-glucans, and (iii) the "β-D-glucosidases" or β-D-

glucoside glucohydrolases, which act to release D-glucose units from cellobiose and soluble cellodextrins, as well as an array of glycosides. Besides these hydrolytic enzymes, the enzyme complexes active in the biodegradation of cellulose frequently also contain oxidatively acting enzyme systems, generally termed gluco-oxidases. They may also play a role in generating sites enhancing hydrolytic enzyme attack, especially in well-ordered regions of the cellulosic substrate (Ryu and Lee 1982).

Comparatively, the enzymatic hydrolysis method offers the potential for higher sugar yields, higher selectivity, low energy costs, and milder operating conditions (50 °C; pH 4.8) than chemically mediated (using mineral acid catalyst) conversion technologies. The general reaction scheme for enzymatic hydrolysis of cellulose is illustrated in Fig. 7. The figure shows the proposed enzymatic cleavage mechanism of cellulose hydrolysis consists of three major steps: (i) adsorption of hydrolytic enzymes onto the cellulose surface, (ii) hydrolysis of cellulose to cellobiose (dimer of two glucose repeating unit) and other oligomers, and (iii) desorption of the enzymes back to the liquid medium. The enzymatic cellulose hydrolysis efficiency is determined by several factors including degree of polymerization, crystallinity, and specific surface area and pore size of cellulose (enzyme accessible surface area). In addition, the content and distribution of the adjacent polymeric materials such as hemicelluloses and lignin in cell wall influence hydrolysis efficiency by providing a matrix cover and irreversible adsorption of enzymes (via hydrophobic ionic bond and hydrogen bonding interactions), respectively (Wyman et al. 2004). For example, Laureano-Perez et al. (2005) have demonstrated how the most influential substrate factors such as cellulose crystallinity and lignin content negatively affect product formation during enzymatic hydrolysis using the specially developed models. In a supporting study, Yu et al. (2014) have recently demonstrated the strong impact of lignin (isolated from softwood and hardwood) on enzymatic hydrolysis and its structure and functional groups on cellulase adsorption using a mimic lignocellulose biomass and reported decreased efficiency of saccharification.

Many studies have concluded that a pretreatment step is needed to remove the enzyme recalcitrance and to make the cellulose more susceptible for hydrolysis

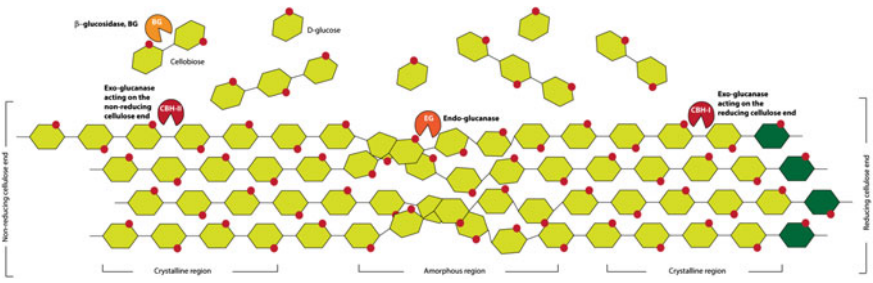


Fig. 7 Proposed synergistic action of cellulase complex during cellulose saccharification reaction (Dutta and Wu 2014)

(Mosier et al. 2005). To date, the most common pretreatment methods available for the lignocellulosic materials are biological (enzyme treatment), physical (mechanical milling), chemical (mineral acid hydrolysis), and physicochemical (steam explosion) treatments. In recent years, researchers have employed ionic liquid (ILs) for the pretreatment of biomass (Sun and Cheng 2002; Kumar et al. 2009). Overall, these methods have shown great impact on modification and/or reconstruction of the substrate material to enhance the enzyme accessibility. However, ILs pretreatment also has drawbacks that prevents commercialization, as its cost and recovery contributes greatly to the operational cost.

Another pretreatment method that has garnered considerable attention is ammonia fiber explosion (AFEX). In a recent study, biomass substrates such as alfalfa, wheat straw, barley straw, corn stover, rice straw, municipal waste, softwood, switchgrass, aspen chips, and bagasse were subjected to the AFEX pretreatment method, which demonstrated enhanced sugar release during subsequent enzymatic hydrolysis (Galbe and Zacchi 2007; Wyman et al. 2005a). Methods to reduce the cost and chemical dispose from this process by recovery and reuse of ammonia have also been considered (Balan et al. 2009).

Another pretreatment approach uses microbes such as brown-, white-, and soft-rot fungi are generally used to degrade lignin and hemicellulose of the lignocellulosics before subjecting them to chemical/enzyme conversion. Recently, Du et al. (2011) have studied the simultaneous pretreatment and saccharification of corn stalk sample using *Irpex lacteus* and reported 82% total sugar yield after 28 days.

According to the mechanistic studies of the enzyme–substrate interaction, irreversible adsorption of cellulase on cellulose and to a lesser extent on lignin, and product (cellobiose and glucose) inhibition significantly decreases the enzyme activity, and in turn, lowers the hydrolysis yield (Yang et al. 2011). Studies have also used nonionic surfactants such as Tween 20 and 80, polyoxyethylene glycol, Tween 81, Emulgen 147, amphoteric anhitole 20BS, etc. along with the enzymes in order to minimize enzymes adsorption onto the solid surface and reported improved glucose yield. Nakagame et al. (2011) have demonstrated that increased carboxyl functional group in lignin significantly reduces the nonproductive binding of cellulases. Additionally, several methods have been developed to reduce the product inhibition including supplementation of β-glucosidase during the course of reaction, use of high concentrations of enzymes, and continuous/intermittent removal of sugars during hydrolysis by ultrafiltration (Yang et al. 2011).

In recent years, the use of synergistic cellulase complex for cellulose hydrolysis strategy has been widely observed, with many combinations reported and proposed, including endoglucanase with exoglucanase, exoglucanase with exoglucanase, endoglucanase with endoglucanase, exoglucanase, or endoglucanase with β-glucosidase, catalytic domain with CBM (cellulose binding module) or two catalytic domains, cellulose-enzyme-microbe synergism, and spatial synergism for cellulase complexes (Yang et al. 2011). Hoshino et al. (1997) have evaluated the synergistic actions of exo-type cellulases with a total of seven different pairs on

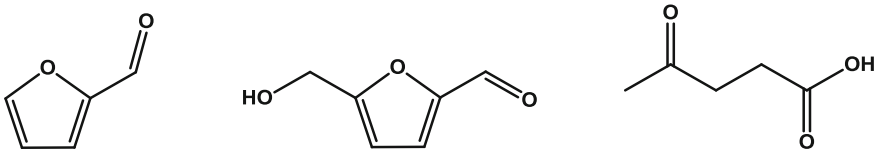
hydrolysis of cellulose having different crystallinities, and reported improved hydrolysis of crystalline cellulose using the enzyme pairs that contained CBH II.

3 Chemical Synthesis of Furanic Chemicals from Lignocellulose

Typically, lignocellulosic biomass-derived molecules contain many functional groups and have large oxygen content (approximately 40–45% by wt. oxygen). In order to produce fuel or fuel precursors, this relatively high oxygen content must be reduced to increase liquid fuel stability and energy density. Among oxygenated compounds of biomass that have the potential for transformation to fuel compounds are furfural, 5-hydroxymethylfurfural (HMF), and levulinic acid (LA) (Huber et al. 2006; Rouilly and Vaca-Garcia 2015). The respective structures of these molecules are represented in Fig. 8.

3.1 Furfural

Furfural (furan-2-carbaldehyde) is synthesized through hydrolysis followed by dehydration of biomass pentosan (mainly xylan), which are often found in significant amounts in the hemicelluloses of some agricultural residues and hardwoods, such as corncobs, bagasse, and softwoods. To date, xylose dehydration to furfural has been extensively studied and optimized. In early 1920s, the Quaker Oats company has commercialized a method for furfural production from oat hulls using sulfuric acid, as catalyst. Furfural, as precursor can be used for the production of wide range of high-value chemicals and fuels (Fig. 9). For example, 2-methylfuran (MF) and 2-methyltetrahydrofuran (MTHF) can be produced via hydrogenation and those are used as gasoline additives. Likewise, levulinic acid ester (ethyl levulinate) can be produced via hydrogenation followed by ethanolysis in the presence of strong acids. Under harsh conditions, furan ring can be further hydrogenated and ring-opened to produce alcohols and diols in the presence of catalyst. In same fashion, furfural and ethylfurfuryl ether (EFE) can undergo ring-hydrogenation to



Furfural 5-hydroxymethylfurfural Levulinic acid

Fig. 8 Chemical structures of furfural, 5-hydroxymethylfurfural, and levulinic acid molecules

deliver tetrahydrofuran (THF) and ethyltetrahydrofurfuryl ether (ETE), respectively, in the presence of catalyst. Direct fuel synthesis from furfural is also possible through sequential aldol condensation and dimerization reactions. Additionally, cyclo-products including cyclopentanol, cyclopentanone can be synthesized in the presence of a catalyst (Cai et al. 2014; Marcotullio 2011; Lange et al. 2012).

Chemical approaches for xylose dehydration involves acidic conditions, using either mineral acids such as sulfuric acid, hydrochloric acid, and phosphoric acid or solid acid heterogeneous catalysts such as zeolites, heteropoly acids, etc. (Cai et al. 2014). Figure 10 depicts the proposed typical reaction mechanism for production of furfural from pentosans. To date, studies have been conducted in monophasic and biphasic solvent systems using homogeneous and heterogeneous catalytic materials containing Lewis and Brønsted acid sites (Hu et al. 2012).

The commonly adapted preparation technologies for furfural production are one-stage and two-stage preparation methods. In industrial processing system, steam stripping and solvent extraction are simultaneously adapted to remove furfural from the reaction system after a predefined time period (Li et al. 2013; Cai et al. 2014). Over the past decades, the production technologies for furfural from xylose have been greatly improved from the initial one-stage batch preparation method to multistage in series for continuous operations using both homogeneous and heterogeneous catalysts. For instance, recently, Mandalika and Runge have demonstrated two-stage integrated conversion of lignocellulosic biomass (hybrid poplar, miscanthus, switchgrass, and corn stover) via hot water hydrolysis followed by homogenous catalysis (sulfuric acid) to produce high yield furfural (87%)

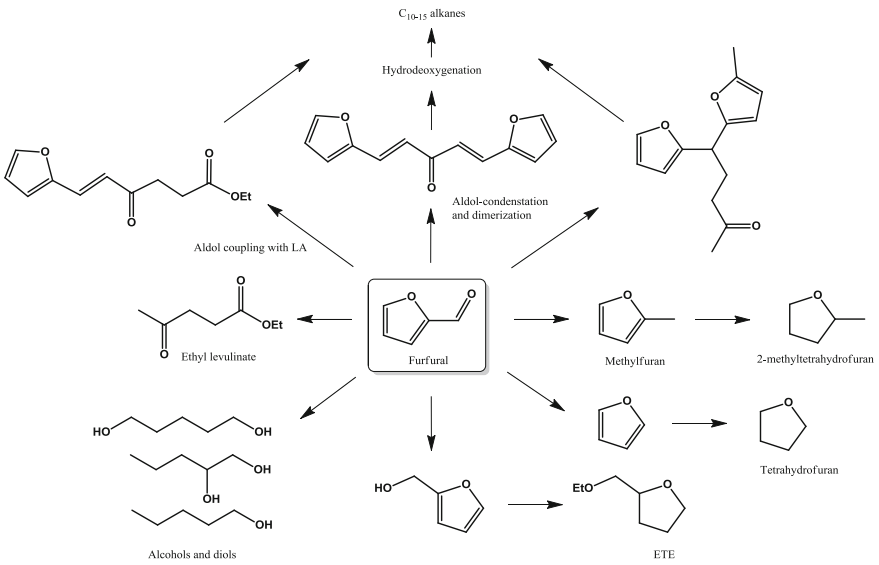


Fig. 9 Outline of potential chemical and fuel derivatives synthesis from furfural by catalytic transformation (Cai et al. 2014)

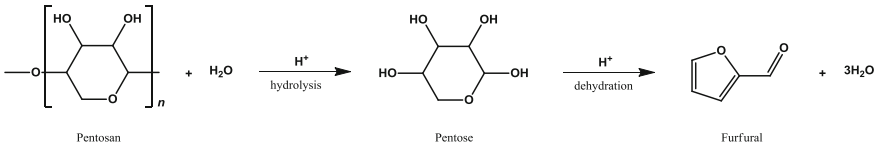


Fig. 10 Proposed mechanism for catalytic reaction of xylose to furfural (Cai et al. 2014)

(Mandalika and Runge 2012). In another study, Binder and Raines (2009) have demonstrated that use of Lewis acid metal chloride catalysts ($CrCl_3$ and $CrCl_2$) for furfural production via isomerization of xylose to xylulose intermediate in a monophasic solvent system (*N*,*N*-dimethylacetamide/LiCl) and reported maximum (56%) furfural yield. Subsequently, Zhang and Zhao (2010) have demonstrated furfural production from variety of biomass substrates such as corn stalk, rice straw, and pine wood using ionic liquids containing $CrCl_3$ under microwave irradiation and reported improved yield within short reaction time (3 min). However, several issues are associated with the homogeneous catalysis systems including metal corrosion and safety problems, difficulty in product separation, excessive waste disposal, extensive side reactions, and low product yield during furfural production, making this processing technology more challenging for commercial production.

Alternatively, Dias et al. (2005) have demonstrated the use of solid catalysts such as heteropoly acids and Nafion for furfural production from xylose in a high boiling point organic solvent dimethylsulfoxide (DMSO) and achieved modest furfural yield of 58–67% mol. with less product selectivity. Undesirable side reactions during xylose dehydration to furfural were responsible for the by-product formation (humic substances) and identified as the key factor for low product selectivity (Bond et al. 2014). Recent studies have employed aqueous biphasic systems (combined water and organic solvents commonly methyl isobutyl ketone, MIBK, or toluene) in order to reduce the humin formation positively by in situ extraction of produced furfural. For example, Stein and co-worker have demonstrated the furfural production from xylose using $FeCl_3{\cdot}6H_2O$ (homogeneous catalyst) in water/2-MTHF biphasic reaction system with NaCl as additive (Fig. 11) and reported improved furfural yield with reduced humin formation (vom Stein et al. 2011).

Chheda et al. (2007) employed biphasic systems using either water/HCl/MIBK/2-butanol or water/HCl/dichloromethane for xylose dehydration and were able to achieve high furfural selectivity (75–90%) via homogenous catalysis alone. In a similar study, Xing et al. (2010) have reported furfural yield as high as 87% in a biphasic reaction system consisting tetrahydrofuran/HCl and NaCl, as phase modifier. In a different study, Moreau et al. (1998) used zeolites such as mordenite and faujasite in biphasic systems consisting of water and MIBK/toluene for the dehydration of xylose to furfural and have reported more than 90% furfural selectivity. On the whole, recent studies have used microporous zeolites, modified mesoporous silica materials, conventional calcium salts of 12-tungstophosphoric acid, and exfoliated transition metal oxides, as new

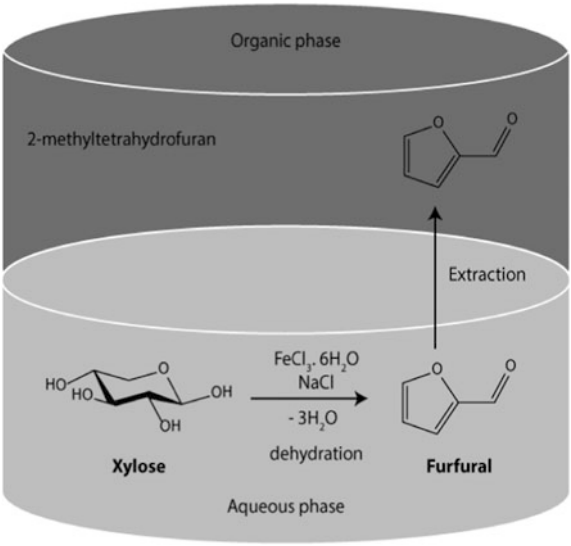


Fig. 11 Furfural production from xylose in a biphasic reaction system (vom Stein et al. 2011)

generation catalyst for the evaluation of xylose to furfural and are in their infancy. In spite of considerable advantages, solid acid catalysts need more characteristic study on both structure for reactivity and stability which leads to catalyst inactivation. Thus, more research is needed for further improvement of product yield, cost-effective method development, and reducing environmental impacts.

3.2 5-Hydroxymethylfurfural

In recent years, the synthesis of 5-hydroxymethylfurfural (HMF) or 5-hydroxymethylfuran-2-carbaldehyde from lignocellulosic biomass was widely researched (Rouilly and Vaca-Garcia 2015). 5-HMF is a heterocyclic furanic molecule substituted in 2,5-position with hydroxide and aldehyde functionalities and is a relatively unsaturated aromatic compound, allowing it to be facilely upgraded to fuel molecules via hydrogenation (Corma et al. 2007). Therefore, 5-HMF is considered as a versatile platform chemical for the production of new generation biofuels (as shown in Fig. 12), for example, dimethylfuran (DMF) can be obtained via selective hydrogenation in the presence of tetrahydrofuran (THF). Similarly, etherification with ethanol in the presence of acid can deliver 5-alkoxymethylfurfural, considered as an excellent additive for diesel fuel. Likewise, 5-HMF can be transformed into many versatile chemical compounds through employing various types of reactions, including 2,5-furandicarboxylic acid (FDCA) (used in polymer industries), 2,5-hydroxymethylfuroic acid (HMFA) and 5,5′(oxy(bismethylene))-2-furaldehyde (OBMF) from oxidation, 2,5-dimethylfuran (DMF) from selective hydrogenation, levulinic acid (LA) from hydration, and

1,6-hexanediol (HDO) from hydrogenolysis. Series of oxidation and hydrogenation reactions can yield adipic acid, caprolactam (monomer for nylon-6 preparation), and caprolactone from 5-HMF in the presence of catalyst (van Putten et al. 2013).

HMF is produced through acid-catalyzed dehydration (loss of three H_2O molecules) of hexose sugars such as glucose or mannose, created during biomass carbohydrate hydrolysis, after the hexose isomerization to fructose as (Takagaki et al. 2009), shown in Fig. 13. Several detailed studies have demonstrated that HMF production is more selective from fructose than it does from glucose. Due to the unstable character of HMF, obtaining of high yield and selectivity from glucose is challenging in a large-scale production. In a typical reaction condition, HMF is more likely to form levulinic acid and formic acid at equal proportion and additionally humin, an undesirable condensation product (Cai et al. 2014).

The traditional strategy for the synthesis of HMF from biomass-derived sugars is either using homogeneous mineral acids or heterogeneous solid acids in monophasic or biphasic reaction systems (Gallo et al. 2013; Corma et al. 2007). In a monophasic, nonaqueous solvent system, dimethylsulfoxide has shown promising yield (>90%) of HMF using acidic resins as catalyst among other solvents by limiting the formation of levulinic acid through rehydration reactions. Although monophasic solvent systems using DMSO or ionic liquids as solvents improves the HMF yield, product separation and purification is still remains challenging along with cost considerations (Lewkowski 2001). Wang et al. (2013) have demonstrated the HMF production from glucose/fructose in DMSO or DMSO-THF catalyzed by

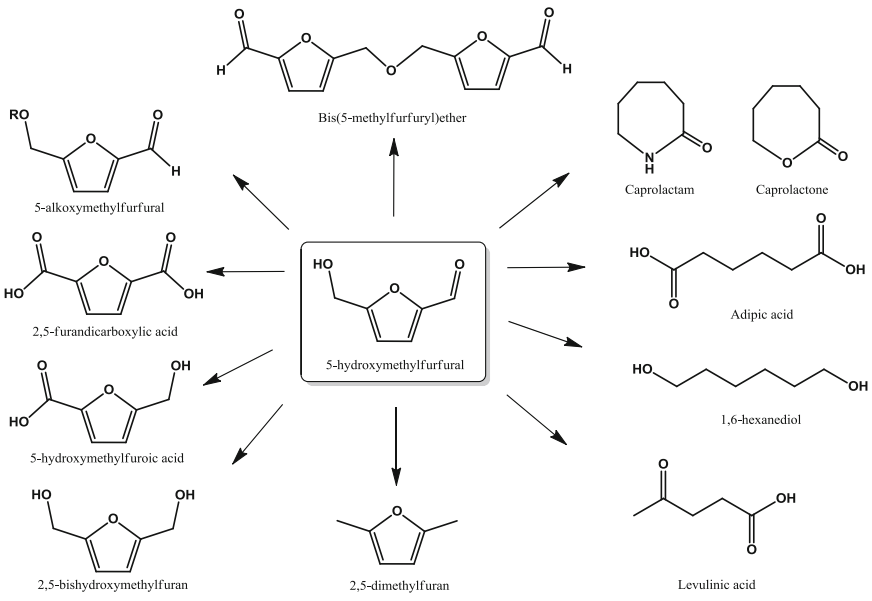


Fig. 12 Outline of potential chemical and fuel derivatives from 5-hydroxymethylfurfural (Krawielitzki and Kläusli 2015)

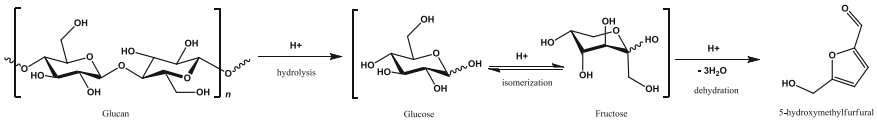


Fig. 13 Proposed three-stage catalytic reaction of glucose to 5-hydroxymethylfurfural (Corma et al. 2007)

sulfonated carbon and reported 91–98% yield (mol. basis). Subsequently, Yan et al. (2009) have demonstrated the glucose conversion using superacids SO_4^{2-}/ZrO_2 (CZS) and $SO_4^{2-}/ZrO_2–Al_2O_3$ (CSZA) in DMSO and could achieve maximum yield of 48% mol. at 130 °C in 4 h. The major drawbacks for industrial-scale production of HMF are: (a) low sugar solubility, which limits the sugar loading to the reaction, (b) product separation from the high boiling point solvents, (c) the possibility of HMF degradation at elevated temperature conditions, and (d) under acidic conditions, DMSO may undergo decomposition reaction resulting in toxic substance formation. In an alternative approach, several studies have adapted biphasic solvent systems comprising a mixture of water and organic solvents, as extracting agent to improve the HMF production yield by simultaneous extraction of sugars and allowing for relatively high loading to the reaction (van Putten et al. 2013).

Synthesis of HMF in biphasic systems have been extensively studied for a number of years using both homogenous and heterogeneous catalysts and demonstrated to be promising. For instance, Yang et al. (2012) have studied HMF production from different biomass substrates including corn stover, pinewood, switchgrass, poplar, and cellulose to HMF in water-tetrahydrofuran biphasic system using $AlCl_3·6H_2O$ and NaCl under microwave heating. Hansen et al. (2011) have demonstrated the catalytic dehydration of glucose using various salts and boric acid in water-methyl isobutyl ketone (MIBK), as extracting organic solvent. Table 5 summarizes the commonly used catalysts (both homogenous and heterogeneous) for the synthesis of HMF from glucose molecule. Recently, studies have also evaluated lanthanide catalysts ($LaCl_3$, $NdCl_3$, $EuCl_3$, $DyCl_3$, and $YbCl_3$), various zirconium-, titanium-, and niobium-based catalysts, etc. for HMF synthesis. These are reported as being highly selective for HMF production but achieving low substrate conversion (Rosatella et al. 2011).

Ionic liquids (ILs) have recently received attentions for the HMF synthesis reaction (Kim et al. 2011). Studies have reported that yields of HMF obtained in ILs are comparable to those obtained in DMSO while HMF can be conveniently recovered by liquid–liquid extraction using MIBK, THF, or 2-butanol. It is noteworthy that the same biphasic system strategy was employed in order to limit the rehydration of HMF to LA. In this case, sodium chloride was generally used to facilitate the extraction of HMF, using the salt-out effect. However, the cost and toxicity of ILs together in addition to problems associated with their long-term recycling capabilities currently hinder their use as large-scale production (Zhao et al. 2007).

Table 5 Common catalyst group for HMF synthesis from hexose sugar molecule

Catalyst group	Example	References
Organic acids	Carboxylic acids, lactic acid, oxalic acid, levulinic acid, maleic acid, *p*-toluenesulfonic acid, boric acid	Cottier et al. (1995), Lewkowski (2001)
Inorganic acids	Sulfuric acid, phosphoric acid, hydrochloric acid, iodine, or hydroiodic acid generated in situ	Lewkowski (2001), Bonner et al. (1960)
Slats	$MgCl_2$, $(NH_4)_2SO_4/SO_3$, pyridine/PO_{4-3}, pyriniden/HCl, aluminum salts, Th and Zr ions, zirconium phosphate ions: Cr, Al, Ti, Ca, In, $ZrOCl_2$, $VO(SO_4)_2$, TiO_2, V-porphyrin, Zr-, Cr-, and Ti-porphyrins, lanthanides ($LaCl_3$, $NdCl_3$, $EuCl_3$, $DyCl_3$, and $YbCl_3$)	Liu et al. (2012), Lewkowski (2001)
Lewis acids	$ZnCl_2$, $AlCl_3$, BF_3	Lewkowski (2001)
Solid acids	Ion exchange resins, zeolites, supported acids, heteropoly compounds	Lewkowski (2001)

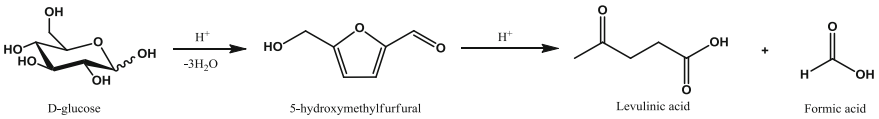


Fig. 14 Proposed general reaction mechanism of glucose to levulinic acid (Bozell et al. 2000)

3.3 Levulinic Acid

Levulinic acid (4-oxopentanoic acid) is a linear C5-alkyl carbon chain containing one carboxylic acid group in position 1 and one carbonyl group in position 4 (Fig. 8). The controlled degradation of hexose sugars (mainly glucose) either using mineral acids or solid acids is still widely used approach to prepare levulinic acid (LA) from lignocellulosic biomass through the intermediate formation of HMF (Fig. 14). In more detail, as proposed, the stepwise reaction mechanism of the transformation of HMF to LA is illustrated in Fig. 15.

As a platform molecule, LA is presently receiving considerable attention; due to its bifunctional nature that enables many catalytic transformation strategies. For instance, LA is used for the sustainable drop-in hydrocarbon fuels production by novel processes, for example, γ-valerolactone and α-angelicalactone (Fig. 16). Historically, homogenous acids such as HCl, H_2SO_4, H_3PO_4, etc. were the most popular catalysts used for the preparation of LA from lignocellulosic materials (Rackemann and Doherty 2011). A more recent system for LA preparation from cellulosic materials is the Biofine process, a two-stage acid-catalyzed reaction system (H_2SO_4) developed by Fitzpatrick (Hayes et al. 2008; Fitzpatrick 1997). This technology makes use of high temperature and short residence time in first reactor and vice versa in the second reactor and could transform 50% of the substrate (biomass) to LA under specified conditions.

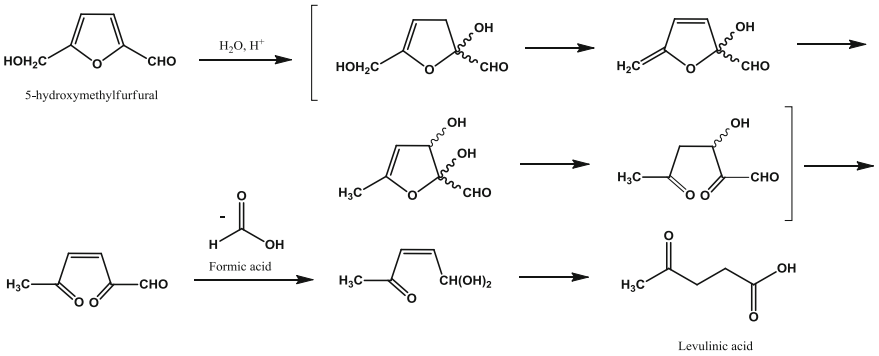


Fig. 15 The proposed stepwise reaction mechanism of 5-hydroxymethylfurfural to levulinic acid (Horvat et al. 1985)

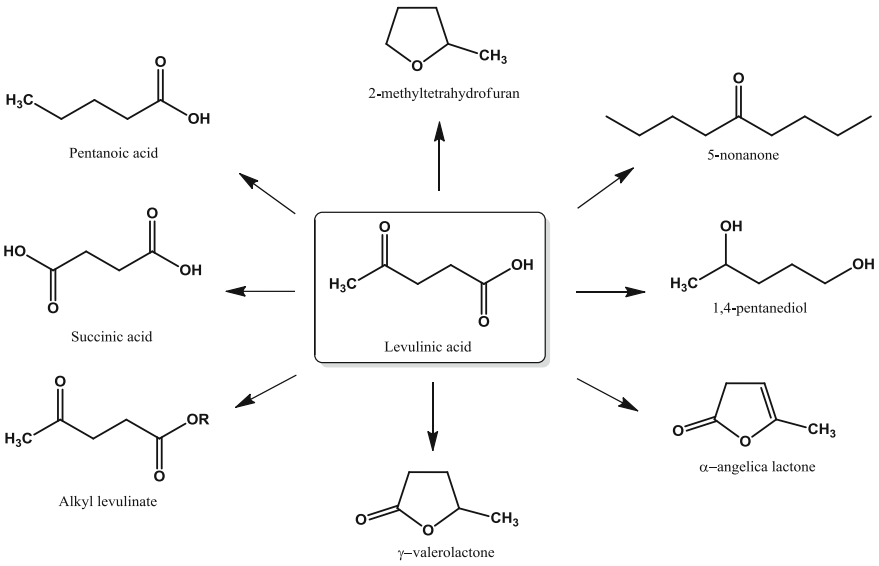


Fig. 16 Outline of potential chemical and fuel derivatives from levulinic acid as precursor (Luterbacher et al. 2014a)

Subsequent studies have evaluated the effectiveness of various acids for the dehydration reaction for levulinic acid preparation and have observed the conversion efficiency decrease in the following order of acids used: HBr > HCl > H_2SO_4 > acetic acid (Ghorpade and Hanna 1997; Girisuta et al. 2013). In another study, Peng et al. (2010) screened the catalytic performance of metal halides and found that $CrCl_3$ effectively catalyzed the cellulose by producing maximum 67% mol. of LA. In another study, Heeres et al. (2009) evaluated

tri-fluoroacetic acid (TFA) as an alternative to mineral acids for LA preparation based on high electronegativity and ionization potential of fluorine as an efficient solvent/catalyst system. This study yielded low LA concentration (57%) when compared to the study using H_2SO_4 (60%). Later TFA was replaced with heptadecafluorononanoic acid as the catalyst in the presence of a soluble solvent such as perflurohexane to overcome the complicated recovery process due to azeotrope formation. A major concern with LA production by this route (homogenously) is the low yields of less than 66% than theoretical, due to the coproduction of formic acid, formation of undesired black insoluble materials called humins, inefficient recovery of levulinic acid due its inherent physical property as well as nonselective nature of catalysts and re-polymerization of products at each step of the reaction pathway. Hence, homogeneous catalysis methods generally cause problems associated with the acid recovery, complicated product separation, undesirable side reactions, and potential environmental issues.

In order to overcome the aforementioned issues and minimize the insoluble by-products formation, heterogeneous catalysis using solid acids for the preparation of LA has been developed and is fairly well established. For instance, studies have been conducted using solid acid catalysts such as ion exchange resins, zirconia and titania, stannous and stannic chloride, $SO_{42}-/ZrO_2–Al_2O_3$ (CSZA), $AlCl_3{\cdot}6H_2O$, Sn-Mont, and zirconium phosphate, etc. These studies suggest that solid super acid, $S_2O_{82}-/ZrO_2–SiO_2–Sm_2O_3$, could be used as a potential catalyst for the preparation of LA from lignocellulosics, e.g., rice straw. Along with other advantages of heterogeneous catalysis over homogeneous catalysis including easy acid separation and reuse, solid superacids offers more acid strength than liquid acids. Recently, Potvin et al. (2011) have reported as high as 70% LA yield from cellulose in aqueous solution using a solid acid-supported catalyst with the addition of 20% NaCl, as an alternative to the use of ionic liquids for cellulose hydrolysis. It has been postulated that the yield and efficiency of LA production could be significantly improved by recovery of the intermediate products at each step of the reaction pathway, such as use of highly selective catalysts which would provide the necessary step change for the optimization of key reactions. A processing environment that allows the use of biphasic systems to allow continuous extraction of products would increase reaction rates, yields, and product quality. Similarly, Runge and Zhang were able to demonstrate higher levulinic acid yields through separation of pentosans and hexosans on biomass to reduce humin production from furfural condensation during levulinic acid production (Runge and Zhang 2012).

Use of ionic liquids for the preparation of LA from lignocellulosics has also been attempted by various researchers, although the technique is marred by high cost of the catalyst rendering it unsuitable for large-scale applications. For instance, Sun et al. (2012) reported one-pot synthesis of LA from cellulose using HPA ionic liquid catalyst in a water-methyl ketone biphasic system with the highest yield of 63% mol. Though the catalyst was separated and reused without appreciable loss of performance, the process was found to still be economically unviable. Lin et al. (2012) recently presented an alternative pathway called Aqueous Phase Partial Oxidation (APPO) for levulinic acid production directly from cellulose with clean

air and water over inexpensive solid metal oxide catalyst (ZrO_2) and achieved nearly 60% mol. yield. Accordingly, compared to conventional acid hydrolysis, APPO was found to be highly selective and environmentally benign process with merits of easy recovery and reuse of heterogeneous catalysts. Overall, the development of production methods has progressed from batch processes to continuous processes incorporating recycling and utilizing multistages to optimize processing conditions to improve the yields from >50 to ~80% of the theoretical limit.

4 Conclusions and Future Perspectives

To date, commercial processes for the production of biomass-derived biofuels are based mainly on bioethanol from corn and sugarcane, and biodiesel from triglycerides. In line, this chapter presents a review on advances in transformation of lignocellulosic biomass toward liquid biofuels synthesis through the formation of several intermediate platform molecules like levulinic acid, HMF, and furfural. These versatile molecules are typically derived from carbohydrates and their derivatives have been proposed for the synthesis of new generation biofuels. For instance, alkyl levulinate, γ-valerolactone (GVL), and 2-methyltetrahydrofuran (MTHF) can be obtained from levulinic acid through series of transformation reactions and possesses many identical properties of an ideal liquid toward synthesis of energy fuel and chemical products particularly GVL. Moreover, GVL exhibit similar behavior to ethanol when blended with gasoline. Also, MTHF is popular as fuel additive that can be blended with gasoline up to 70% vol. proportions without engine modification (van Putten et al. 2013). Likewise, HMF and other furfural derivatives can be upgraded to form hydrocarbon fuel via aldol condensation reaction with ketones and followed by undergoing hydrogenation/dehydration reactions (Beerthuis et al. 2015). In majority, these high-value molecules and its derivative chemicals are prepared through heterogeneous catalysis owing to its reaction specificity and improved yield. Indeed, these precursor synthesis processes are still far behind from being commercial stage operations mainly due to its high cost of operation. Continued research and development can further improve the commercial considerations including the probable methods like tailoring of catalyst to perform the reaction in less steps and avoiding intermediate product separation could positively facilitate implementation of sustainable biorefining processes.

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Biodiesel Synthesis: Use of Activated Carbon as Support of the Catalysts

Aida Mireya Ramírez-Arias, Liliana Giraldo and Juan Carlos Moreno-Piraján

Abstract Biodiesel synthesis was performed by means of a transesterification reaction, for which soybean oil and palm oil were used as raw materials. Homogeneous processes, heterogeneous catalysis, and biocatalysis, using *Lipase type II from porcine pancreas* as a biocatalyst, as alcohol, methanol, were used to carry out the reaction. With regard to the heterogeneous catalysts, it is used activated coal and gamma alumina like support. Once the process was finished and biodiesel was purified, the chemical and physical properties like: density, humidity, acid value, saponification, cetane index, and calorific value were determined according to ASTM (American Society for Testing and Materials) standards and the methyl esters were quantified by gas chromatography, where it was in more percentage C18 and C16. The best conditions found for each transesterificación were: 0.7% KOH, at 60 °C, 100 rpm, a molar ratio of alcohol: oil of 6:1 and 90 min for homogeneous catalysis. Heterogeneous catalysis 3.0 wt% catalyst, a molar ratio of alcohol: oil of 9:1, the temperature of 60 °C, at 100 rpm for 90 min, the catalyst that presented higher yields of biodiesel 95 and 96% soybean oil (SO) and crude palm oil (OP) was when pelleted activated carbon (PAC) was used as support. In the biocatalysis conditions were 40 °C for 6 h, 6.0% lipase and 300 rpm agitation.

Finally, it is concluded that the biodiesel yields have a direct relation with the surface area of the catalyst, being a greater area in PAC and with this also the higher yields of biodiesel in each one of the different catalysis. Activated carbon is a material that produced good yields in the synthesis of biodiesel shows potential for transesterification of FFA. The synthesis of biodiesel from the transesterification of soybean oil and palm oil complies with the standards stipulated by ASTM to be used as fuel and thereby generate alternative sources of energy.

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1 Introduction

In this century, the human population must confront the problems generated by the increase in energy demand, the depletion of fossil fuels, rising oil prices, and environmental pollution by greenhouse gases produced largely by oil use. These gases are responsible for much of the climate change around the globe, with adverse effects seen on human health. It is therefore necessary to look for alternative energy sources based on renewable processes. From this perspective, research into the production of biofuels is very important (Banerjee and Chakraborty 2009; Banerjee et al. 2002; Björkling et al. 1991; Bommarius and Riebel 2004). One of the sectors in the industry with increased demand for liquid fuels such as gasoline and diesel is the automotive sector. This unit produces two disadvantages: oil shortage and increased emission of pollutants generated in the incomplete combustion of these liquids (Gomez 2007).

The approval of law 693 marked the entry of Colombia into the new era of biofuels, used for many decades, especially bioethanol, due to the economic attractiveness, according to the Kyoto Protocol, and the dynamic oil prices. Preparation processes of biofuels are intended to diversify the energy mix using new energy alternatives that create a positive impact on economic, social, and environmental levels (Gomez 2007; Hossain et al. 2008).

Biofuels are substances that are produced from biomass, and serve as a source of renewable energy, reducing CO_2 production, closing the carbon cycle, through photosynthesis, transforming in carbohydrates, like sugars and starches (Diaz and Balkus 1996). The authors of the present work show recent results obtained in your laboratory which involved preparing biodiesel, from soybean oil and palm oil by heterogeneous and homogeneous catalysis, where activated carbon and gamma alumina was used as a support for catalysts in transesterification reactions. The activated carbon used is obtained from coconut shell, in granular and pelletised form, with a good surface area (A_{BET} 842–1131 $m^2\ g^{-1}$), a residue that is not given an adequate use.

The biodiesel obtained is characterized, taking into account its physical and chemical properties and the percentage of methyl esters, where it is a biofuel and must comply with certain standards already established by ASTM for its commercialization. Results of three types of catalysis (homogeneous, heterogeneous, and biocatalysis) are presented, in order to determine which process is more viable for biofuel production, taking into account the type of catalyst and the price factor.

2 Theoretical Basis

2.1 Biodiesel

The biodiesel Fatty Acid Methyl Esters (FAME) is a synthetic, clean, nontoxic, and high-quality biofuel, comprising a mixture of monoalkyl esters of long chain fatty acids derived from sources of triacylglycerides such as vegetable oil residues from the food industry and animal fats. For those produced from plant materials, emissions of carbon dioxide (CO_2) are lower than those produced by fossil fuels, in the combustion process. Biodiesel properties vary according to the raw material; among the most outstanding and attractive are its biodegradability and non-toxicity (Boz and Kara 2009; Cadena agroindustrial Etanol 2004).

In contrast to diesel, biodiesel has several advantages: (1) it is a renewable and biodegradable energy (degrades four times faster than diesel); (2) during combustion, it produces fewer emissions of pollutants (carbon dioxide, sulfur oxides, nitrogen, and metals) because of its oxygenated state (Bommarius and Riebel 2004); (3) it has lubricating properties that minimize engine wear; and (4) it is safe for storage and handling due to its low volatility and high flash point (100–170 °C) (Bommarius and Riebel 2004).

The technology for the production of biodiesel has been known for more than 50 years in the United States and it has been primarily made from soy oil. Other sources of biodiesel in the world are canola oil, animal fat, palm oil, corn oil, canola oil, Jatropha oil, coconut oil, waste cooking oil, and algae oil, which have potential to displace diesel, taking into account their oil yield and surface area required (Table 1) (Chisti 2007; Idris and Bukhari 2012; Meher et al. 2006).

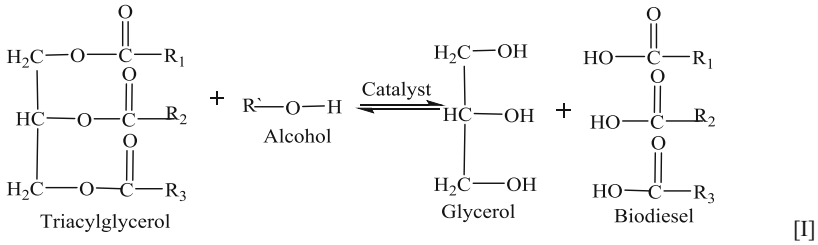
Biodiesel is produced from a transesterification reaction, based on the transformation of a triacylglycerides in Fatty acid methyl esters (FAMES) in the presence of an alcohol (methanol or ethanol) and an acid, with the most common basic catalyst, glycerol, being obtained as a by-product (see Fig. 1) (Demirbas and Demirbas 2011).

In the first reaction [II], diacylglyceride is obtained from triacylglycerides, then monoacylglycerides [III], and finally glycerine [IV]. During the process, three

Table 1 Comparison of some sources of biodiesel (Meher et al. 2006)

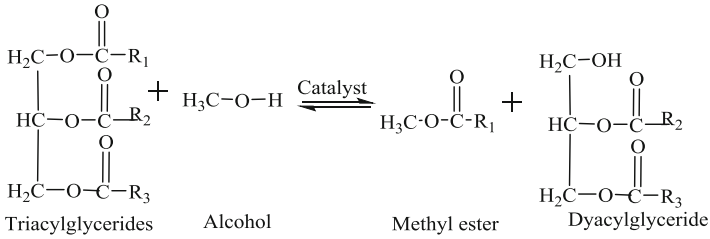
Cultivation	Oil yield (L ha^{-1})	Surface area required (m ha)[a]
Corn	172	1540
Soy	446	594
Canola	1190	223
Jatropha	1892	140
Coconut	2689	99
Palm oil	5950	45

[a]To fulfill 50% of all transport fuel necessary for the United States

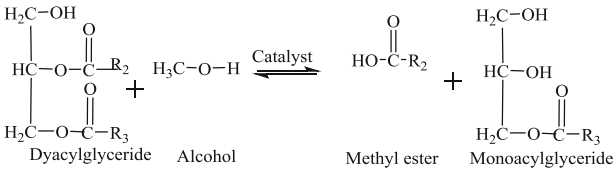


[I]

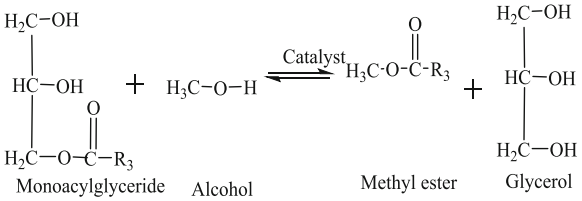
The global process involves a sequence of three reversible reactions:



[II]



[III]



[IV]

Fig. 1 Process for the synthesis of biodiesel

molecules of methyl esters are produced from an excess of alcohol to displace the reaction towards the production of methyl esters (Ebert 2008).

In transesterification, the variables that have the greatest influence are: purity and quality of the reagents, alcohol/oil molar ratio, alcohol type, catalyst type and amount, temperature, agitation and time. For this reason, it is essential to control these variables (Ebert 2008).

2.1.1 Synthesis of Biodiesel

Transesterification Basic Medium

The base-catalyzed transesterification is a classic example of a nucleophilic acyl substitution by an addition–elimination mechanism. The alkoxide ion is highly nucleophilic, attacking the ester carbonyl and subsequently the alkoxide ion which the ester was traveling to, and thus the transesterification occurs (see Fig. 2).

This transesterification is about 4000 times faster than the acid-catalyzed reaction (Fukuda et al. 2001). It commonly uses sodium hydroxide and potassium at a concentration of 1% by weight of oil as catalysts. Alkoxides such as sodium methoxide are even better catalysts. The transesterification is carried out at 60 °C, at atmospheric pressure and takes approximately 90 min to complete (Fukuda et al. 2001).

Transesterification in Acidic

The acid-catalyzed reaction is similar to the above mechanism, only the proton alcohol group, being less nucleophilic, facilitates further transfer given protonation of the oxygen carbonyl group, which behaves as a presented Lewis base due to its lone pairs. The protonation of oxygen gives more character to the carbon electrophile attack alcohol, resulting in the formation of a tetrahedral intermediate which is a deprotonated alcohol group of another molecule. A subsequent protonation provides adequate oxygen, making this group a better leaving group than the unprotonated form; when oxygen enters the electron pair, a molecule of alcohol is ejected, leaving a protonated version of the final product (see Fig. 3) (Fukuda et al. 2001).

2.2 Homogeneous Catalysis

2.2.1 Homogeneous Alkaline Catalysis

Homogeneous catalysis is the best known method for the synthesis of biodiesel, with the following being commonly used as catalysts: sodium hydroxide (NaOH),

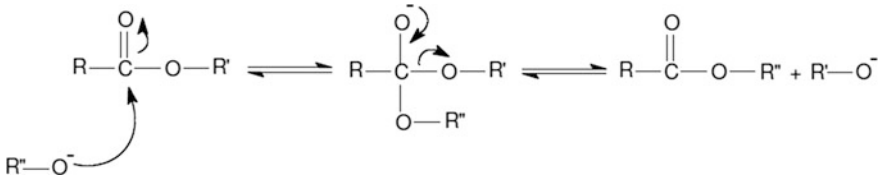


Fig. 2 Transesterification in a basic medium

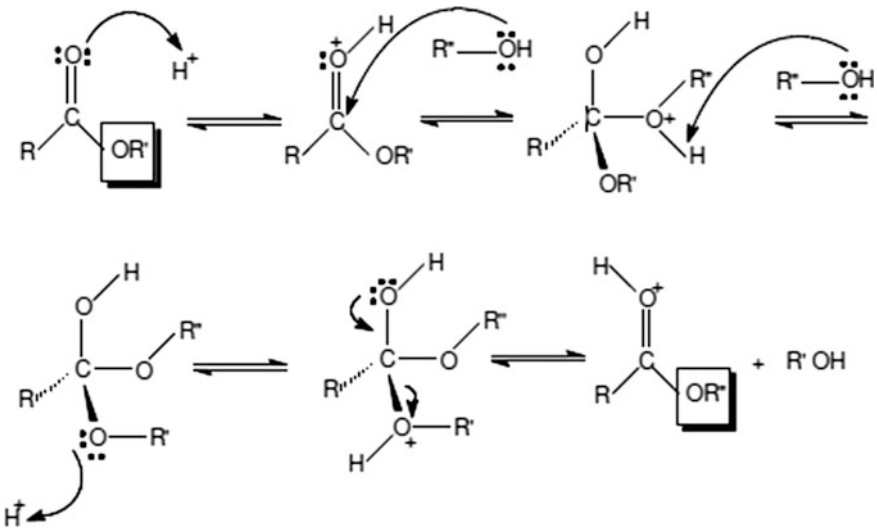


Fig. 3 Acid catalyzed transesterification

potassium hydroxide (KOH), and sodium methoxide ($NaOCH_3$); these soluble catalysts are inexpensive and generate the transesterification reaction at short times, using a lower temperature, moderate pressure and a 3:1 oil:alcohol ratio for the production of methoxide to react with the oil to produce biodiesel and glycerol. Despite these advantages, the preparation and catalyst recovery is tedious (Kok et al. 2011), since it is not easy to separate the catalyst from the reaction products, which makes it necessary to perform extensive washings with water, producing large quantities of water alkaline waste which has to be treated for proper disposal (Knothe et al. 2005). Also, if the starting material (oil) contains a high proportion of free fatty acids (FFA), or water, soap FFA is formed, which can affect the performance of biodiesel (Kok et al. 2011).

2.2.2 Homogeneous Acid Catalysis

In such catalysis, the most frequently used catalysts are sulphuric acid, hydrochloric acid, and phosphoric acid. In contrast to the basic homogeneous catalysis, this uses a higher temperature, increased reaction time, higher oil:alcohol ratio, and more alcohol. No side reactions occur in the transesterification process, with high percentages of fatty acids (greater than 3%) and water (Kok et al. 2011).

However, homogeneous reactions have several disadvantages that make them economically unattractive, such as the homogeneous phase between the catalyst and products, which makes separation and purification steps more complicated. Alkaline water and oily residues generated in the separation increase the production

costs of biodiesel; in addition, glycerine purification is difficult due to the solubility of methanol (Knothe et al. 2005).

2.3 Heterogeneous Catalysis

The use of heterogeneous catalysts reduces the problems presented by homogeneous catalysts, including avoiding the complex separation procedures required in homogeneous catalysis. Here, the catalyst and products are in different phases; heterogeneous catalysts can also be easily recovered and reused, reducing the cost of the production of biodiesel [16.17]. This catalysis is not affected by the presence of high fatty acid content (greater than 3%) in oil. However, compared with homogeneous catalysis, heterogeneous catalysis proceeds at a slower rate due to the three-phase system: oil, alcohol and solid catalyst (Kok et al. 2011). To avoid the problem of mass transfer, porous supports with catalysts that can provide a relatively high specific surface area are used; the presence of pores can react with triacylglycerides (Kok et al. 2011; Zabeti et al. 2009). Also, ɤ-Al_2O_3 has been widely used as a support in catalysis processes due to the thermal and mechanical stability, specific surface area and pore volume (Zabeti et al. 2009; Yun et al. 2011) (Table 2).

Table 2 Characteristics of heterogeneous acidic and basic catalysis

Heterogeneous basic catalysis (Boz and Kara 2009; Yun et al. 2011)	Heterogeneous acidic catalysis (Zabeti et al. 2009; Yun et al. 2011)
✓ The catalyst is solid and the reactants and products are liquid ✓ The catalyst can be fully recovered and reused ✓ The catalyst is easy to separate and purify, typical of homogeneous processes catalyzed by bases.	
Less explored than basic homogeneous catalysis	The solid acid catalysts in heterogeneous transesterification are useful when the oil has a high content of FFA and water
Requires less temperatures than acid catalysis	Require high temperatures for the reaction to complete the transesterification process
Most of the basic solid catalysts have a higher activity and reaction rate with respect to solid acids in transesterification processes	The catalytic activity of solid acid catalysts, it is necessary modify the surface, to increase the pore size, density of active sites (Boz and Kara 2009; Bengoagorostiza 2012)
Between the basic solid catalysts are: NaOH/ɤ-Al_2O_3, K/ɤ-Al_2O_3, KOH/NaY, KOH/ɤ-Al_2O_3 and zeolites	Among the solid acid catalysts are: SO_4^{2-}/ZrO_2, SO_4^{2-}/SnO_2, sulphonated amorphous carbon, ZrO_2, and WO_3

2.4 Biocatalysis

In recent decades, biotechnology has achieved progress in various areas of science; in particular, enzymes have become important tools for catalysis, reaching a broad spectrum of industrial applications, among which chemical synthesis, the food industry, and the pharmaceutical industry should be mentioned, as these present clear advantages over non-biological conventional catalysts, greater specificity and selectivity, working better under pressure, temperature and moderate pH and reaching speeds similar to the chemical catalyst reactions should be mentioned. Within the wide variety of industrially important enzymes are lipases (acyl ester hydrolases), which catalyze the hydrolysis and formation of lipids, with applications in the oleochemical industry, and fats, as in biodiesel production. This is one of the most interesting factors for the transesterification of oils, because it has the most efficient mechanism, there is no need for pretreatment of the raw material, since the catalyst is selective, and it consumes less power, generating little waste and making it environmentally attractive (Refaat 2010).

2.4.1 Lipases

Lipases (acyl ester hydrolases) are enzymes that catalyse the hydrolysis of triacylglycerols, especially long chain, which give monoacylglycerides, diacylglycerides, free fatty acids, and glycerol in an equilibrium reaction controlled by water content in the reaction medium. They modify the reaction conditions, and are also able to catalyse other reactions such as esterification, transesterification, and interesterification (Naranjo et al. 2010), as illustrated in Fig. 4.

These enzymes are mainly characterized by being water soluble and insoluble substrates and acting on aggregates, which operate together to form organic–aqueous interfaces; these are the only enzymes capable of this, unlike esterases, which hydrolyse soluble esters, working without the need for an interface. Most lipases are esterases, although not all esterases are lipases. The author (Verma et al.

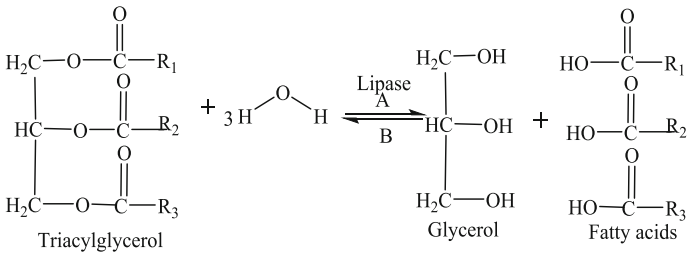


Fig. 4 Catalytic reaction of natural lipase. A triacylglycerol can be hydrolysed to glycerol and fatty acids (A), or the reverse reaction combining glycerol and fatty acids to form triacylglycerol (B)

2012) presented as a fold structure of polypeptides composed of eight β-sheets, connected by six α-spirals. The triad Ser-His-Asp/Glu is completely covered by a lid which must be fully opened to access the substrate; the catalytic triad is embedded in a consensus region Gly-X-Ser-X-Gly. Its mechanism of activation may be open or closed: (1) Open or active: the polypeptide chain is displaced and the active site is exposed to the reaction medium; and (2) Closed: The polypeptide chain closes the active site, forming the top "lid", causing inactivation of the enzyme (Verma et al. 2012).

2.4.2 Transesterification and Lipase Interesterification

The transesterification reaction is a process in which the acyl donor is an ester and can be classified via glycerolysis and alcoholysis, as glycerol or an alcohol, and the acyl receptor (see Fig. 5).

The transesterification reaction is reversible and an excess of alcohol is used to drive equilibrium towards the formation of esters; chemically, the enzymatic transesterification mechanism comprises three consecutive reversible reactions: the triacylglycerides becomes sequentially diacylglycerides, monoacylglycerides, glycerol, and methyl esters (see Fig. 6) (Naranjo et al. 2010).

Interesterification is the reaction between two esters exchanging their acyl groups, while acidolysis is the reaction between an ester and a carboxylic acid, which proceeds by replacement of the acyl ester group by the free acid (see Fig. 7) (Björkling et al. 1991).

Despite the advantages of using lipase as a biocatalyst, the process of isolation and purification is costly; when they are isolated from their natural environment, the structure is unstable. Moreover, being soluble in water and operating in homogeneous reaction systems, contaminate the product and cannot be recovered for reuse, which becomes an economic problem because the enzymatic activity is lost (Mateo et al. 2007). The enzyme action is increased when immobilized on a suitable support; in the industrial process in which is used, in order to improve properties such as operational stability, thermal stability, activity, specificity and selectivity, reduced inhibition of the reaction products is also possible. Therefore, the above

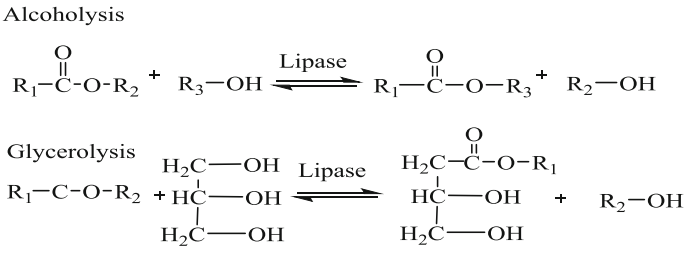


Fig. 5 Transesterification type with lipase

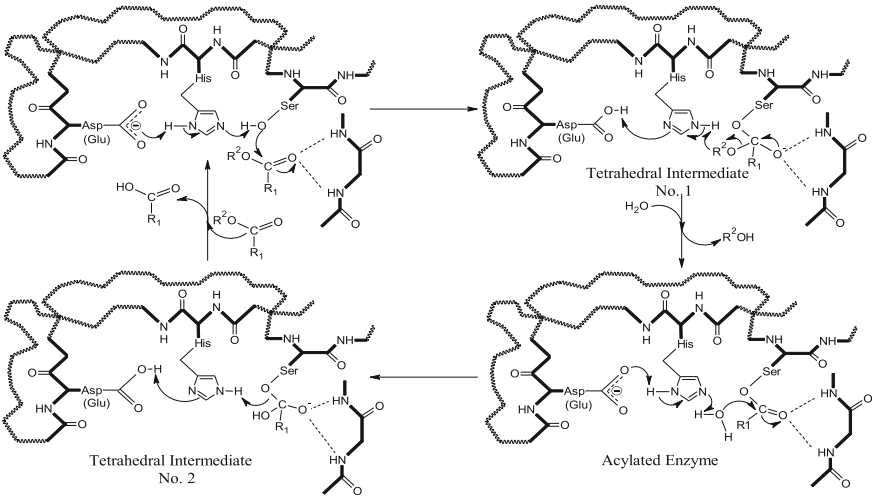


Fig. 6 Reaction mechanisms of lipases (taken from Naranjo et al. 2010)

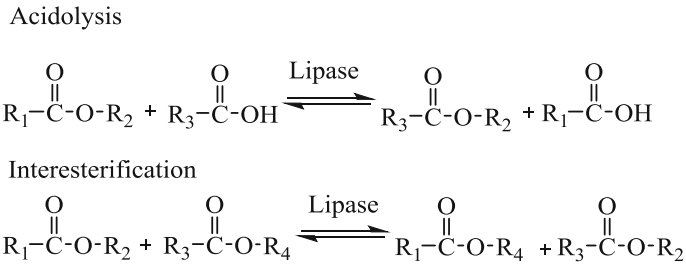


Fig. 7 Types of interesterification with lipases

depend heavily on the support and the locking protocol that is used (Verma et al. 2012; Mateo et al. 2007).

2.4.3 Immobilization of Enzyme

Immobilization is understood to be anchoring of the enzyme to an insoluble solid support, wherein the movement of the enzyme in the space is restricted or partially complete, resulting in a heterogeneous reaction system with the immobilized enzyme. This enables reactions, continuous enzymatic control, and product formation to be carried out, and facilitates the removal of the enzyme reaction mixture. Comparing the methodology when enzymes are free with when they are immobilized, the latter are more robust and more resistant to environmental changes (Saxena et al. 1999).

In the scientific literature, there is a method that is "standardized" for the immobilization of enzymes; this is due to its composition, differences in chemical characteristics, the different properties of the substrates, products and product applications, as well the advantages and disadvantages of each of the methods of restraint. Therefore, conditions for the investigation of an enzyme and its application are usually set based on the physical and structural properties of the support, the physical and chemical properties of the enzyme and the process specifications for the catalyst, to ensure the highest possible retention of enzyme activity and consider their performance, durability, cost and toxicity for immobilization reagent immobilization (Bengoagorostiza 2012; Saxena et al. 1999). The authors of this work have analyzed the most crucial variables in the process of immobilization of the *Lipase type II from porcine pancreas* and has found the most suitable conditions for immobilization to allow its use in the transesterification of fatty acids for the synthesis of biodiesel. Conditions, procedures, and results will be shown in this chapter. Immobilization was performed on granular and pelletised activated carbon made from coconut husks by thermal and chemical pretreatment to develop specific mesoporosity and proper surface chemistry that allows the high adsorption of lipase. The use of immobilized lipase in the transesterification reactions and subsequent synthesis of biodiesel has become a novel process due to its high specificity.

Methods of Immobilization by Chemical Bonding

- Covalent bonding

The covalent bonding methodology is based on the chemical activation of groups on the support by a specific reagent, which enables reactions with amino acid residues on the surface of the enzyme. The advantage of this method is the strength of unity and stability as a result of immobilization, whereas the disadvantages are the high costs and low yields, because the conformation of the enzyme and its activities are strongly influenced by the covalent union (Bommarius and Riebel 2004; Idris and Bukhari 2012; Bengoagorostiza 2012).

Methods of Immobilization by Physical Retention

- Adsorption

In adsorption, the enzyme is attached to an unfunctionalized support through reversible interactions, such as van der Waals forces and hydrogen bonds. It is the simplest, cheapest, and fastest method; chemical changes to the support or enzyme are not needed but this method has the disadvantage of loss of enzyme from the

support, steric hindrance occurring inside the support and the lack of specificity for this type of immobilization (Idris and Bukhari 2012; Bengoagorostiza 2012; Naranjo et al. 2010).

- Capture

In this method of immobilization, the enzyme is free in solution, but is limited in its movement by the lattice structure of the gel used for this procedure, which prevents the release of the protein without preventing penetration of the substrate. The immobilization process is carried out by suspending the enzyme in a monomer solution. Subsequently, the polymerisation is conducted by a temperature change or by adding a chemical reagent. The capture method has the disadvantage of the support acting as a barrier to mass transfer (Bommarius and Riebel 2004; Idris and Bukhari 2012).

- Encapsulation

The encapsulation method involves trapping the enzyme in various forms of spherical semi-permeable membranes with diameters ranging between 10 and 100 μm. Enzymes are physically contained within the membrane and the substrate and product molecules must diffuse through it freely if their sizes are small enough for this to be feasible. A disadvantage is the problem of acute dissemination, but an advantage may be the co-immobilization of different enzymes for special applications (Bommarius and Riebel 2004; Idris and Bukhari 2012; Naranjo et al. 2010).

Activated carbon includes a broad spectrum of materials consisting essentially of carbon, and is specially prepared to have high internal surface and high porosity, which allows different compounds to adsorb, both solids and liquids. Chemical analysis has shown that in addition to carbon, hydrogen and oxygen are present in its structure, allowing the formation of functional groups such as carbonyls, phenolics, esters, and carboxyl groups, among others, generating acidity or basicity in the coal, depending on their composition.

Activated carbon possesses a laminar structure consisting of microcrystalline parallel layers of carbon atoms arranged in regular hexagons; crystal regions are about 100 times lower than graphite and have a random orientation, allowing the existence of pores of different sizes to retain molecules of a gaseous medium or dissolution (Rodriguez 2003).

2.4.4 Supports for Immobilization

The physical and structural properties of the support and the physicochemical and chemical properties of the enzyme are critical for use as a guide in the selection of a suitable immobilization process, and in order to achieve a specific enzyme-support catalyst system with physicochemical properties and kinetics that are completely different from the free enzyme.

A wide variety of potentially viable supports are available for immobilizing an enzyme, using natural, synthetic, organic and inorganic compounds that differ in size, shape, density, and porosity. These take the form of sheets, tubes, fibers, and cylinders, but the most commonly used form is spheres; these should provide the system with permeability and have a suitable biotransformation surface area. Any material considered for the immobilization of enzymes must have certain characteristics, such as mechanical strength, high affinity for proteins, microbial resistance, the availability of reactive functional groups to direct reactions with the enzyme and/or chemical modifications, thermal stability, chemical durability, regenerability, biodegradability, and low cost (Mateo et al. 2007).

2.5 Carbon Activated as Catalytic Support

Activated carbon is a group of porous carbons obtained from the reaction of a carbonized material with oxidizing gases or by the carbonization of lignocellulosic materials impregnated with chemical dehydrating agents (Gómez et al. 2010). Its main characteristics are determined by its physical and chemical properties, such as: porous structure and its adsorption potential. Activated carbons are specially prepared to have a high internal surface and high porosity. Due to its textural properties, surface chemistry and mechanical resistance is used as a catalyst (Santos et al. 2006) and support for catalysts that allows to perform chemical processes under optimum conditions of environmental operation (Gómez et al. 2010).

The activated carbon as the carrier disperses the active phase (noble metals or transition metals) along its surface, generating a high active surface per gram of catalyst. This support must allow the diffusion of the reagents to the active phase, resistance to surface poisoning, good catalytic activity and migration of products; taking into account the type of process, gives the textural properties to the carbon (Gómez et al. 2010).

The activated carbon used as catalyst, has been used in processes of degradation of phenols (Omri and Benzina 2014), derivatives in aqueous effluents (Santos et al. 2006; Matsumura et al. 2002), hydrogenation (Merabti et al. 2010) and transesterification processes (Malins et al. 2015; Baroutian et al. 2010).

3 Methodology

The method used for the preparation of biodiesel from soybean and palm oils using activated carbon made from coconut shell and in the presence of lipases is presented in Fig. 8.

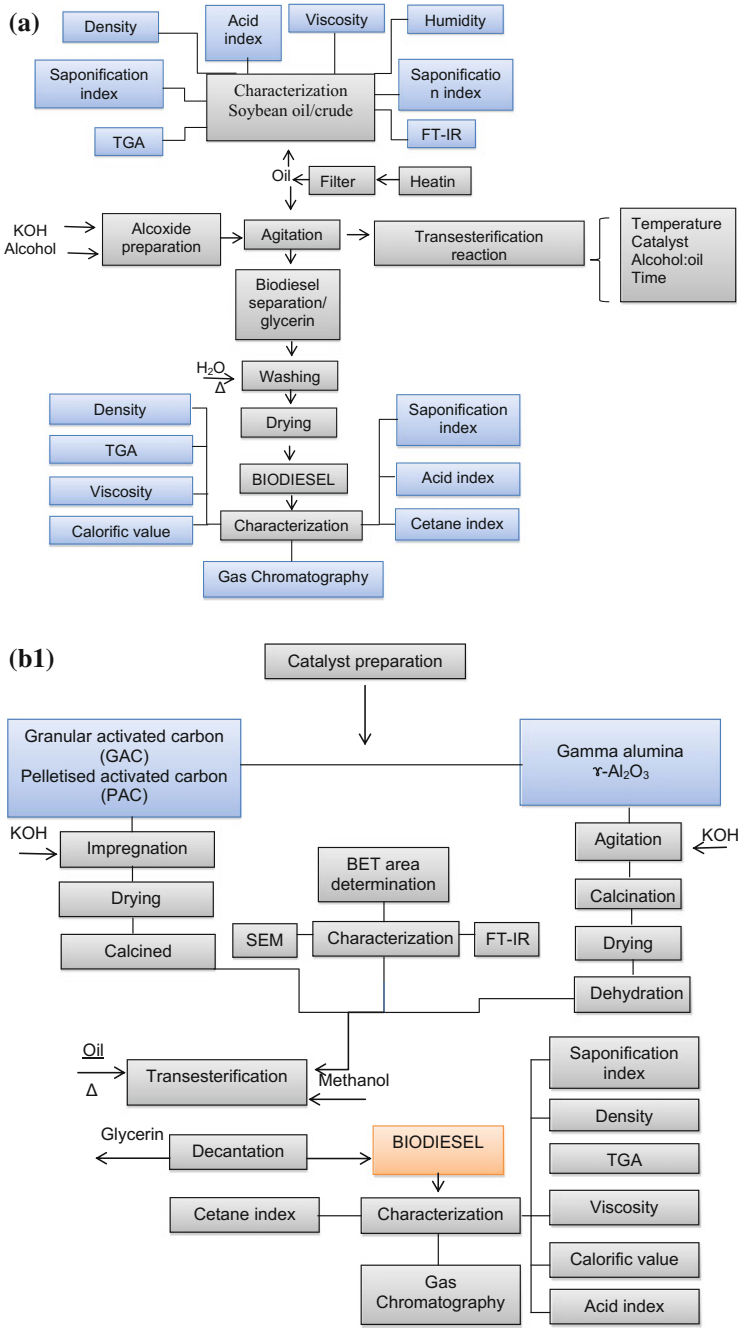


Fig. 8 Flow chart of procedures: **a** Homogeneous catalyst; **b1** and **b2** Heterogeneous catalysts

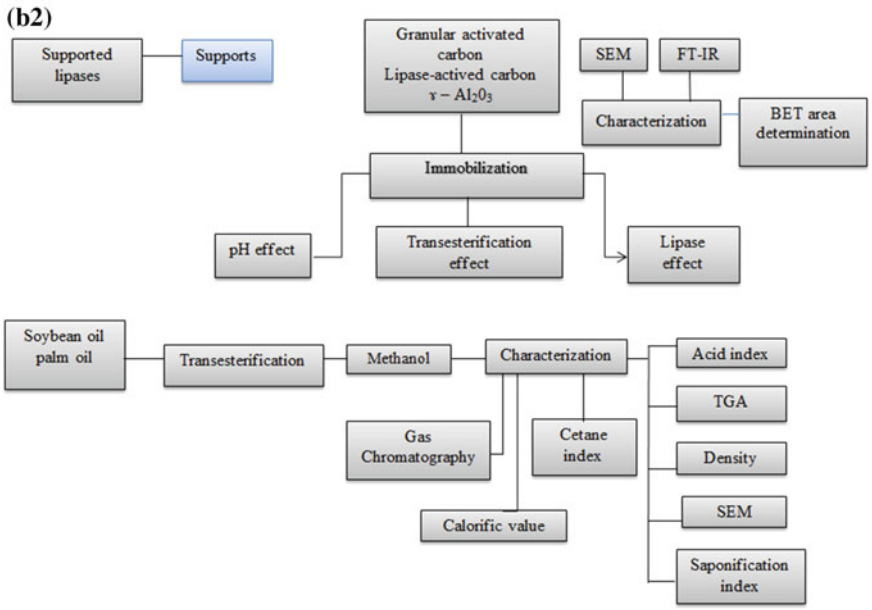


Fig. 8 (continued)

3.1 Homogeneous Catalysis

3.1.1 Characterization of Oil

Soybean and crude oil were characterized, measuring: density, humidity, acid index, viscosity, and saponification index. A thermogravimetric analysis and FT-IR were performed.

3.1.2 Preparation of Alkoxide

For the preparation of the alkoxide, the specific amount was weighed in each catalyst experiment (KOH) and the amount of alcohol was added, and then mixed in a reflux system, until complete dissolution, forming the alkoxide.

3.1.3 Preparation of Oil

The amount of oil for each test is measured, is heated to 110 °C for 30 min, and filtered. Subsequently, it is allowed to reach the desired study temperature, it is allowed to reach the desired study temperature.

3.1.4 Transesterification

To the alkoxide formed, the oil is added and left at reflux for 90 min by controlling the temperature and stirring at 100 rpm.

Effect of Temperature

The reaction was developed using a molar ratio of methanol to triglyceride of 6–1, of catalyst 0.7% (potassium hydroxide), the effect of the temperature in the range of 30–100 °C was studied.

Catalyst Effect

A molar ratio of alcohol:oil 6:1 at 60 °C was used, with stirring 100 rpm, for 90 min and the catalyst concentration varying 0.2–3.0%.

Molar Ratio of Alcohol:Oil

The molar ratio of alcohol:oil of 3–10, at 60 °C, was varied with 100 rpm of stirring, 0.7% of catalyst (KOH).

3.1.5 Separation of Biodiesel and Glycerine

After the reaction time, the resulting solution was transferred to a separatory funnel and allowed to stand for 24 h. Then, the two phases visualized in the lower part (glycerin) and in the upper (biodiesel) were separated.

3.1.6 Washing

Once the biodiesel is separated from the glycerin it must be washing because it may have contents of catalyst, alcohol, soaps, and glycerides without reacting, washes are performed until the residual water is clear and a pH near 7.0

3.1.7 Drying

The biodiesel previously washing is dried by adding 25% by weight of anhydrous sodium sulfate for 4 h, then filtered and was placed in the oven at 110 °C for three hours to completely ensure the presence of water.

3.2 Heterogeneous Catalysis

Three supports were used for the heterogeneous catalysis: granular activated carbon (GAC), pelletized activated carbon (PAC) and gamma alumina (ɤ-Al2O3), which were impregnated with potassium hydroxide (KOH); as alcohol methanol and the source of triglycerides: soybean oil and crude palm oil.

Preparation of the catalyst

3.2.1 KOH/Granular and Pelletized Carbon

All catalysts were prepared by impregnation with 15 mL of different concentrations of potassium hydroxide in aqueous solution (1–5% w/w), is left in constant agitation for 24 h, then the catalyst is dried at 110 °C for 24 h and calcined at 210 °C for 3 h. Finally the catalysts are characterized by FT-IR, BET and SEM. Its basic strength (H_) and basicity were determined by means of a Hammett titration.

3.2.2 KOH/ɤ-Al_2O_3

The alumina was impregnated with a solution of potassium hydroxide (1–5% w/w) for 24 h, with constant stirring. Then was calcined KOH/ɤ-Al_2O_3, a heating rate of 3 °C/min, were dried at 110 °C for 1 h, then dehydrated at a temperature of 250 °C for a period of 1 h, thereafter at a rate of 3 °C/min to 500 °C and left for 1 h. Finally, it was characterized by FT-IR, BET, SEM and its basic strength (H_).

3.3 Biocatalysis

3.3.1 Optimizing the Immobilization of Lipase on Granular Carbon, Alumina and Pelletized

In order to immobilize the lipase from porcine on different supports, immobilization conditions were optimized. To determine the effect of pH on immobilization of porcine lipase was used as 20 mM sodium phosphate buffer at pH 6.0; 7.0; 8.0, and 9.0. For the effect of temperature 125 mg support (granular carbon, pelletized, and aluminum oxide) were suspended in the lipase solution (100 mg porcine lipase dissolved in 0.500 mL of buffer 20 mM sodium phosphate pH 8.0) at different temperatures (4, 25, 37, 60, and 70 °C) for 6 h, then centrifuged at 10,000 rpm, the residue volume was 95% compared to the original solution of the lipase. The effect of incubation time on the immobilization of lipase was also studied, for that small aliquots were withdrawn during a time interval, the solutions were centrifuged and the residue (lipase) is stored at 5 °C.

3.3.2 Preparation of the Enzyme Tuned

100 mg of porcine pancreatic lipase was weighed and dissolved in 0.5 mL of 20 mM sodium phosphate buffer pH 8.00. Subsequently it is frozen at −20 °C and lyophilized for 48 h.

3.3.3 Preparation of Immobilized Enzyme

(a) 100 mg of porcine lipase was dissolved in 0.500 mL buffer 20 mM sodium phosphate pH 8.00 and mixed with 125 mg support (granular carbon, pelletized carbon, and aluminum oxide). After 24 h with occasional stirring, the support is removed; the residual liquid was stored for evaluating the amount of protein. Washes were performed with sodium phosphate pH 8.00 for one to two minutes each wash session. Then, the biocatalyst (lipase activated carbon) stored in refrigeration at 5 °C. Wash solutions were stored to determine your protein content.
(b) Enzyme activators carbon system was characterized by scanning electron microscopy (SEM).
(c) The amount of immobilized enzyme on the support was evaluated by performing the measurement of the concentration in the initial enzyme solution (initial mg protein) and concentration in the residue and wash solutions (final protein mg) using the method of Bradford (Naranjo et al. 2010).

3.3.4 Enzyme Activity

The lipase activity was determined using as substrate p-nitrophenyl palmitate (pNPP), a reaction mixture was realized, which contained 75 μL of pNPP (20 mM), 5 μL of the enzyme and 20 mM sodium phosphate buffer pH 8.00 to a volume of 3 mL, this was done for all the three supports. Subsequently, it incubated at 37 °C for 15 min and the reaction was terminated by adding 1 mL of 0.2 M sodium carbonate. The p-nitrophenol liberated was assessed by 410 nm spectrophotometry. One unit of enzyme activity is defined as the amount of enzyme capable of releasing 1 μmol of *p*-nitrophenol per minute (Soham et al. 2011).

3.3.5 Transesterification Catalyzed by Porcine Lipase

Soybean and palm oil (0.500 g) with methanol at molar ratio 1:6 (mol mol^{-1}) into vial. To this mixture is added 100 mg of enzyme preparation (tuned and immobilized) and incubated at 60 °C with an agitation of 300 rpm. The course of the reaction is performed by taking 200 μL aliquots and analyzing by gas chromatography (Naranjo et al. 2010).

3.4 Quantification of Biodiesel

Fuel characteristics were verified by EN and ASTM, based on this, determined properties such as: acidity, pH, density, calorific value, humidity, and percentage of esters.

Methyl esters (biodiesel) were analyzed using a gas chromatograph (GC) coupled to mass brand Shimadzu QP2010S Series with DB-225 MS column Agilent serial NºUS5268413H dimensions (20 m × 0.1 mm × 0.1 μm). The conditions set for the analysis were: injector temperature 220 °C, using helium as carrier gas with a flow of 1.0 mL/min. The sample injection 1.0 μL, automatic injection AOC 20s (Split mode, using a ratio 30:1. The temperature program in the oven was followed: initial temperature 60.0 °C for 1 min, then heating of 10 °C/min to 195 °C, after reaching this temperature heating of 3.0 °C/min to 205 °C and finally 8.0 °C/min to 220 °C, maintaining this temperature 30 min before giving for finishing the analysis.

4 Results and Analysis

The rate of chemical reactions is affected by many variables, so it is necessary for this kind of study prior to the synthesis of biodiesel. In this chapter, the authors present the results obtained in their laboratory to analyze the effect of five factors on the reaction yield following alkyl preparation for homogeneous, heterogeneous, and enzymatic transesterification from crude palm oil and soybeans.

4.1 Homogeneous and Heterogeneous Catalysis

4.1.1 Homogeneous Catalysis

In transesterification using basic solid catalysts, the first reaction step is the elimination of protons from the basic sites of the catalyst to form alkoxide; then an ester alkoxide attacks a carbonyl group of the triacylglycerides, forming a tetrahedral intermediate, which is subsequently divided into fatty acid alkyl esters and the corresponding anion of the diacylglyceride. Then, the monoacylglycerides and diacylglycerides are converted by the same mechanism as triacylglycerides, into a mixture of alkyl esters and glycerol (Jiang et al. 2010).

Before performing any type of reaction, the oil must be heated and filtered, in order to remove moisture and suspended solids. Then, the physical and chemical properties of oils are determined (see Table 3).

The data in Table 3 show that there is less than 1% acid in the case of vegetable oil (soybean), so pretreatments are not necessary, while palm oil has a high acid

Table 3 Physical and chemical properties of vegetable oil and palm oil used in the transesterification

Property	Vegetable oil (Soybean) (value)	Palm oil (value)
Density 15 °C (kg cm^{-3})	910	890
Viscosity 40 °C (cp)	38.0	37.5
Humidity (%)	0.08	0.04
Acid index (%)	1.00	3.10
Saponification index	185	190

content, i.e., has a higher percentage of free fatty acids, which is a problem when performing homogeneous alkaline catalysis, since the catalyst (KOH) neutralizes free fatty acids, promoting the saponification reaction, which in turn can also promote the generation of stable emulsions, preventing the separation of FAMES and glycerine (Jiang et al. 2010).

Tests with different concentrations of catalyst from 0.2 to 3.0% were performed, in which soap formation and the formation of FAMES was not evidenced. To avoid this, esterification was performed in two steps: in the first step, FFA methyl esters were formed with an acid (sulphuric acid) catalyst, and the percentage of acidity was redetermined, obtaining a value of 0.5%; in the second stage, an alkaline transesterification process was carried out. Once it was determined that the saponification reaction was not favored, the different parameters affecting catalysis were studied. Although the acid value obtained (3.10%) allows homogeneous acid catalysis, transesterification was not performed in this way because the acid catalysis has been reported to have a low reaction rate and high alcohol:oil molar ratios (Titipong and Ajay 2014), which prevent costs from being reduced in the production of biodiesel.

Effect of Time

This type of test was used to find the minimum time for the total conversion of fatty acids to methyl esters.

Figure 9 shows the evolution of conversion along with transesterification, expressed as a percent of triacylglycerides (TG) to fatty acid methyl esters. The minimum time to ensure 100% conversion was 90 min, so tests were conducted allowing this reaction time.

Effect of Catalysts

Of the possible catalysts used, due to cost and by the catalytic activity was more efficient than acidic catalysts, NaOH and KOH were assessed; was decided to use KOH due to the glycerine generated being less toxic than when prepared with sodium, and this product is used in the preparation of fertilizers.

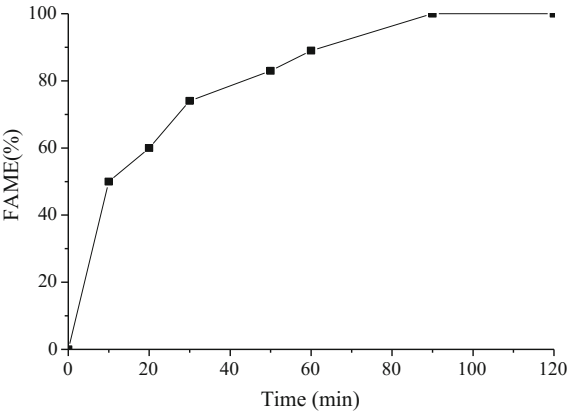


Fig. 9 Conversion of methyl esters from fatty acids as a function of time, with a molar alcohol:oil ratio of 6:1, with 0.7% KOH to vegetable oil (soybean)

Table 3 summarizes the results showing that the higher percent yield were obtained when 0.7% catalyst was used for both palm oil and soybean oil. For values lower than 0.7%, a worse performance was noted because there is a decrease in the reaction rate in the intermediate stages. For larger values, a significant decrease in reaction yields was evidenced as a result of the secondary saponification reaction for triacylglycerides oil, producing soaps which are dissolved in the glycerol phase because this is the more polar phase. These reaction yields were quantified by measuring the mass of methyl esters resulting from the transesterification process (Table 4).

Effect of Molar Ratio of Alcohol:Oil

The results of these studies are shown in Fig. 10; it can be observed that even a molar ratio methanol:oil of 6, presented an increase in performance for both oils, displacing of the reaction towards product formation. Furthermore, at higher values of 6 the yield of methyl esters decrease especially for the oil palm, because the high alcohol content affects the separation of glycerine due to increased solubility; here

Table 4 Percentages of catalyst used in the basic homogeneous transesterification at 60 °C for 90 min, molar ratio methanol:oil 6:1

Molar relation oil:alcohol	Vegetable oil (Soybean)		Palm oil	
	% Catalyst KOH	% Yield	% Catalyst KOH	% Yield
6:1	0.2	80	0.4	82
	0.4	85	0.7	94
	0.7	96	1.1	91
	1.1	92	1.6	82
	2.1	90	2.1	76
	3.0	59	3.0	49

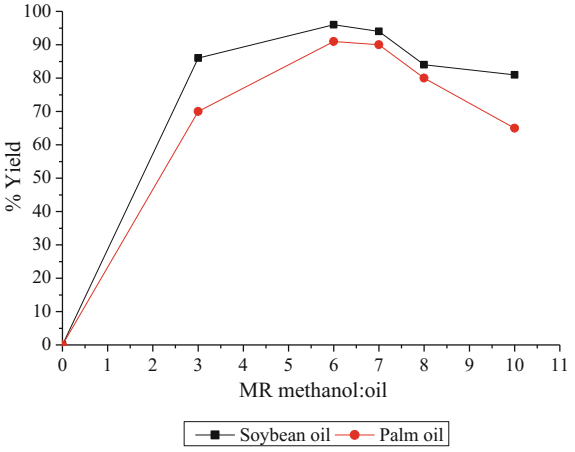


Fig. 10 Effect of methanol molar ratio (MR):oil in a homogeneous basic transesterification process, at 60 °C for 90 min, with 0.7% KOH and 100 rpm agitation

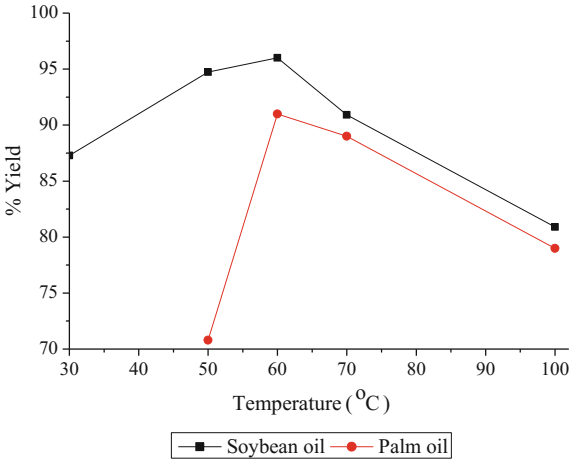


Fig. 11 Effect of temperature on the basic homogeneous transesterification process, for 90 min, with a molar methanol:oil ratio of 6:1, with 0.7% KOH and 100 rpm agitation

the glycerine solution has a shift in equilibrium towards the formation of reactive species (left), finally creating a mixture of triacylglycerides, glycerine and FAMES.

Effect of Temperature

This study shows that palm oil at a temperature of 30 °C did not react, since oil at temperatures below 40 °C is still solid, while a significant increase in performance occurs from 50 to 60 °C, which is higher compared to vegetable oil (soybean). It was found that the highest yield for both vegetable oil (Soybean Oil) and palm oil was seen at 60 °C, the boiling point of methanol. At higher values, performance decreases in a linear manner (see Fig. 11).

It is interesting to note that although the process of homogeneous alkaline transesterification is relatively fast and has a high conversion rate, the catalyst (KOH) cannot be reused because it requires neutralization and purification steps; also, it generates important polluting effluents at the end of the reaction, a process that ultimately has technical and ecological disadvantages, generating overruns in the separation and purification of the end-product (Knothe et al. 2005).

Figure 12a, b show the results obtained using the technique of differential thermogravimetric analysis (TGA-DTA) for the soybean and palm oil samples used

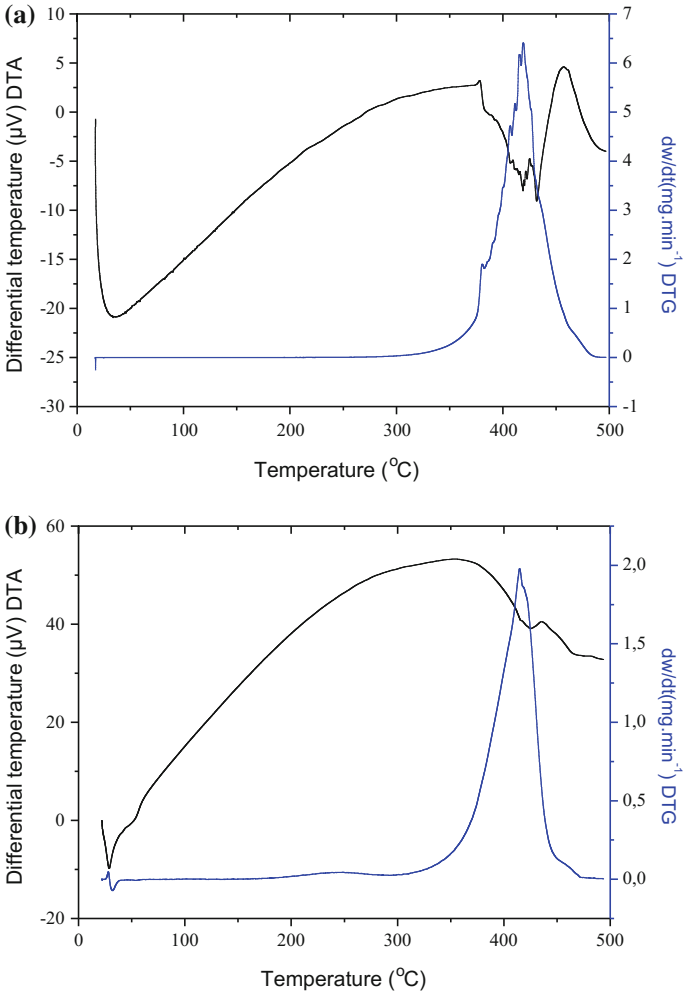


Fig. 12 **a** Thermogravimetric analysis of palm oil, up to 500 °C with a nitrogen flow of 100 mL min^{-1} and a heating rate of 10 °C min^{-1} and **b** Thermogravimetric analysis of soybean oil to 500 °C with a nitrogen flow of 100 mL min^{-1} and a heating rate of 10 °C min^{-1}

in a typical transesterification reaction in our laboratory. The mass starts to decrease after 290 °C for palm oil and 180 °C for soybean, continuing down until all oil present in the sample was completely decomposed at temperatures of 420 and 410 °C for palm oil and soybean oil, respectively. The two oils present endothermic processes, which is more noticeable for palm oil than soybean oil.

Differential thermal analysis (DTA) shows maximum peak decomposition in the temperature range between 210 and 500 °C for palm oil, which is attributed to the decomposition of palmitic acid. For soybean oil, this occurs in a range of 220–460 °C, which is attributed to the decomposition of stearic acid. Greater decomposition occurs in soybean oil compared to palm oil, because palm oil is mainly saturated fatty acids (C16) while soybean has a higher level of unsaturated fatty acids (C18), therefore requiring less energy to break the carbon–carbon single bonds (Muhammad and Tat 2013).

Figure 13a shows the infrared spectrum corresponding to soybean oil. This spectrum shows representative bands of low intensity at 3470 cm^{-1}, corresponding to O–H stretching due to oil moisture; medium intensity 3007 cm^{-1}, indicating HC=CH vibration; intense bands at 2924 and 2853 cm^{-1}, associated with C–H aliphatic stretch; an intense band at 1746.51 cm^{-1}, associated with C=O ester stretching; a medium intensity band at 1464 cm^{-1}, denoting bending in the H–C–H plane; a high intensity band at 1163 cm^{-1}, associated with C–O stretching and a low intensity band at 722 cm^{-1}, linked to C=C–R in alkenes.

On the other hand, Fig. 13b shows the corresponding infrared spectrum for palm oil which displayed the following characteristic bands: medium intensity bands at 2917 and 2850 cm^{-1}, associated with aliphatic C–H stretching; a medium intensity band at 1737 cm^{-1}, associated with C=O unsaturated aliphatic ester stretching; a medium intensity band at 1470 cm^{-1}, corresponding to flexion in the H–C–H plane;

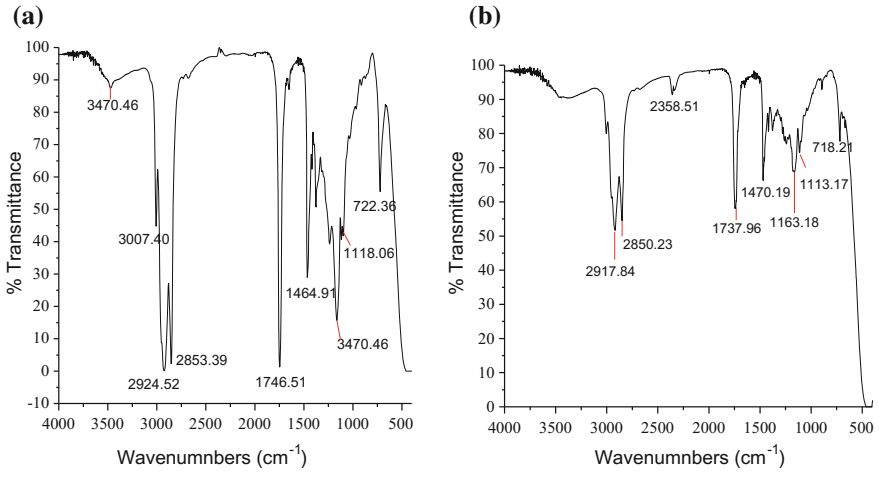


Fig. 13 The corresponding oils used in our investigation. **a** Soybean oil and **b** oil palm

a low intensity band at 1163 cm^{-1}, corresponding to C–O stretching; and a low intensity band at 718 cm^{-1}, corresponding to C–C–R in alkanes.

4.1.2 Heterogeneous Catalysis

Catalysts Characterisation

Table 5 shows the textural properties of the catalysts prepared in our laboratory. The supported catalyst pelletised activated carbon (PAC) has the largest surface area and pore volume, followed by granular activated carbon (GAC) and finally ɤ-lumina, where there is a decline in both the area and total pore volume due to the addition of potassium hydroxide in all cases. This reduction is mainly caused by the preparation process of each solid, which alter the pore volume, apparent surface area and pore distribution according to the starting material and both the chemical and heat treatments used. Decreasing the area is additionally associated with locked pores and the interaction of metal cations with the catalyst support (Ebiura 2005). With a major area, the probability of having active sites is increased and the catalytic reaction type can be adjusted.

Figure 14 shows SEM micrographs taken at our laboratory of heterogeneous catalysts; it can be seen that there is higher porosity in coals in ɤ-Al_2O_3. Activated carbon (called GAC and PAC) has a non-uniform porosity. In the catalysts prepared (called KOH/GAC and KOH/PAC), there is reduced porosity with respect to GAC and PAC, due to thermal and chemical actions to which they are subjected during preparation. Alumina (ɤ-Al_2O_3) has a non-uniform appearance and granular KOH/ɤ-Al_2O_3 has a particle size less than when ɤ-Al_2O_3 is used as precursor.

Table 6 shows the results for the elemental analysis by SEM; some catalysts in which the ɤ-Al_2O_3 decreases the percentage of aluminum and carbon content develop calcination products. Regarding GAC and PAC, the carbon percentage decreases due to a merger of salt, thus increasing the surface coverage of the support. Detections match the nature of the sample, but a complete comparison cannot be performed regarding the composition of all catalysts used, as this analysis was performed on a given area of the sample, which cannot allow the composition of the entire sample to be generalized.

Table 5 Textural parameters of the catalysts prepared from the calculated isotherms of N_2 at −196 °C

Catalyst	A_{BET} ($m^2\ g^{-1}$)	V_p ($cm^3\ g^{-1}$)	D_p (nm)
ɤ-Al_2O_3	144	0.49	1.2
KOH/ɤ-Al_2O_3	121	0.42	–
Granular activated carbon (GAC)	842	0.34	1.7
KOH/GAC	830	0.31	
Pelletised activated carbon (PAC)	1131	0.59	1.9
KOH/PAC	1120	0.45	–

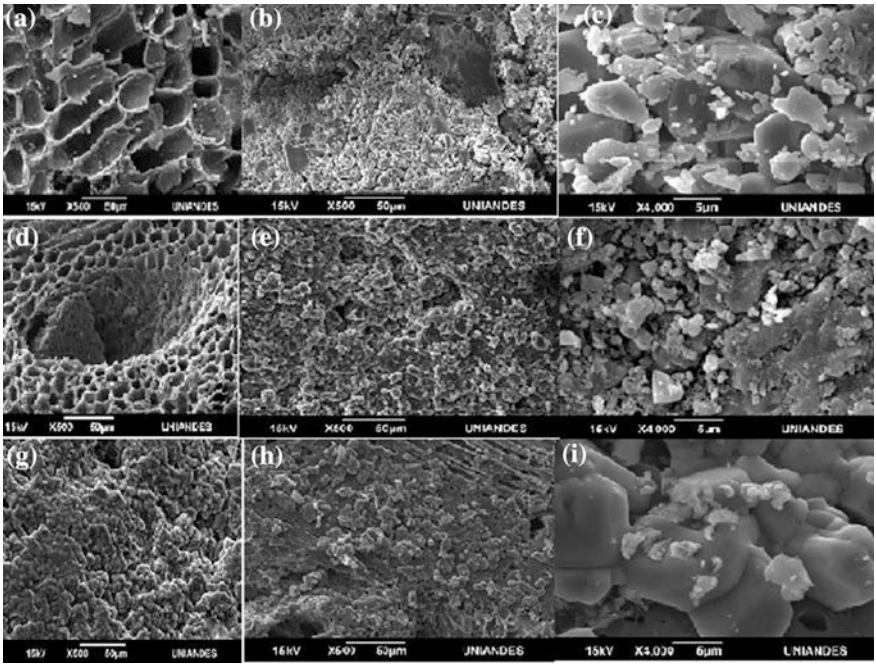


Fig. 14 SEM catalysts prepared, **a** GAC, **b** and **c** KOH/GAC, **d** PAC, **e** and **f** KOH/PAC, **g** ɤ-Al_2O_3, **h** and **i** KOH/ɤ-Al_2O_3

Table 6 Chemical analysis by dispersive energy of some heterogeneously prepared catalysts

Sample	% Weight			
	C	O	Al	K
GAC	100	–	–	–
PAC	100	–	–	–
ɤ-Al_2O_3	–	58.10	41.99	–
KOH/GAC	71.33	27.66	–	1.01
KOH/PAC	71.30	28.13	–	0.57
KOH/ɤ-Al_2O_3	15.20	56.21	27.55	1.04

The basic strength and the basicity of the prepared catalysts were determined using the Hammett titration method. In general, all sites have a basic strength between $7.2 < H^+ < 9.3$. In heterogeneous catalysis, for the reaction between the liquid reactant (methanol) and the solid catalyst (KOH/ɤ-Al_2O_3, KOH/GAC, KOH/PAC), thermodynamically viability is essential in certain stages, such as (Ebiura 2005):

(1) Diffusion of reactive molecules to the solid surface
(2) Chemisorption of some reactive species on the surface

(3) Chemical reaction on the surface
(4) Desorption of surface products
(5) Diffusion of products to dilute phase

The slowest step determines the reaction rate. Steps (1)–(5) depend on the pressure, temperature, and gas viscosity and are usually fast, while steps (2), (3), and (4) are slow and thus determine the reaction rate.

Step (2) is usually rapid, since it is a process with zero energy or close to zero activation. In contrast, (4) needs some activation energy $E_{a,des} = E_{a,adsP} - \Delta H_{adsP} = E_{a,adsP} + \Delta H_{desP}$ (where ΔH_{desP} is enthalpy of desorption of the product P, a normally positive, and ΔH_{adsP} is enthalpy of adsorption of the product P); this step is not determinant of the process but may present inhibition if the catalyst is blocked by non-desorption. Finally, step (3) is the determining step, which results in the conversion of reactants to products (Ebiura 2005).

As shown in Fig. 15, the greatest percent yield in the production of biodiesel was reached at 90 min for the two catalytic systems, both homogeneous and heterogeneous. The homogeneous system has higher yields using soybean oil (SO) and crude palm oil (OP) compared to the heterogeneous system. In the heterogeneous system prepared using different catalysts (KOH/ɤ-Al_2O_3, KOH/GAC and KOH/PAC), higher yields were presented for KOH/PAC and lower yields for KOH/ɤ-Al_2O_3; these results are associated with the surface area catalysts, where PAC is the best support for catalyst preparation and is suitable for transesterification, since it has more active sites available. The ɤ-Al_2O_3 has less A_{BET}, thus generating low yields in the production of biodiesel. After 90 min, the conversion does not increase, but favors the backward reaction (hydrolysis of esters) reducing of product yield (Leung and Guo 2006).

It is also established that, under these experimental conditions, crude palm oil has lower yields compared to soybean, because of the amount of free fatty acids which are reacting with the catalyst, generating side reactions which influence the

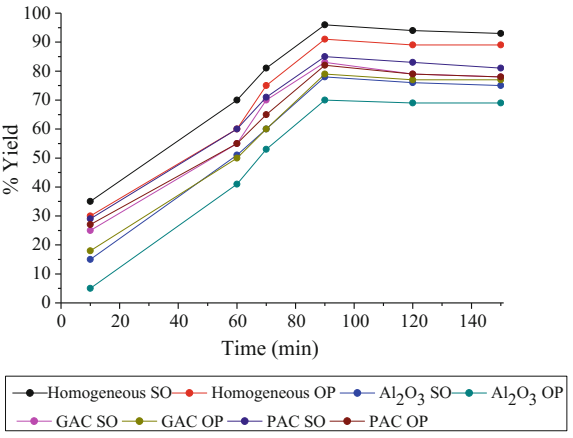


Fig. 15 Effect of reaction time on the yield of biodiesel produced in homogeneous and heterogeneous catalysis using as ɤ-Al_2O_3 support, pelletised activated carbon (PAC) and granular activated carbon (GAC) with KOH, at 60 °C and 100 rpm. The molar ratio of alcohol:oil was 6:1 and 0.7% catalyst was used for palm oil (OP) and soybeans oil (SO)

separation of the two phases (FAMES and glycerol) and thus the overall performance.

Each study for the synthesis of biodiesel is very particular, so it is necessary to investigate each parameter. In this study, the reaction conditions employed were found to be optimal in homogeneous catalysis, meaning that it was necessary to optimize the heterogeneous system to maximize the yield of biodiesel.

It is necessary to point out that in the case of the homogeneous catalysis compared to the heterogeneous, presents higher yields to the same reaction conditions, because the presence in solid basic catalysts in transesterification, the reaction mixture is three-phase catalyst-methanol-oil, where the methoxide which is thought to be the active species in the transesterification are formed upon adsorption of methanol on the catalyst surface, and the transesterification reaction becomes mass transfer-controlled (Dossin et al. 2006; Xie and Peng 2006).

Effect Molar Ratio of Alcohol:Oil

By stoichiometry (Fig. 1), it can be seen that the transesterification requires a molar ratio of methanol:oil of 3:1; as it is a reversible reaction, the effect of the molar ratio of methyl ester on performance is very important. The authors of this work conducted research into such parameters to assess the molar ratio of oil:methanol in the range from 3:1 to 12:1.

It should be noted that the transesterification by heterogeneous catalysis had limited mass transfer, resulting in a low yield of biodiesel, as shown in Fig. 16. The results show that for each catalyst (KOH/ɤ-Al_2O_3, KOH/GAC, KOH/PAC), when the molar ratio of methanol:oil was increased to 9:1, the yield of biodiesel increased, obtaining a yield between 86 and 84% for soybean oil (SO) and oil palm oil (OP) on ɤ-Al_2O_3; 91 and 89% for soybean oil and crude palm oil on GAC; and finally 92 and 91% for soybean oil (SO) and crude palm oil (OP) on PAC. Beyond the molar ratio of 9:1, the excess amount of methanol has a negative effect on

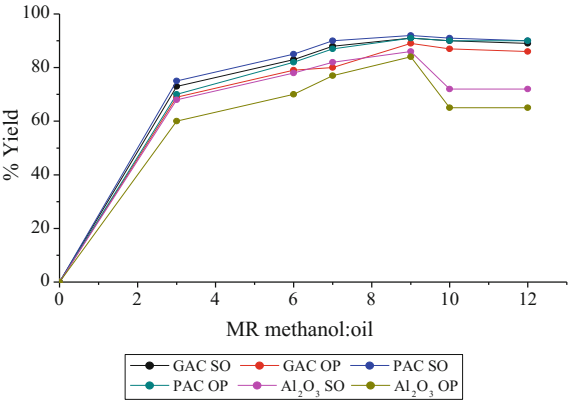


Fig. 16 Effect of molar ratio (MR) of methanol:oil in the basic heterogeneous transesterification process, at 60 °C for 90 min, with 0.7% KOH and 100 rpm agitation. Results for crude palm oil (OP) and soybean oil (SO) using different catalysts

performance, which makes the amount of biodiesel decrease; this is because the high dissolution of oil in alcohol and the high percentage of water content in the alcohol that reduce the percentage FAMES (Kim et al. 2004).

As the transesterification reaction is a balance where an excess of alcohol is required or by separation of any of the products from the reaction mixture, to handle the reaction to the right. However, a high proportion of alcohol:oil interferes in the separation of glycerine, due to an increase in solubility. When glycerin remains in solution, it helps to drive equilibrium back to the left, lowering the yield of esters (Meher et al. 2006).

The literature reports in other studies, with residual sunflower oil, methanol to oil ratio of 6:1 as an optimum proportion corresponding to a conversion of 89.8%, similarly using waste rapeseed oil presents higher yields at an optimum oil ratio alcohol:oil of 7:1 (Yuan et al. 2008). Another study with frying oil reveals that the optimal conditions are given in molar ratio of alcohol:oil between 5:1 and 8:1, after which there is already a constant behavior (Leung and Guo 2006).

Effect of Catalysts

To determine the influence of the amount of catalyst on the yield of biodiesel, it was varied in the range from 1 to 5% by weight. The results obtained are shown in Fig. 17.

It is evident that when there is an insufficient amount of catalyst, biodiesel yields are low; for example, when the amount of catalyst in the reaction was 1%, the following yields were obtained: 78 and 70% for crude soybean oil and palm oil on ɤ-Al_2O_3; 83 and 79% when soybean oil and crude palm oil, respectively, on GAC, and 85 and 82% for SO and OP, respectively, when PAC is used. By increasing the

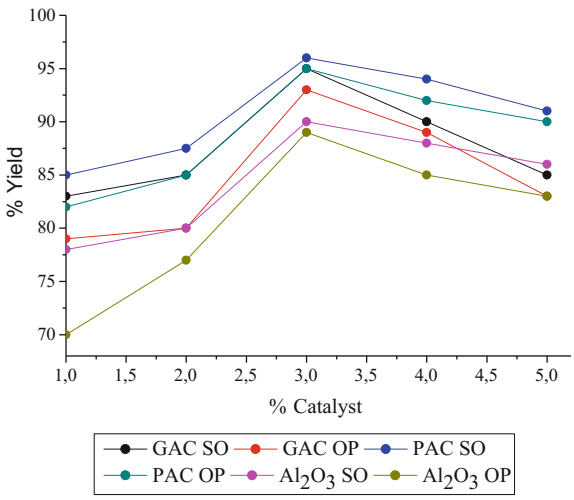


Fig. 17 Effect of catalyst on Transesterification, at 60 °C for 90 min, 100 rpm agitation and MR 9:1

catalyst amount up to 3% by weight, the biodiesel performance for all systems increased. Above 3%, the reagent mixture became too viscous, resulting in emulsification, promoting salt formation, and creating problems of mass transfer, thus decreasing biodiesel performance. By increasing the amount of catalyst in the transesterification, increases the amount of basic sites and the possibilities of contact with the reactants, increasing the yield of biodiesel. Comparing the % catalyst optimal with the literature, studies have shown that the maximum conversion (98%) using vegetable oil is 1% by weight of the alkaline catalyst concentration and then tends to constant values (Leung and Guo 2006).

Effect of Temperature

The rate of conversion to product in the transesterification reaction is greatly influenced by temperature. Despite this, the reaction can be carried out within a wide temperature range as long as the reaction time is sufficient to achieve good performance (Banerjee and Chakraborty 2009).

Transesterification is present at different temperatures depending on the type of oil and alcohol; the results are shown in Fig. 18. As the temperature increases, the yield and reaction time increase until maximum conversion is reached, due to the higher energy state of the molecules, generating greater collisions and higher solubility of the reactants, increasing the rate of reaction (Xie and Peng 2006).

When exceeding the boiling point of methanol (65 °C), performance decreases, as it is vaporized to produce bubbles that limit the reaction between the catalyst–methanol–oil–biodiesel interface. The catalytic process has lower relative yields of 89 and 90% for OP and SO respectively, generated using the catalyst KOH/ɤ-Al_2O_3, which is associated with fewer active sites than the highest yields

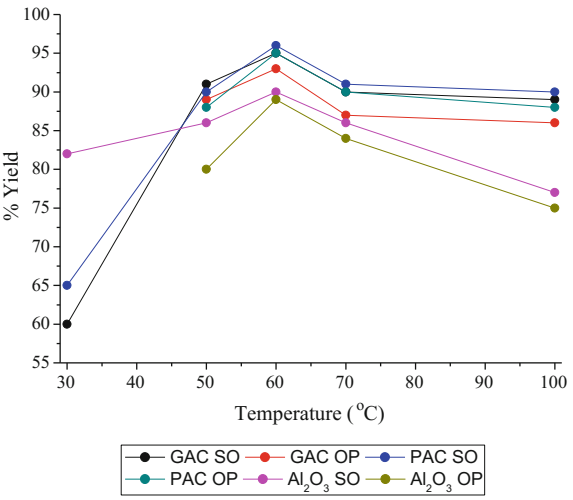


Fig. 18 Effect of temperature heterogeneous basic transesterification process, for 90 min, with a molar ratio methanol:oil of 9:1, with 3.0% KOH and 100 rpm agitation

for the catalyst KOH/PAC, generating yields of 95 and 96% using palm oil and soybean oil, respectively. The results are consistent if one considers that this catalyst (Table 5) has a greater surface area (Marchetti et al. 2007; Jagadale and Jugulkar 2012). Additionally, the result (65 °C) coincides with that reported in the literature, where the maximum yield of esters occurs in the temperature range 60–80 °C, at a molar ratio of alcohol:oil 6:1 with 1% catalyst in a time of 60 min (Cvengros and Cvengrosova 2004), but according to previous studies of optimum operating temperature, it can be concluded that the transesterification can be carried out at various ranges of temperature from room temperature to the boiling point of each alcohol used. For example, in transesterification studies of waste sunflower oil the reaction occurred efficiently at 55 °C, whereas waste rapeseed oil is used without changing the other conditions, the maximum yield occurs at 48.5 °C (Yuan et al. 2008).

No yields were recorded at 30 °C for cases where palm oil was used as it is still in the solid phase at temperatures below 40 °C.

When comparing the basic transesterification processes homogeneous catalysis and basic heterogeneous catalysis, and when using the same catalyst for both types of oil, homogeneous catalysis generates higher yields of biodiesel, but removal of the catalyst is technically difficult and is not necessary for heterogeneous catalysis. Here, the separation of catalysts (KOH/GAC, KOH/PAC, and KOH/ɤ-Al_2O_3) from the reaction products is easily achieved by using a filtering process. The catalyst has the advantage that it can be reused, although an additional cost is incurred by recovering their activity; additionally, when using heterogeneous catalysis in the transesterification, cleaner products (FAMES and glycerol) are obtained by simplifying the separation and purification steps, but heterogeneous catalysis for the synthesis of biodiesel requires more reagents and energy, increasing production costs. Additionally, presents diffusion processes as the reagents and the catalyst are in different state (Dossin et al. 2006).

4.2 Lipase

Lipase type II from porcine pancreas (Sigma-Aldrich; L3126) was used and the transesterification process was carried out with the free enzyme and the supported

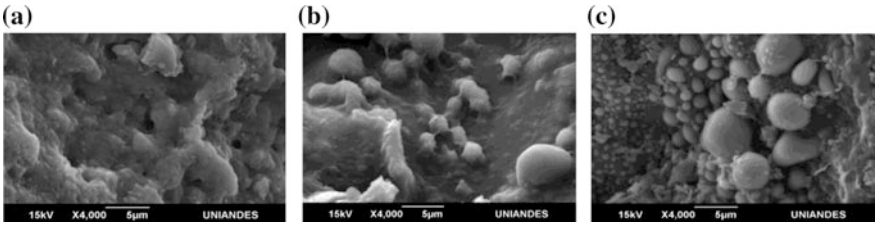


Fig. 19 Study of scanning electron microscopy. **a** ɤ-Al_2O_3-immobilized porcine lipase, **b** GAC-immobilized pig lipase and **c** PAC-immobilized porcine lipase

enzyme for this study: activated carbon, coal and pellet ɤ-Al_2O_3; as a solvent for the process, ethanol, methanol, isopropanol, and isobutanol were used, and palm oil and soybean oil were the raw materials.

In Fig. 19a–c, scanning electron photomicrographs are shown in which *Lipase type II from porcine pancreas* was adsorbed on the respective support (ɤ-Al_2O_3, GAC, and PAC), showing a greater amount of lipase supported on PAC. This is in good agreement with the results obtained in the textural characterisation of each type (Table 5), since PAC has a larger pore size, which allows the lipase to more easily reach the pores, and also enables greater interaction and thus a larger amount of supported lipase.

The optimal conditions for the free lipase were: 37 °C and pH 8.0. It is determined that the amount of protein absorbed (Pg) to GAC is 20 mg g^{-1}, to PAC is 18 mg g^{-1} and to ɤ-Al_2O_3 is 9 mg g^{-1}; i.e., GAC is supported more appropriately compared with PAC and ɤ-Al_2O_3, a result which was unexpected since PAC has a pore diameter that is greater than that of GAC and ɤ-Al_2O_3, which should facilitate its adsorption. This is due to the number of points on the surface hydrophobic interaction and the morphology of PAC (Diaz and Balkus 1996).

4.2.1 Effect of Time

Figure 20 shows that the yield of methyl esters increases as a function of time up to 360 min for all cases. At higher times, yields are constant; for that reason, 360 min was selected as the optimal time for transesterification. In transesterification with no catalyst and a set temperature, the time is important, since the process may be stopped at an intermediate step if the time is not optimal, preventing the complete conversion of triacylglycerides to methyl esters.

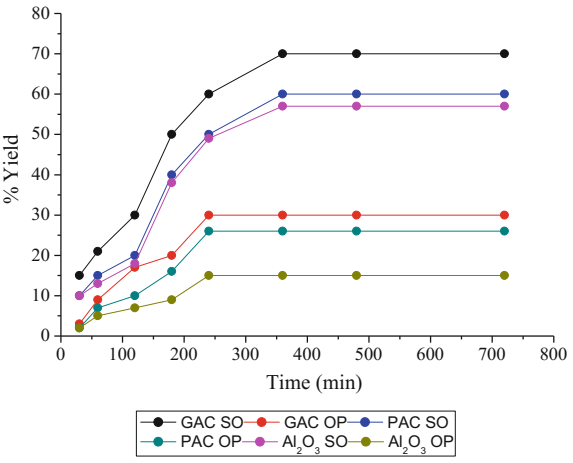


Fig. 20 Effect of time in the process of biocatalysis, with a molar ratio

4.2.2 Catalysts

Increasing the amount of lipase to 6% by weight of oil increases the amount of methyl esters; the yield for the corresponding systems is shown in Fig. 21. Further increases show no significant effect, because after the saturation point of the substrate is reached, the addition of more enzymes will not enable the substrate to serve as a reactant (Torres and Otero 2001). A relatively low enzyme concentration of 1–2% causes inhibition, and can result in low conversions (see Fig. 21).

4.2.3 Effect of Temperature

In this case, unlike homogeneous and heterogeneous catalysis, at temperatures below 60 °C, where the miscibility between oil and alcohol occurs, is quite low. As evidenced, an increase in the yield of methyl esters to a temperature of 37 °C occurs, which is then diminished appreciably because the optimum temperature of the enzyme is 37 °C; after this, the lipase begins to denature. It should be noted that at high temperatures, the system attempts to find a balance, as at low temperatures, again causing a decrease in the yield of methyl esters. Furthermore, the vibration and movement of the enzyme caused by high temperatures affects hydrogen bonds and other lipase bonds, altering the primary and tertiary structure. As shown in Fig. 22, in the case where palm oil (OP) is used, no results were obtained at temperatures below 40 °C as palm oil is still solid at this temperature.

It should be noted that although the optimal temperature is 37 °C, the temperature used in this study was 40 °C as the palm oil was liquid at this temperature and it was therefore possible to compare the two processes. When palm oil is used, lower yields were obtained than with soybean oil, which may be due to problems with mass transfer between the interfaces.

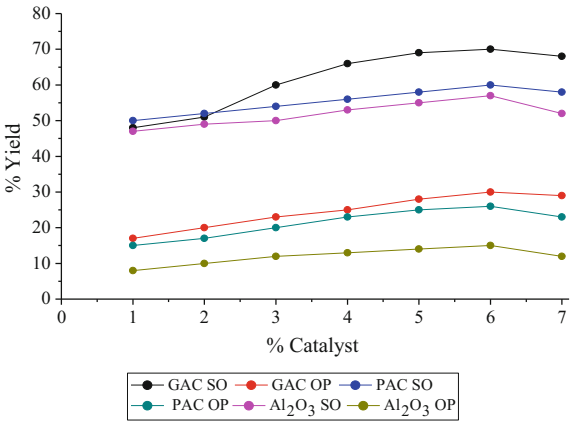


Fig. 21 Effect of the amount of enzyme in the process of biocatalysis, with a molar ratio of methanol:oil of 6:1, with 6.0% by weight of oil lipase, 300 rpm agitation at 40 °C and a 6 h reaction time

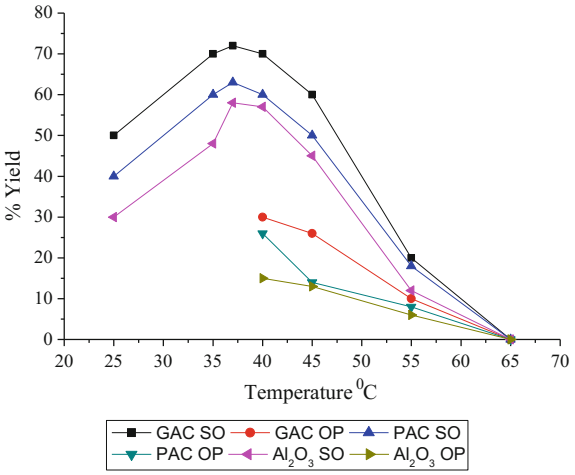


Fig. 22 Effect of temperature on the process of biocatalysis for 6 h, with a molar ratio of methanol:oil of 6:1, with 6.0% lipase by weight of oil and 300 rpm agitation

4.2.4 Oil:Alcohol Molar Ratio

The results of a study of this type are shown in Fig. 23. The highest yield was obtained at a molar ratio of 6:1, which then decreased due to the fact that excessive alcohol destabilizes the enzyme (Shimada et al. 2002), which sometimes hinders the interaction of the enzyme with the substrate (Kanasawud et al. 1992). Increased performance for GAC with soybean oil of 70% was obtained, while yields were lower when using palm oil due to the temperature of this study (40 °C).

Lipases as biocatalysts have high-potential transesterification, but have the disadvantage that the amount of lipase adsorbed on the support is less than 50% the initial amount that was dissolved, meaning that a greater amount of spent lipase does not yet occur, leading to high costs when compared with other catalysts. The

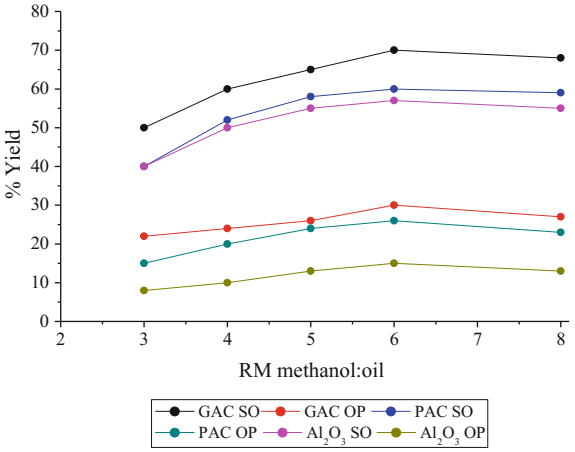


Fig. 23 Effect of methanol: oil molar ratio, the transesterification process for biocatalysis, at 40 °C, for 6 h, with 6.0% lipase and 300 rpm agitation

ability to select catalysts for the transesterification allows the products obtained to be pure and saves costs in purification and washing. Comparing the homogeneous and heterogeneous basic catalysis, using KOH provides a method wherein pre-treatment of the oil is not required, since the catalyst is selective (lipase), it consumes less energy and causes less waste, making it environmentally more attractive.

To remedy the problems of the adsorbed amount of lipase, further studies are needed, where the conditions of the supports will be varied, providing the surface with greater affinity to the lipase; as a result, it will be fully immobilized and recovered and reused, reducing the costs associated with biodiesel production.

5 Conclusions

Higher yields in the homogeneous transesterification were obtained for 0.7% KOH, at 60 °C, 100 rpm and 90 min. The heterogeneous catalysts KOH/ɤ-Al_2O_3, KOH/GAC, and KOH/PAC can be used in the transesterification of crude palm oil and soybean oil, presenting high yields (greater than 85%) under optimum conditions of 3.0 wt% catalyst, a molar ratio of alcohol:oil of 9: 1, the temperature of 60 °C, at 100 rpm for 90 min.

The heterogeneous catalytic process generates lower yields of 89 and 90% for palm oil (OP) and soybean oil (SO), respectively. Therefore, KOH/ɤ-alumina should be used as a catalyst, with KOH/PAC having a higher performance of 95 and 96% for palm oil and soybean oil, respectively. Biodiesel yields are directly related to the surface area of the catalyst, with PAC having a greater area and thus also the highest yield of biodiesel of 95% for palm oil and 96% for soybean oil.

Biocatalysis is a method that generates significant returns, and as a selective method is a huge draw for the production of biodiesel. Optimal conditions for biocatalysis are: molar ratio of methanol:oil of 6:1, 6.0% lipase by weight of oil, a temperature of 40 °C and 300 rpm.

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Utilization of Biodiesel in Compression Ignition Engines

K. A. Subramanian

Abstract As biodiesel has higher ignition quality than conventional petrodiesel, it could be one of the best alternative and renewable fuels for compression ignition engines. The high cetane number fuels would have benefit in terms of better engine's cold startability, less white smoke emission during engine's warm-up period, smooth engine running and better transient operation as compared to conventional diesel-fueled engines. In addition, the smoke/particulate matter (PM) along with CO and HC emissions in compression ignition engines decrease drastically due to biodiesel fuel having nearly negligible amount of sulfur and aromatic substances and its embedded inherent oxygen with the hydrocarbon molecule. However, the engine has a problem with biodiesel such as higher NO_x emission, power drop, and wall impingement. But, these problems can be addressed using suitable technologies.

Keywords Biodiesel fuel quality · Compression ignition engine
Performance · Emissions · CO and HC emissions · NO_x emission
Smoke/particulate matter · Wall impingement

Biodiesel is a renewable fuel which can be derived from a variety of feedstock including vegetable oil, algae, and animal fats. Biodiesel is degradable and high flash point. It has better fuel quality such as higher cetane number, lower sulfur content, and lower aromatics than that of petroleum diesel. Biodiesel can be blended with diesel at any ratio. The notation "B" denotes biodiesel. For example, B20 means 20% biodiesel by volume and rest petrodiesel. Biodiesel utilization in diesel engines would lead to reduction in the emissions including polycyclic aromatic hydrocarbon (PAH), nitrate polycyclic aromatic hydrocarbon (nPAH), sulfate emission (Subramanian et al. 2005). CO_2 emitting from biodiesel-fueled engine would be recycled by the crop plant resulting to no new addition into the

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atmosphere. The analysis of fuel quality of biodiesel with a comparison of the base diesel is given in the first section. Then, based on the IIT Delhi research work, the effect of biodiesel on performance (power and torque, brake thermal efficiency) and emissions characteristics (CO, HC, NO_x, and smoke) of diesel engines are described below.

1 Fuel Quality of Biodiesel

The effects of its properties on the performance of the engine are described below.

1.1 Cetane Number

It denotes ignition quality of the fuel for compression ignition engine. This is a dimensionless number derived from CFR Engine or Ignition Quality Tester as per ASTM standards. The cetane number as much as higher is preferable for smooth operation of compression ignition engine. The cetane number is indirectly proportional to activation energy of the fuel as cetane number increases, the activation energy decreases and vice versa (Heywood 1988). The typical cetane number of diesel fuel is generally in the range of 45–51. If it is lower than 45, the engine combustion may proceed with knocking due to relatively more fuel accumulates before start of combustion. In addition to this, the cold startability problem would also arise as the low cetane number fuel decrease the reaction rate of fuel with air resulting in slow initiation of ignition process. While starting the engine with low cetane number fuel, an engine may get misfiring resulting in more white smoke emissions. It means the bonds of high cetane number fuel at relatively less temperature by chemical reaction would be dissociated easily and then ignition and combustion proceed fast. The transient performance of the engine would affect with the low cetane number fuel. But, biodiesel has higher cetane number in the range of 45–56 (Graboski and McCormick 1998). So the biodiesel-fueled engine will run smoothly with better transient engine performance and emission reduction. The rate of pressure rise will decrease with high cetane number fuel leading to an increased life of the engine. So, biodiesel which is the high ignition quality (cetane number), is a better fuel for compression ignition engine compared to low cetane number diesel fuel.

1.2 Density

As biodiesel has relatively higher density than that of base diesel. As per ASTM standard, the specific gravity of diesel and biodiesel at 60 C should be 0.85 and

0.88 respectively. It would affect mass flow rate of fuel per cycle as fuel flow rate will increase per cycle if it is used in conventional engine. The higher density of the biodiesel would affect negatively as it will lead to larger break-up length, higher Sauter mean diameter, higher penetration distance, lower spray cone angle, and lower air entrainment process resulting in relatively lower mixing rate of air and fuel. However, the cetane number is higher with biodiesel that will more dominate on ignition than the shortcoming of biodiesel's high density for ignition.

1.3 Viscosity

The viscosity of the biodiesel is higher than that of diesel. As per ASTM standard, the viscosity of diesel and biodiesel should be from 1.3 to 4.1 mm^2/s and 1.9 to 6.0 mm^2/s respectively. The injection system needs slightly more work input for injection of the higher density fuel. On the other hand, it reduces the fuel leakage in the fuel pump. The Sauter mean diameter is relatively higher with biodiesel than that of base diesel resulting in relatively less mixing rate of air and fuel.

1.4 Distillation Characteristics

This is a measure of percent evaporation of fuel vapor with respect to the temperature. The minimum percentage of fuel has to be vaporized while starting of the engine, cruise, and high speed. The 10% fuel evaporation (T10) could give the information about easy cold starting of the engine and 50% fuel vapor (T50) could provide good warm up and cold weather driveability whereas 90% evaporated fuel vapor (T90) could give insight into possible deposits on combustion chamber of the engine. The temperature of diesel and biodiesel vapor are 210 and 352 C at T10, 260 and 351 C at T50, 315 and 355 C at T90 respectively (Graboski and McCormick 1998). Even though a biodiesel-fueled engine does not have startability problem due to its high cetane number, the distillation characteristics of biodiesel indicate the engine could have problem of the fuel dilution with lubricating oil at low load, its associated deposits on combustion chamber, etc.

1.5 Calorific Value

The calorific value indicates the specific energy content. The calorific value of biodiesel (for example, 40.7 MJ/kg for karanja biodiesel) (Lahane and Subramanian 2015) is lower than that of base diesel (42–46 MJ/kg). The lower calorific value of biodiesel would lead to more power and torque drop of the diesel engines.

The injection duration increased as relatively more amount of biodiesel fuel has to be injected in order to maintain same power output.

1.6 Sulfur Content in Biodiesel Fuel

Sulfur in the fuel is the main source of particulate matter formation in a diesel engine as the sulfur will oxidize to oxides of sulfur (SO_x) during combustion. It again deposits on the periphery of soot particles and then it is called as particulate matter. If any after-treatment device is used for emission reduction, the sulfur level should be negligible or very less otherwise, it may lead to sulfur poisoning of the catalyst resulting in ineffectiveness and poor conversion efficiency. In addition to this, the sulfur is to be oxidized by the catalyst leading to more SO_x emission. The sulfur oxidation to sulfuring acid, sulfate, etc. would lead to corroding of the engine components especially the fuel injection system would severely be affected. It may be noted that the sulfur in the commercial diesel as per BSIV is about 50 ppm.

1.7 Aromatic Content

Diesel fuel is blend of different hydrocarbon chain (C8–C18) and molecular structures (paraffins, cycloparaffins, olefins, aromatics, and alcohols). Typical aromatic compound in diesel is about 20–30%. Among the molecular structure, a fuel with more aromatic is a source for PM as well as polyaromatic hydrocarbon (PAH) emission from the engine. As biodiesel has negligible or less aromatic, the PM emission in diesel engines would relatively be lower than that of base diesel.

1.8 Bulk Modulus

The fuel property "bulk modulus" indicates the compressibility of the fuel that would effect on injection characteristics of diesel engines. The start of injection timing of the diesel engine advanced when the engine is fueled with biodiesel due to its higher bulk modulus than base diesel. For example, the bulk modulus of diesel and linseed biodiesel at 40 C is in the range of 13,000–15,000 bar and 16,000–21,000 bar respectively (Tat and Van Gerpen 2003).

1.9 Cold Flow Properties: Cold Filter Plugging Point (CFPP)

The CFPP is the lowest temperature at which fuel will still flow through a specific filter. When fuel is cooled, the temperature at which wax is first seen to crystallize is known as the cloud point and it is defined as the temperature where wax crystals first appear. Pour point is defined as the lowest temperature where fuel is observed to flow. CFPP, cloud point, and pour point of diesel and biodiesel are −42, −31, −46 to 5 and −9 to 13, −9 to 13, −3 to 14 C respectively (Kent Hoekmana et al. 2012). It could be observed from the data that all cold flow characteristics for biodiesel are above 0 C as compared to base diesel which is in negative temperature. In general, the CFPP, cloud point, and pour points are lower with biodiesel than that of base diesel and this would lead to a major technical issue of biodiesel storage and handling during cold period or cold climate regions.

1.10 Oxidation Stability

It is one of the important pysico-chemical properties and indicates the fuel's oxidation ability by itself while it is storing for long period. The oxidation stability of biodiesel is lower than conventional diesel fuel as the biodiesel which reacts itself due to contaminants (alcohols, acids, etc.), aerobic process, and thermal decomposition (excess heat exposure). Biodiesel has a lower resistance capacity to oxidation and can easily be affected by air-oxidation during long-term storage.

Closure: Cetane number of biodiesel which is higher than petrodiesel, indicates more suitability of its utilization in a compression ignition engine. Even though other physicochemical properties of biodiesel is similar as compared to base diesel, but slight variation in density, viscosity, and bulk modulus would largely affect the injection and spray characteristics resulting in necessary of the engine calibration. Biodiesel has poor cold-flow properties which need to be addressed. In general, Biodiesel which has high cetane number, less sulfur, and aromatic content of biodiesel, emerges as a new generation biofuel for compression ignition engines.

2 Introduction of Compression Ignition Engine

Compression ignition (CI) engines are used as prime movers for mass and passenger transportation, power generation, off-road transportation for agriculture, and building construction industries. The available CI engine power rating in the market is available in the varied range of 1 kW to several megawatts. The engine speed is generally varied from 1000 to 5500 rpm. The small rated power output CI engines are with air cooled engine whereas large rated power engines are with water cooled

engine. The large capacity engines are having turbocharger which gets power input from the waste exhaust gas. The engines with turbocharger would have higher specific power output compared to naturally aspirated engine.

The modern CI engine is generally designed with four strokes such as suction, compression, expansion, and exhaust. The fuel injection designed for modern diesel engine called common rail direct injection (CRDI) is up to 2200 bar.

The air is inducted through intake manifold during suction stroke. The working fluid "air" is compressed during compression stroke. The fuel is directly injected into the engine's combustion chamber using a high injection system at end of compression stroke. The fuel injection pumps supply the correct quantity of fuel with respect to load and the fuel is injected by injector. Due to high fuel injection pressure, the fuel gets fine atomized and mixing with the surrounding air which is at high swirl/squish velocity. The diesel engine has generally a bowl type piston and mixing of fuel which is to be taken in the bowl as its shape is designed in such a way to induce turbulence intensity and hence high mixing rate. The fuel is mixed with air and then ignition process starts and combustion proceeds further. The fuel accumulated during ignition delay period takes ignition and combustion called as premixed combustion phase. In this phase, the pressure and temperature of the combustion products rise up resulting in peak heat release rate. Then the remaining amount of fuel is injected in later phase called diffusion combustion phase where the combustion proceeds with the fresh injected fuel with the rest of air and combusted products. The chemical energy of the fuel is converted into heat energy through combustion process. The heat energy at high temperature and pressure is converted into the useful work during expansion stroke. The burned products are expelled to the atmosphere during exhaust stroke. Thus, a cycle is completed and the similar process continues again and again till stopping of the engine. Compression ignition engines are operated in a dual thermodynamic cycle which comprises of two isentropic processes and one constant volume and pressure heat addition, and heat rejection at constant volume. The CI engine operates relatively at higher compression ratio as compared to spark ignition engine resulting in higher thermal efficiency. Diesel engines provide higher torque due to higher volumetric efficiency of air due to no throttling operation. The higher thermal efficiency and torque of the engine attract the usage of the engine as a prime mover for mass and passenger applications. However, these engines suffer high level of particulate matter and oxides of nitrogen as the petrodiesel has high level of sulfur and aromatic. In diesel engines, CO and HC emissions are lower due to lean mixture compared to spark ignition engines. Diesel engines operate with heterogeneous mixture as an air–fuel ratio in combustion chamber is varying from region to region. The overall equivalence ratio is lean (more air than stoichiometric amount) but localized equivalence ratio is rich due to heterogeneous nature of air–fuel distribution. As a localized rich mixture, particulate matter emission will shoot up. On the other hand, the diesel engines are operating with high in-cylinder

temperature due to stoichiometric or rich mixture zones, oxides of nitrogen (NO_x) emission formation is high. So, diesel engines suffer dual emissions of high PM and NO_x emissions. Simultaneous reduction of NO_x and PM is still a research challenge.

As the emission norms are stringent from time to time, these engines are facing the challenges to reduce the emission level to the desired or acceptable level. In this aspects, the biodiesel offers solution to the diesel vehicles to bring down the PM, CO, and HC emissions to the desired levels. Some of the finding based on our research work carried out in IIT Delhi (Lahane and Subramanian 2014, 2015; Subramanian and Lahane 2013) are described below.

3 Performance and Emissions Characteristics of Biodiesel-Fueled Diesel Engine

3.1 Spray Characteristics

The main spray characteristics are break-up length, spray cone angle, Sauter mean diameter, penetration distance, and air entrainment. Break-up length for biodiesel/biodiesel–diesel blends is generally higher due to higher density of biodiesel than base diesel. Spray cone angle is lower with biodiesel due to higher viscosity and density of biodiesel. Penetration distance is higher with biodiesel due to higher fuel injection pressure. Air entrainment with biodiesel is influenced by either higher penetration distance or lower cone angle. A summary of spray characteristics of biodiesel-fueled compression ignition engine is given in Table 1.

Table 1 Effect of biodiesel on spray characteristics of a compression ignition engine

Spray characteristic	Main function	Remarks
Break-up length	Fuel density, air density, diameter of nozzle	Higher due to higher fuel density
Spray cone angle	Fuel density, air density, area of nozzle, length of nozzle, diameter of nozzle	Higher due to higher fuel density
Sauter mean diameter (SMD)	Viscosity of air and fuel, Fuel density, air density, diameter of nozzle, Reynold number, Webber number	Higher due to higher viscosity and density of fuel
Penetration distance	In-line fuel pressure, density of air, diameter of nozzle, injection time	Higher due to higher in-line fuel pressure
Air entrainment	Spray cone angle, density of air, spray penetration	Uncertain

3.2 Power Output

The power output of the engine may drop at higher loads in unmodified conventional diesel engine as the calorific value of the biodiesel (38 MJ/kg) is lower than base diesel (44 MJ/kg) and the injection duration of the fuel increases with biodiesel as more fuel relatively have to be injected in order to maintain the same power output. The change in mass flow rate of biodiesel is about 15%. It indicates 15% more amount of biodiesel fuel has to be injected for maintaining same power output. But, the engine's injection system may not be able to inject the required amount of fuel resulting in power drop. It is reported in the literature that there is a power drop at high loads. But, these issues may be resolved by optimizing the injection system. Torque of the engine would decrease relatively as brake mean effective pressure decreases as it is a function of thermal efficiency, volumetric efficiency, fuel–air ratio, density of air, and calorific value. If it is assumed that all parameters are same, the caloric value of the fuel is less resulting in lower BMEP and Torque.

3.3 Brake Thermal Efficiency

Brake thermal efficiency of the engine fueled with biodiesel is lower as the combustion pattern alters due to increase in injection and combustion duration. The start of dynamic injection timing gets advanced due to biodiesel having higher bulk modulus. The start of combustion also advanced due to the early injection and less ignition delay due to high cetane number. Some useful work may be lost due to the earlier occurrence of start of combustion which needs to be optimized for better thermal efficiency. Brake thermal efficiency decreased marginally with lower biodiesel–diesel blends (up to B25) whereas it decreased significantly with higher biodiesel–diesel blends. The brake specific energy consumption of 7.4 kW diesel engine at 1500 rpm engine speed with the rated load decreased from 17.5 MJ/kW-h with base diesel to 12.7 MJ/kW-h with karanja biodiesel (B100). It may be mainly due to biodiesel has higher density, viscosity, and surface tension results in poor atomization and mixture formation (less spray cone angle) with air. If the injection and spray characteristics are optimized, the thermal efficiency could be improved to alteast base diesel level.

3.4 CO and HC Emissions

These emissions decreased with all biodiesel–diesel blends due to the higher oxygen content. As the adiabatic flame temperature of biodiesel is higher than that

of base diesel, in-cylinder temperature is high. Carbon-monoxide emission forms due to mainly deficiency of oxygen/air and less in-cylinder temperature. The oxygen content in the biodiesel leads to better oxidation of CO into CO_2 emission. In case of biodiesel, CO and HC emissions generally decreased as the oxygen in the combustion chamber increased through air and fuel containing oxygen. HC emission is a function of over-leaning of air–fuel mixture, less in-cylinder temperature, crevice volume, flame quenching, etc. CO and HC emissions at the rated load decreased from 2.68 and 0.02 g/kW-h with base diesel to 0.6 and 0.005 g/kW-h with B100 respectively.

3.5 NO_x Emission

Atmospheric nitrogen and oxygen do not react with each other at normal atmospheric temperature or standard temperature and pressure (STP) condition. If the temperature is high in a combustion chamber of heat engines, the nitrogen molecule or nitrogen atom will react with oxygen molecule or oxygen atom to form nitrous oxide (NO). The formation mechanism of NO via nitrogen and oxygen is described by Zeldovich mechanism as shown in equations from 1 to 3 (Heywood 1988).

$$N_2 + O \rightarrow NO + N \quad (1)$$

$$N + O_2 \rightarrow NO + O \quad (2)$$

$$N + OH \rightarrow NO + H \quad (3)$$

Prompt NO_x is defined as the emission forms through HCN reaction with the flame. Nitrogen-containing biodiesel would oxidize into NO emission. NO_x can also form through lubricating oil which has nitrogen based additives. In general, NO emission is higher than that of NO_2 emission.

NO_x emission increased from 7.39 g/kW-h with base diesel to 8.07 g/kW-h with B100. This is one of the major problems for use of biodiesel in diesel engine. NO_x emission formation is due to biodiesel having oxygen molecule, advanced injection timing, and high in-cylinder temperature. NO_x emission for lower biodiesel–diesel blends (up to B15) increased marginally at all loads whereas it increased significantly for higher biodiesel–diesel blends (B20, B25, B50, and B100) at all loads. High NO_x emission can be reduced using suitable techniques including exhaust gas recirculation, retarding of injection timing, and optimization of fuel rate shaping and injection system.

3.6 Smoke/PM Emissions

Smoke forms during combustion wherever the rich pockets are present in a combustion chamber. Smoke is a visible indicator of carbonaceous particle which can be suspended in air column. Soot can be defined as carbonaceous particle impregnated with tar material. Particulate matter (PM) is either liquid or solid particle that comprises of organic (soot, smoke, hydrocarbon, etc.) as well as inorganic matter (sulfate, minerals from lubricating oil, ketones, etc.). Particulate matter forms mainly during diffusion combustion phase in which the combustion proceeds along with injecting fuel and partially and fully oxidized particles which are burned during premixed combustion phase. The main reason for PM emission formation is due to rich air–fuel mixture present in a combustion chamber. The degree of homogenous mixture is 100% when the localized air–fuel ratio is equal to global air–fuel ratio (or equivalence ratio), however, diesel engines operate with heterogeneous mixture as less time for mixture formation is relatively available compared to spark ignition engines. The diesel fuel has less volatility and diffusivity resulting in less degree of mixing formation. Soot forms in the core of the spray as air entrainment is relatively less as compared to periphery of the spray. The degree of mixing of injected diesel with the surrounding air can be enhanced by optimizing spray dynamics (high fuel injection pressure, optimization of numbers, and diameter of injector holes, etc.) and aerodynamics (enhancing swirl speed, squish velocity, piston geometry). Even though the advanced technologies are available to reduce particulate matter in diesel engines, it is difficult to reduce the desired levels at source level in order to meet the stringent emission norms. Particulate matter decreased significantly in a biodiesel-fueled diesel engine. The main reasons for the emission reduction are absence of sulfur and aromatic and oxygen inherent in biodiesel fuel. As the adiabatic flame temperature is higher with biodiesel than diesel, the in-cylinder temperature of the engine is higher with biodiesel. The high in-cylinder temperature increases better oxidation of soot particles resulting in less PM emission. Smoke emission decreased from 22% with base diesel to 15.4% with B100. The value of CO, HC, NO_x, and smoke emissions of the engine is given in Table 2.

Table 2 Quantitative data comparison of emission characteristics of a compression ignition engine (7.4 kW rated power output) for base diesel and biodiesel (B100)

Emission characteristic	Quantitative data of emission from diesel-fueled compression ignition engine	Emission from biodiesel-(B100) fueled compression ignition engine	Emission Percentage change (%)
CO (g/kW-h)	2.68	0.6	−77.6
HC (g/kW-h)	0.021	0.005	−76
NO_x (g/kW-h)	7.39	8.07	+9.2
Smoke (% opacity)	51.9	15.4	−70.3

3.7 Wall Impingement

Spray wall impingement is an important process in spray combustion. Spray penetration is a measure of the depth of the spray. The probability of wall impingement is more with higher biodiesel–diesel blends (B25, B50, and B100) due to increased spray penetration. The penetration distance with biodiesel increases due to increase in fuel injection pressure. If the spray penetration is too long, wall impingement could occur. This will result in wetting of the combustion chamber wall and/or piston crown leading to durable issues. The probability of wall impingement is less with B0–B15, critical at B20, and more beyond B25. The wall impingement problem may be minimized by the optimization of nozzle holes, fuel injection pressure, piston bowl geometry, and deflection of spray by enhancement of swirl and squish intensity. It is reported in the literature that the modification of nozzle configuration (number of holes) is a solution to reduce NO_x emission of biodiesel-fueled diesel engine at source level and the chance of wall impingement is also minimized along with tangible benefits of HC, CO, smoke, and BSEC reduction (Lahane and Subramanian 2014). NO_x emission of the engine fueled with B20 fuel decreased from 7.4 g/kW-h with the base nozzle configuration to 6.6 g/kW-h with modified nozzle configuration due to reduction in in-cylinder temperature, retarding in dynamic injection timing, and reduction in spray penetration distance.

A summary of the effect of biodiesel on performance and emission characteristics of diesel engines along with reasons and remedial measures are given in Table 3.

4 Summary

On the whole, it can be concluded that utilization of biodiesel–diesel blends up to B15 in unmodified diesel engine does not have problem of wall impingement but increase in NO_x emission is moderate. However, higher biodiesel–diesel blends would give undesirable results such as higher NO_x and BSEC, and more probability of wall impingement as compared to base diesel operation. A notable conclusion emerged from this study is that biodiesel-fueled diesel engine with modification of nozzle configuration could provide better results of performance improvement and emission reduction as compared to other techniques (retarded injection timing, pump's plunger modification, change in nozzle opening pressure).

Table 3 Summary of performance and emissions of biodiesel-fueled diesel engines

Description	Trend as compared to base diesel	Reasons	Remedial measures
Power and torque output	Drop	Lower calorific value, more fuel has to be injected	Increase the injection duration, injection rate, higher capacity injection pump and injector, super charging/turbocharging with the modified injection system
Brake thermal efficiency	Lower	Change in injection timing and duration and poor spray characteristics	Optimise the injection timing and injection system, improvement of spray characteristics (SMD, air entrainment)
Carbon monoxide	Lower	Inherent oxygen content in biodiesel and higher in-cylinder temperature	Nil
Hydrocarbon	Lower	Inherent oxygen content in biodiesel and higher in-cylinder temperature	Nil
Smoke/soot/particulate matter	Lower	Absence of aromatic, sulfur content in biodiesel and inherent oxygen content in the fuel	Nil
NO_x	Higher	Automatic advancement of injection timing due to high bulk modulus, inherent oxygen content and high in-cylinder temperature	Retarding of injection timing, exhaust gas recirculation, optimization of fuel rate shaping
Wall impingement	Higher	Higher penetration distance due to higher density and bulk modulus of biodiesel	Optimization of nozzle hole and injection pressure, enhancement of swirl and squish velocity

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Potential Role of Halophile in Crude Glycerol Based Biorefinery

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Abstract Biorefinery includes microbial fermentation processes which could utilize glycerol as raw material for the production of bio-derived building block compounds and polymers. The recent expansion in biodiesel market has resulted in a remarkable transformation in availability and subsequent cost of glycerol, which is generated at 10% of total biodiesel produced. Being produced in excess, crude glycerol price has suffered a major decline, thereby affecting the economics of biodiesel industry. Purification of crude glycerol for use in cosmetics and pharmaceutical industry increases the production cost and hence not considered as a viable option for disposal of such huge amount of glycerol, which also poses an environmental concern. Thus the crude glycerol based refinery concept is being explored whose objective should be to actualize technologies for valorization of waste glycerol. The major challenge thwarting the development of such biorefinery is obtaining microbial strains tolerant of crude glycerol along with its impurities. However, concentrated crude glycerol has rarely been used for microbial conversion to value-added products. High usage of portable water is required to dilute concentrated crude glycerol for crude glycerol based biorefinery. In this chapter, the recent attempts to explore microbial assimilation of glycerol has been summarized. Besides how halophiles can be considered as a viable alternative for valorization of crude glycerol is presented.

Keywords Crude glycerol · Halophiles · Biorefinery

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1 Introduction

Glycerol or 1, 2, 3 propanetriol, is a conventional, commercially valuable feedstock for the production of useful chemicals. Swedish chemist Carl Wilhelm Scheele discovered glycerol in 1783 by transesterification of natural oils with alkali materials (Behr et al. 2007).

Glycerol, a trihydric alcohol with three hydroxyl groups (Fig. 1) is viscous and hygroscopic in nature. It is water-soluble, clear, almost colorless, odorless liquid with a high boiling point. It is sweet-tasting and nontoxic.

Glycerol undergoes catalytic oxidation and microbial fermentation to form derivatives, which find uses in diverse sectors.

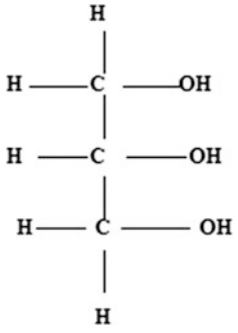
The oxidation of different hydroxyl groups present in glycerol yields glyceric acid, tartronic acid, dihydroxyacetone, and ketomalonic acid, all of which are commercially useful compounds. Dihydroxyacetone is the principal active constituent in all sunless tanning cream (Pagliaro et al. 2007). Poly(ketomalonate) is an excellent building block for household detergents (Pagliaro et al. 2007).

Glycerol is also used in pharmaceutical industries where it is commonly utilized as humectants and tablet holding agent (Ayoub and Abdullah 2012). Another glycerol derivative, nitroglycerin is used to treat ischemic heart disease (Pagliaro et al. 2007). Nitroglycerin is an active component in dynamite and other propellants (Pagliaro and Rossi 2008) Glycerol's nontoxic nature renders it a viable alternative to ethylene glycol as antifreeze agents (Pagliaro et al. 2007).

2 Crude Glycerol Production and Challenges

Glycerol is generated as a by-product of oleochemical industries, soaps, oils, detergents, and ethanol fermentation. Among all the glycerol producing industries, the biodiesel industry has a maximum share in glycerol production (Leoneti et al. 2012). Transesterification of vegetable oil with a primary alcohol catalyzed by an

Fig. 1 Structure of glycerol



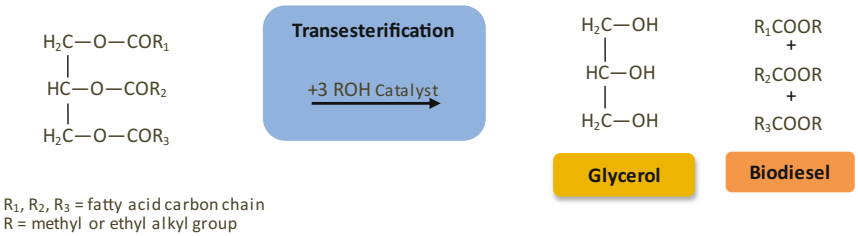


Fig. 2 Transesterification reaction for vegetable oils: fatty acid methyl ester is the main product while glycerol is a by-product. Acid or base can be used as catalyst (Leoneti et al. 2012)

acid/ base leads to the formation of biodiesel with glycerol as a major by-product Fig. 2 summarizes transesterification of vegetable oils to glycerol.

One of the major limitations with biodiesel is its production cost which can be reduced if its by-products are converted into valuable coproducts Zhang YHP (2007). Transesterification of every 10 kg of various oils into biodiesel generates 1 kg of glycerol as a reaction by-product (Rivaldi et al. 2009; Wen et al. 2009a) which is only 50–60% pure and contains impurities such as methanol, sodium, and potassium salts. Composition of crude glycerol varies from one industry to another on the basis of oil source and reaction conditions, as shown in Table 1.

Overproduction of this low-grade crude glycerol is affecting the comprehensive economy of biodiesel production plant and net market price. The high biodiesel production cost and low glycerol market price are the major motivating factors for researchers, to discover a large spectrum of value-added applications for crude glycerol in order to overcome glycerol glut in the market.

The traditional areas of glycerol utilization include animal feed, pharmaceutical, and cosmetic industry where high quality purified glycerol is required. But before crude glycerol can be utilized in these areas, it has to undergo several pretreatments to lower its alkalinity, salt content, and removal of toxic methanol. After pretreatment, the glycerol content of crude glycerol is increased up to 99%. The purification process is expensive and energy intensive, which is economically unfavorable for sectors involved in the production of lower value-added products, the purification procedure is summarized in Fig. 3. With an annual growth of 42% in the biodiesel industry, it is estimated that the biodiesel market will reach to 37 billion gallons by 2016 which will produce 4 billion gallons of crude glycerol as

Table 1 Composition of crude glycerol Kovcas (2011)

Crude glycerol composition	%
Glycerol	50–60
Methanol	10–30
Alkali catalyst	8–20
Soap	5–15
Water	5
Polar compound	1

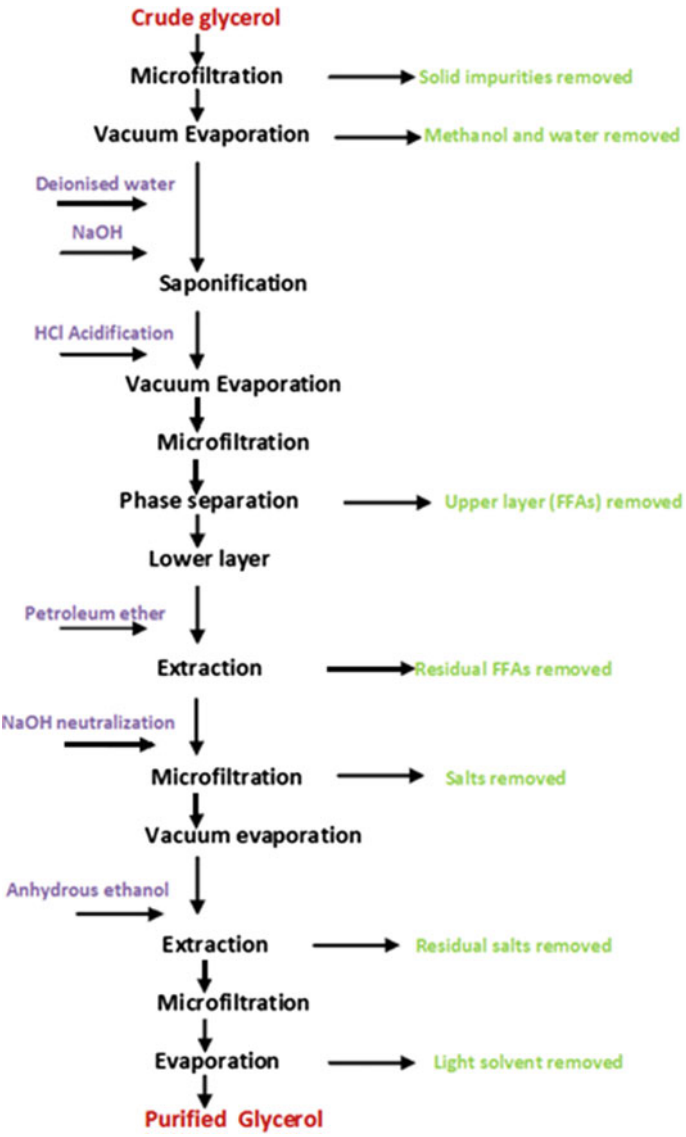


Fig. 3 Brief procedure for crude glycerol purification (Yang et al. 2013)

a by-product (Garlapati et al. 2016). With the developing glycerin glut in the market, there is an increasing urge to explore economical ways of crude glycerol valorization without the necessity of purification. An effective use of crude glycerol is required to limit the concomitant supply of glycerol in the market by biodiesel production.

Disposal of glycerol containing waste poses economical as well as environmental concerns. Therefore, other industrially feasible and viable options such as crude glycerol based biorefinery process should be considered. This can represent an "environmental-friendly" strategy, possessing the potential to improve the economics of biodiesel industry. It can be considered as an alternative way to circumvent the problem associated with accumulation of glycerol waste streams (Singhbandhu and Tezuka 2010; Yazdani and Gonzalez 2007; Saxena et al. 2009). This would yield more profit to the industry, along with reduction in the treatment and disposal cost. The question still remains which process should be adopted that can not only convert glycerol to value-added product but also environmentally non-taxing.

3 Crude Glycerol Utilization

Crude glycerol owing to its dwindling market price along with an associated disposal cost is affecting biodiesel industry dearly. An ample increase in the amount of crude glycerol production is likely to cause consequential environmental concerns in the forthcoming future. Thus it has become extremely necessary to explore environmental-friendly uses for waste glycerol. With the increasing production of glycerol, its price is dropping steadily. Therefore, there is a pressing need for valorization of low-value product by physical or chemical transformation or microbial fermentation.

Physical transformation with an increase in the price of corn and maize, glycerol is used as an alternative in feed ingredient for ruminants and nonruminant diet since years. Glycerol is an attractive feed supplement as it has high-absorption rate and energy content, but quality offered should be monitored properly as impurities present in crude glycerol feed can cause potential threats to animals. Excess of salts can cause electrolyte imbalance in animals and high-level of methanol can lead to methanol poisoning resulting in central nervous system injury and related side effects (Dorman et al. 1993). When studied in broilers, laying hens, and swine, crude glycerol proved to be an excellent energy source (Kerr et al. 2007; Donkin 2008). Feed for lactating cows containing crude glycerol up to 15% was used without any deleterious effect on feed intake and milk production. In a study conducted on broiler chicken, increased intake of crude glycerol in feed led to increased feed conversion without any effect on growth and digestibility (McLea et al. 2011).

Chemical transformation Glycerol can be completely transformed into various useful oxygenated chemicals, hydrogen gas, and branched oxygen-containing compounds. *Oxygenated chemical* like (2, 2-Dimethyl-1,3-dioxolan-4-yl) methyl acetate is synthesized using crude glycerol and used as biodiesel supplement to improve viscosity. *Acrolein* an important starting material for the synthesis of detergents, acrylic acid, and super absorbent polymer is produced by vaporizing liquid crude glycerol over fluidized bed reactor and then reacting over a tungsten

doped zirconia catalyst (Sereshki et al. 2010). *Hydrogen* a clean energy source is produced by gasification of crude glycerol via thermal decomposition mechanism.

Chemical catalysis process involves high temperature, pressure, and specific catalyst. Impurities present in crude glycerol may affect catalyst efficiency, increase char production, and influence product yield thus can cause a problem with chemical transformation of crude glycerol (Yang et al. 2012).

Due to constraints in physical and chemical transformations now the ultimate objective is to develop biorefineries in close relationship with biodiesel plants that can coproduce high-value products along with biofuels.

3.1 Crude Glycerol Biorefinery

Glycerol-based biorefinery is the sustainable processing of waste glycerol stream. It is achieved by integrating microbial fermentation with technologies into concomitant production of a spectrum of marketable biofuels, chemicals, and materials, preferably of added-value. Biological conversion is being extensively explored as it could aid overcoming the drawbacks of chemical catalysis, such as low product specificity, high pressure or temperature requirement, and catalyst poisoning. Glycerol is reducing in nature, its three hydroxyl groups provide additional advantages over other sugars regarding the ease of microbial utilization.

Microbial glycerol fermentation has led to the production of several highly reduced compounds such as 1, 3-propanediol (Szymanowska Powałowska and Leja 2014), 2, 3 butanediol (Petrov and Petrov 2009), mannitol (Khan et al. 2009), erythritol (Rymowicz et al. 2009), arabitol (Koganti et al. 2011), citric acid (Rymowicz et al. 2010), lactic acid (Hong et al. 2009), glyceric acid (Habe et al. 2009), succinic acid (Carvalho et al. 2014) etc. In most cases, the yield of bacterial biomass was low due to high osmotic pressure exerted by high concentration of glycerol. In some cases, the presence of salt also contributed to the inhibition of bacterial growth (Koganti et al. 2011; Jitrwung and Yargeau 2011; Campos et al. 2014). Bacterial cultures studied in these cases were found to divert metabolites into different metabolic pathways leading to the formation of unnecessary by-product (Sattayasamitsathita et al. 2011; Petrov and Petrova 2009; Malaviya et al. 2012; Rymowicz et al. 2009, 2010). End product toxicity, pH fluctuations, high cost of by-product removal were among other problems plaguing the successful utilization of crude glycerol as a feedstock, details are given in Table 2.

Biofuels are fuels produced through contemporary biological processes. Biodiesel, biohydrogen, and bioethanol are categorized under the same. *Biodiesel* is recyclable, renewable, and clean fuel derived from transesterification of plant-based lipid source or lipid produced by *oleaginous microorganisms*. Several strains of bacteria, yeast, fungi, or microalgae which can accumulate lipid 20% or more of their cell dry weight can be categorized as an oleaginous organism.

In oleaginous microorganism, lipid accumulation occurs under stress conditions, when there is an abundance of carbon source and one of the other nutrients present

Table 2 Value added opportunities for crude glycerol using microbial fermentation

Products Chemicals	Uses	Organism	Crude glycerol conc g/L	Problems associated with use of crude glycerol	References
1,3 propanediol	Adhesives, fragrances and perfumes, personal care products, paint coating, polyesters and polyurethanes	*Clostridium butyricum* DSP1	80	High glycerol concentration increased osmotic pressure which in turn decreased the biomass growth	Szymanowska Powałowska and Leja (2014)
		Clostridium butyricum DSM 5431	87.8	Pretreatment with hexanol was required	Rehman et al. (2008)
		Klebsiella pneumonia SU6	200	Ammonium phosphate, 2,3 butanediol were produced as by-products	Sattayasamitsathita et al. (2011)
2,3 butanediol	Plastics, antifreeze, solvents, additive to fuel	*Klebsiella pneumoniae* G31	70	Acetic acid accumulation and pH fluctuation affected yield	Petrov and Petrov (2009)
Butanol	Solvent in paint and varnish industry, production of resin, plasticizer, and fuel	*C. pasteurianum* MBELGLY2	82	End product toxicity	Malaviya et al. (2012)
Mannitol	Food and pharmaceutical preparations	*C. magnoliae* (NCIM 3470)	100	Media volume was a limiting factor, could be problem in scale up	Khan et al. (2009)
Erythritol	Food and pharmaceuticals industry as biological sweetner	*Yarrowia lipolytica*	300	Unwanted by-product formation with pH fluctuation	Rymowicz et al. (2009)
Arabitol	Natural sweetner	*D. hansenii strain SBP-1* (NRRL Y-7483)	150	Growth was inhibited due to salt	Koganti et al. (2011)
Citric acid	Food and pharmaceutical industry	*Yarrowia lipolytica* A-101-1.22	250	Mannitol, erythritol accumulation	Rymowicz et al. (2010)
Lactic acid	Production of acrylic acid, 1,2 propanediol, polyester resins, polyurethanes, antifreeze, food industry	*E. coli* AC-521	30	Low glycerol consumption, lower biomass concentration	Hong et al. (2009)
Glyceric acid	Polymer, surfactants	*G. frateurii* NBRC103465	250	High cost of removal of by-product dihydroxyacetone	Habe et al. (2009)

(continued)

Table 2 (continued)

Products Chemicals	Uses	Organism	Crude glycerol conc g/L	Problems associated with use of crude glycerol	References
Succinic acid	Production of plastics, resins, drugs, food additives	*A. succinogenes*	50.78	Glycerol consumption is limited because of a redox imbalance during cell growth, DMSO requirement	Carvalho et al. (2014)
Biodiesel	Transportation, cleaning up oil spills, generating electricity, heating fuel in domestic and commercial boilers, adds lubricity to diesel, industrial solvent for metal cleaning, paint and grease removal	Yeast			
		Yarrowia lipolytica ACA-DC 50109	50	At high dilution rate citric acid was the major product in place of single cell oil	Papanikolaou and Aggelis (2009)
		Cryptococcus curvatus ATCC 20509	214.2 in batches	Growth was suppressed at high crude glycerol concentration due to impurities, more growth was with pure glycerol	Cui et al. (2012)
		Rhodotorula glutinis TISTR 5159	85	Higher glycerol concentration inhibited growth and product formation	Saenge et al. (2011)
		Trichosporanoides spathulata JU457	100	Higher glycerol in initial phase was inhibitory to cell growth, therefore, fed-batch strategy was applied	Kitcha and Cheirslip (2013)
		Yarrowia lipolytica Q21	100	Crude glycerol promoted biomass production but not lipid yield a possible explanation for this may be presence of proteins and peptide impurities which supported growth	Poli et al. (2014)
		Metschnikowia pulcherrima	100	NA	Santamauro et al. (2014)
		Rhodosporidium toruloides	50	High glycerol content decreased cell growth and lipid formation, high methanol concentration was also found to be toxic	Yang et al. (2014)

(continued)

Table 2 (continued)

Products Chemicals	Uses	Organism	Crude glycerol conc g/L	Problems associated with use of crude glycerol	References
		FUNGI			
		Mortierella isabellina ATHUM 2935	100	High glycerol concentration decreased growth	Papanikolaou et al. (2008)
		Cunninghamella echinulata ATHUM 4411	80	High glycerol concentration decreased growth	Fakas et al. (2009)
		*Aspergillus niger*LFMB 1	60	High glycerol concentration decreased growth	Andre et al. (2010)
		*Aspergillus niger*NRRL 364			
		Thamnidium elegans CCF 1465	90	NA	Chatzifragkou et al. (2011)
		Lipomyces starkeyi DSM 70296 *Rhodosporidium toruloides* NRRL Y27012	180	Very high glycerol concentration lead to substrate inhibition	Tchakouteu et al. (2015)
		Microalgae			
		Schizochytrium limacinum SR21	23	High glycerol concentration caused substrate inhibition, methanol poisoning, lower biomass production	Liang et al. (2010)

(continued)

Table 2 (continued)

Products Chemicals	Uses	Organism	Crude glycerol conc g/L	Problems associated with use of crude glycerol	References
Biohydrogen and Bioethanol	Ethanol: transportation supplement, MTBE (methyl tert-butyl ether) replacement, substrate for biodiesel production Biohydrogen: clean, recyclable, and efficient energy carrier	*E. aerogenes* (ATCC 35029)	25	Study indicated that use of simplified media with less salt could improve growth in crude glycerol	Jitrwung and Yargeau (2011)
		Klebsiella sp. HE1	50	Concentration of glycerol used was low	Kenjer et al. (2011)
		Kluyvera cryocrescens S26	90	High concentration up to 100 g/l decreased growth	Choi et al. (2011)
Polymer	Polyhydroxyalkanoates: disposable, biodegradable, biocompatible polyester, packaging, medical devices	*Cupriavidus necator* DSM 545	20	Glycerol concentration above 20 g/l may lead to sodium ion accumulation, slower growth rate, lower yield, chain termination	Cavalheiro et al. (2009), Nuppatol and Jian (2012)
		MMC	100	Reactors suffered from foaming, due to the presence of surfactant contaminants in crude glycerol	Dobroth et al. (2011)
		Cupriavidus necator	15	Higher crude glycerol concentration decreased the amount of PHA produced due to the presence of sodium chloride as impurities	Campos et al. (2014)
		Zobellella denitrificans MWI	15	High PHB accumulation in the presence of NaCl	Ibrahim and Steinbuchel (2009)

in a limited amount. Production of microbial oils is gaining attention over oilseeds since it is not associated with "food versus fuel" debate, has a short lifecycle, no significant requirement for agricultural land, unaffected by locale or climate, and ease in scale up.

In microbial oil production traditionally starch, glucose, xylose are used as carbon sources. To make the process economically viable more renewable and affordable carbon sources with zero or negative value are required which can decrease the overall fermentation cost. Although utilization of crude glycerol over pure glycerol can offer remarkable advantages and reduce the cost of production, only a few reports are available in the literature.

Yeast strains *Yarrowia lipolytica* (Papanikolaou and Aggelis 2009), *Cryptococcus curvatus* (Cui et al. 2012), *Rhodotorrula glutinis* (Xu et al. 2012), *Candida* sp. (Duarte et al. 2013b) can successfully valorize crude glycerol to lipids. But the major drawback is that variation in glycerol concentration can shift metabolism from lipid production to citric acid, succinic acid or 1,3 propanediol formation and high glycerol concentration limited the cell growth and product formation. The presence of impurities and high salt concentration may be a possible reason for this.

In *Mortierella isabellina* ATHUM 2935 (Papanikolaou et al. 2008) and *Cunninghamella echinulata* ATHUM 4411 (Fakas et al. 2009), no effects on growth and product formation were observed with the use of pure or crude glycerol but cell growth was decreased with high glycerol concentration (Fakas et al. 2009). In *C. echinulata* CCRC 31840 when glycerol was used as carbon source, caused a reduction in microbial growth and lipid accumulation. There can be three possible explanations for the diminished production of biomass and lipid accumulation. First the enzymes involved in the initial metabolic steps of glycerol assimilation (Glycerol kinase and glycerol-3-phosphate dehydrogenase), can be poorly regulated. Second, there can be a decrease in the activity of gluconeogenic enzymes and finally slow activity of the NADP+ - malic enzyme that can severely decrease glycerol uptake and lipid accumulation (Papanikolaou et al. 2008).

Hydrogen is a clean fuel and has potential to be considered as an alternative future energy carrier molecule. Its combustion releases a high amount of energy. Hydrogen is either derived chemically from fossil fuels or by electrolysis of water. Biological production of hydrogen using raw glycerol as substrate has potential to solve energy problems. Biohydrogen is produced either by anaerobic fermentation or by photofermentation. Crude glycerol is reported as a potential substrate in hydrogen production due to its high-energy content and no prior treatment is required to use it as a carbon source in biohydrogen refinery (Saurabh et al. 2012). Most investigated microorganism for hydrogen production is *Enterobacter aerogenes* (Jitrwung and Yargeau 2011). (Guillaume and Patrick 2009) first used crude glycerol for hydrogen production using *Rhodopseudomonas palustris*; a photofermentative hydrogen-producing bacteria. Effect of toxicity at high glycerol concentration was not studied, but the yield of 6 mol H_2/mol glycerol was quite promising.

Ethanol is used as a fuel for transportation, solvent, and chemical. *E. aerogenes* (Marques et al. 2009), *Klebsiella sp.* (Oh et al. 2008) can metabolize glycerol to ethanol, however, yield was relatively low as ethanol was a by-product of fermentation. *Kluyvera cryocrescens* S26 (Choi et al. 2011) can utilize up to 90 g/L crude glycerol with an ethanol yield of 27 g/L. Glycerol concentration higher than 100 g/L resulted in decreased growth.

Polyhydroxyalkanoates (PHAs) are biodegradable, renewable, and biocompatible polyesters which were first discovered by Lemoigne (1926). It is naturally synthesized in many bacteria as a storage carbon source under nutrient limited conditions just as biodiesel. Commercial production of PHB is limited due to its high production cost than that of petrochemical plastics. Strategies like cheap carbon source, economical recovery processes, and more efficient fermentation strategies can remove the obstacles in the path of successful PHB commercialization. Crude glycerol being a cheap source based on the current market scenario could be an alternative route to the economical production of PHAs.

PHB production by using crude glycerol was studied in *Paracoccus denitrificans,* and *Cupriavidus necator* JMP 134 (Mothes et al. 2007) polymer obtained was similar as those with glucose, but yield was significantly reduced from 70% of dry cell mass with pure glycerol to 48% using crude glycerol. The PHB yield was assumed to be reduced, due to osmoregulation (Mothes et al. 2007). *C. necator* DSM 545 was used for large-scale PHB production but cell growth was hindered due to the presence of NaCl as an impurity (Cavalheiro et al. 2009). *Zobella denitrificans* MW1 can valorize crude glycerol for biomass and PHB production (Ibrahim and Steinbuchel 2009). A comprehensive overview of the products formed using a high concentration of crude glycerol is summarized in Table 2.

4 Halophiles: An Overview

The major bottleneck observed in the biological valorization of crude glycerol is the presence of impurities such as methanol, sodium, potassium salts which create a harsh environment for survival and growth of the microorganism. To combat such conditions, extremophiles can be considered as viable machinery. Extremophiles are defined as organisms capable of thriving in adverse conditions. Halophiles which are classified under extremophiles are salt-loving (prokaryotic and eukaryotic) organisms that inhabit saline environments. According to their salt tolerance limits, halophiles are categorized under slightly, moderately, or extremely halophilic. Halophiles can counterbalance osmotic stress caused by the presence of salts in two ways (a) by accumulating compatible solutes (b) by accumulating equal amount of KCl inside their cells. To survive in high saline environments halophiles produce an acidic protein which increases their solvation and prevents its aggregation, precipitation, and denaturation. Apart from thriving in high salinity, some of them can

also handle high alkaline condition, elevated temperature, and stability (DasSharma and DasSarma 2012).

Glycerol is a common osmoregulatory compound produced by *Dunaliella*, a unicellular green algae that are found in saline environments (Mezghani et al. 2012). Owing to their unique properties, halophiles can be expected to be capable of utilizing this glycerol. Surprisingly, only few strains, *Halanaerobacter jeridensis* (Roush et al. 2013) *Halanaerobium hydrogeniformans* (Anniina et al. 2010), and *Halanaerobium saccharolyticum subsp. senegalensis* (Lillo and Rodriguez 1990) have been reported to ferment glycerol.

4.1 Glycerol Valorization by Halophilic Biorefineries

Crude glycerol, a by-product of biodiesel industry contains high salt impurity and has high pH owing to their unique characteristics to thrive in high salinity (2–5 M) and alkalinity (6–10 pH), halophiles are potential alternative for valorization of crude glycerol via microbial fermentation.

Haloferax, *Haloarcula*, *Haloquadratum*, *Halorubrum*, *Halobiforma*, *Halorhabdus*, *Halalkalicoccus*, *Halobacterium*, *Natrianema*, *Halostagnicola*, *Natrinema*, *Natronobacterium*, *Natronorubrum*, *Haloterrigena*, *Halopiger*, and *Halococcus* genera are known to synthesize PHA by utilizing cheap carbon sources (Don et al. 2006; Nicolaus et al. 1999; Waino et al. 2000; Hezayen et al. 2002; Xu et al. 2005; Romano et al. 2007; Burns et al. 2007; Hezayen et al. 2010; Legat et al. 2010; Quillaguaman et al. 2010; Han et al. 2010; Kaeata and Aiba 2010). Strains of halophilic genera *Halomonas* sp KM-1 (Anupama et al. 2010), *Hydrothermalis* SMP3 M (Melanio et al. 2015), *Bacillus sonorensis* (Melanio et al. 2015), *Yangia* sp ND199 (Oren 2005) have shown to successfully ferment crude glycerol to PHA. When *Halomonas* sp KM-1 and *Yangia* sp ND199 strains were used, no prior media sterilization was required this can further cut the cost of production (Anupama et al. 2010; Oren 2005). Halophilic *Hydrothermalis* SMP3 M, *B. sonorensis*, *Yangia* sp ND199 can achieve high cell density by directly fermenting the waste glycerol stream with no prior requirement of pH adjustment or other treatments. Also, recovery of polymer from cellular inclusion was easier because the cell can be easily lysed using low salt concentration or distilled water (Melanio et al. 2015), Oren (2005). This resulted in lower solvent consumption and energy saving.

H. hydrogeniformans is known to ferment up to 184 g/L crude glycerol to 1, 3 propanediol (Kivisito et al. 2012), *Halanaerobium saccharalyticum* subsp. senegalensis can produce H_2 up to 1.6 mol/mol of glycerol and was found to be a potential strain for large-scale production (Lillo and Rodriguez 1990) Some value-added products formed by crude glycerol as a feedstock using halophiles are summarized in Table 3.

There are numerous reports on the use of non-halophilic culture for valorization of crude glycerol to various valuable products. However, in all the cases, the

Table 3 Value-added product formation using crude glycerol by halophilic fermentation

Products Chemicals	Organism	Crude glycerol concentration g/L	Advantages	References
1,3-Propanediol (1,3-PD)	*Halanaerobium saccharolyticum* subsp. *saccharolyticum* strain DSM 6643	2.5	Reduced need for pH neutralization and dilution of residual salts in	Das Sharma DasSarma (2012)
	Halanaerobium hydrogeniformans	184		Kivisito et al. (2012)
Biohydrogen Polymer	*Halanaerobium saccharolyticum senegalensis*	2.5	It was found to be a potential strain for H_2 production, the process can be easily scaled up	Lillo and Rodriguez (1990)
PHA	*Halomonas sp.* KM-1	30	Cheap media components, resistance to contamination, no requirement for medium sterilization	Anupama et al. (2010)
poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV)	*Yangia sp.* ND199	20	Cells grew faster, higher PHA accumulation, no inhibition of cell growth and polymer production, growth in unsterile conditions	VanThuoc et al. (2015)
PHB	*B.sonorensis* SMP1S	10	Increased cell growth and PHA accumulation, no pH adjustments or preliminary treatment of crude glycerol required	Melanio et al. (2015)
	*Hydrothermalis sp.*SMP3 M	10		

concentration of glycerol was low and thus necessitated increased usage of portable water as the glycerol concentration in crude glycerol ranges from 50 to 60% Kovcas (2011).

Commercial exploitation of crude glycerol using microbial process is limited due to this increased usage of portable water. In this respect, halophile can be a good choice as it can grow in low water activity and high NaCl concentration. However, limited literature on crude glycerol valorization using halophile indicates more attention is needed in this direction.

5 Conclusion: Challenges to Glycerol Fermentation and Potentials of Halophilic Biorefinery

1. Increased level of crude glycerol and its impurities mainly salts leads to an increase in osmotic pressure with simultaneous lowering of water activity of medium. This hinders the growth of organisms when high glycerol concentration is used in the medium. As halophiles are capable of thriving in low water activity, they can be an interesting alternative.
2. In many studies, high glycerol concentration has proved to be toxic for cell growth. Cell growth and product yield were better in pure glycerol than crude glycerol. Probable reason for this is the presence of impurities such as salt and high pH of glycerol feedstock which hinders the growth of organisms. Using halophiles for valorization of crude glycerol can solve the problem as halophiles can grow in high salt and alkaline condition.
3. Glycerol kinase phosphorylases glycerol to glycerol-3-phosphate with subsequent formation of Dihydroxy Acetone Phosphate (DHAP). DHAP can be integrated into pyruvate metabolism easily. Glycerol kinase is widely distributed among halobacteria (Oren and Gurevich 1994). Thus utilization of glycerol by halophiles can be more frequent as compared to non-halophiles.
4. In the pursuance of crude glycerol utilization as feedstock in industrial setting pretreatments such as neutralization, desalination, and temperature control are required. Due to haloalkaliphilic and halotolerant capabilities, halophilic microorganisms can utilize nonneutralized feedstocks, saving energy and capital.
5. Industrial glycerol often contains impurities like heavy metals along with sodium, potassium salts, and methanol which are putative growth inhibitors for microbes. Halophilic bacteria possess capability for industrial glycerol valorization as high heavy metal tolerance has been reported in several halophilic strains and their salt utilization potential is well known (Neito et al. 1989).
6. A significant challenge in microbial bioprocesses is the maintenance of axenic culture. This is especially a challenge for bioprocesses utilizing pure cultures that require closed sterile systems. Halophilic microorganisms are advantageous for process biotechnology because hypersaline environment suppresses the growth of non-halophiles and therefore, sterilization costs can be reduced.

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Bio-jet Fuel

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Abstract The crude oils are processed in a refinery to make a host of useful products; including gasoline, diesel, jet fuel, petrochemicals, and asphalt components. Kerosene is produced as a straight run product but is also produced through hydroprocesses, especially from heavier crude oil feedstocks. Kerosene jet fuel is a hydrocarbon fuel composed almost entirely of hydrogen and carbon elements. The hydrocarbon composition consists mainly of paraffins (iso and normal), cycloparaffins (naphthenes), and aromatics. Aviation jet fuel produced from different feeds and processes will have different ratios of these hydrocarbon components. Combustion of Aviation Turbine fuel or jet fuel (Jet-A1) for aviation purpose has contributed to "global warming" leading to a proposed blending of "Biojet" to reduce the carbon footprint. In 2009 a new ASTM specification (D7566-09, *Standard Specification for Aviation Turbine Fuel Containing Synthesized Hydrocarbons*) was developed for aviation turbine fuels. The specification allows for a maximum of a 50% blend of Biojet with conventional jet fuel. While Bioethanol and Biobutanol, a proven biofuel for the automobiles, were found unsuitable biofuel for aviation purpose due to a mismatch in ASTM D7566-09 specifications. Several technological options have emerged on intensive R&D efforts globally. Such technologies used plant seed oil, waste cooking oil, animal fat, agricultural residues, and MSW as feedstock to produce renewable hydrocarbon fraction as drop-in fuel known as "Biojet". Basic advantage of using plant or agricultural waste based feedstock instead if crude oil is the minimization of carbon footprint in the aviation fuel. However, several challenges have emerged to meet the stringent specifications of aviation fuel and challenges being addressed to ascertain Biojet as sustainable, cost-effective, and green aviation fuel.

Keywords Aviation Turbine Fuel (ATF) · Jatropha oil · HEFA DSHC · ASTM · Bio-Jet

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1 Introduction

Air travel has changed our world for the better quality of life, enabling better relationship of communities, fuelling commerce, facilitating the exchange of goods that could not have existed for previous generations. While air travel makes our world a smaller and better place, there are significant costs associated with the energy needed for flight. The passenger, cargo, and military air transportation place significant demands around on very specialized jet fuel (Jet-A-1) derived from crude oil. Increase in air traffic globally at 5% per annum has set high demand 200 million tons per annum of jet fuel in the aviation industry (Davidson et al. 2014). Thus, combustion of traditional fossil fuel (Jet-A) in large quantity by air carriers poses a global threat to high altitude propagation making 2% of total greenhouse gases emission by total transportation sector. As a measure to curb the "Global Warming" some countries have proposed to impose "carbon-tax" on the airlines using fossil fuel based aviation fuel such as Jet A-1. The members of the International Air Transport Association (IATA) have pledged the following goals:

- To improve fuel efficiency by 1.5%/year over the decade to 2020
- To make all aviation industry growth carbon neutral by 2020
- To reduce net CO_2-e emissions by 50% by 2050, against 2005 levels.

1.1 Growth of Aviation Industry

Indian aviation industry is the fastest growing sector with CAGR of 18% by 2020. India has the highest fuel costs and taxes in the world and if not it is among the top two or three which hits the profitability of smaller airlines.

Domestic and international air traffic in Indian civil aviation sector has also been increased around 20% in last five years (Fig. 1).

The main greenhouse gas (GHG) emissions generated by air transport during flight are carbon dioxide (CO_2), nitrogen oxides (NO_X), water vapor (H_2O), and particulate matter (PM) and hence causes global air pollution.

Essentially an urgent need for green fuel, alternate to fossil fuel based jet A-1 fuel for aviation sector is of high demand. Second generation Biofuels could be potential alternative and can be mixed with fossil fuel based Jet A-1 which would lead to minimizing the net carbon dioxide (CO_2), nitrogen oxides (NO_X), water vapor (H_2O), and particulate matter (PM) footprint in the environment simultaneously could meet up demand of aviation fuel partially. These fuels can be partially mixed with aviation fuel using existing refueling infrastructure providing the easy supply chain system globally. Each kilogram of fuel saved reduces carbon dioxide (CO_2) emissions by 3.16 kg. However, there are several challenges exists in application of Biofuels in the aviation purpose as compared to other transportation mode such as automobiles. One of the major challenges of application of Biofuels

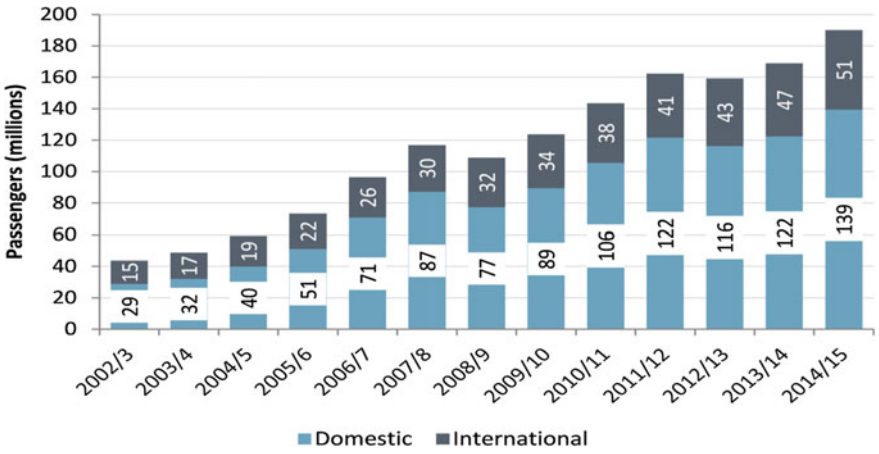

Fig. 1 Indian domestic and international passenger traffic. *Source* www.steerdaviesgleave.com/news-and-insight/Indian-airport-passenger-trafic-continues-to-grow

for aviation purpose is the meeting of stringer fuel specifications as compared to that of automobiles.

Main requirements for sustainable alternative jet fuels:

- Can be mixed with conventional jet fuel,
- Can use the same supply infrastructure and do not require adaptation of aircraft or engines (drop-in fuel),
- Meet the same specifications as conventional jet fuel, in particular, resistance to cold (Jet A: −40 °C, Jet A-1: −47 °C),
- High energy content (min 42.8 MJ/kg)
- Meet sustainability criteria such as lifecycle carbon reductions, limited fresh water requirements, no competition with food production, and no deforestation.

The stringent specifications of aviation fuel as required by present aircraft industry certainly need to comply with any alternate fuel (Table 1).

1.2 Sustainable Aviation Biofuels

Bioethanol and Biodiesel are most sustainable Biofuels propagated now for the automobiles used as surface transport. Bioethanol is mixed with gasoline at 5–10% (E5–E10) and used in the traditional cars in India, EU, and the USA whereas a mixture of 85% bioethanol in gasoline (E85) is used in Flexi cars in Brazil. Diesel vehicles run successfully with 20% biodiesel in petro-diesel (B20) globally. Lignocellulosic biomass and nonedible oils extracted from plant seeds are most sustainable feedstocks used for large-scale production of Bioethanol and Biodiesel

Table 1 Aviation fuel specification compliance (Jet A-1)

Cold flow properties, pour point °C	<44
Energy density	44 MJ/kg
Fuel composition	Proper ratio of *n*-alkanes, iso-alkanes, Cyclo-alkanes and aromatics (25 vol.%) Selective hydrocarbons *n*-dodecane (C_{12}) 43% Iso-cetane 27% Methylcyclohexane 15% 1-methylnaphthalene 15%
Density at 15 °C	0.779–0.840
Viscosity at −20 °C Cst max	8.0
Kinematic viscosity at 40 °C	1.2
Smoke pt. mm min	19
Thermal stability JFTOT ΔP (mm Hg) max	25.0
Existent gum (mg/100 mL) max	5.0–7.0
Ignition, extinction, and flammability	Within limits
Compatibility issues	Materials in jet engine and additives

Table 2 Limitation of specification of bioethanol and biodiesel as aviation fuel

	Bioethanol	Biodiesel
Energy density MJ/kg	26.4	37.27
Kinematic viscosity cSt at 40 °C	0.80	1.9–6.0
Pour pt °C	Within limit	13–16
Flash point		130 °C higher
Compatibility	Corrosive with moisture	Non compatible
Impurities		Glycerol, acids

respectively. Technologies for large-scale production of these two Biofuels have been licensed to several commercial farms in USA and Brazil. The Life Cycle Analysis (LCA) of these two Biofuels showed that both have a significant low carbon footprint as compared to gasoline and diesel. Thus, Bioethanol and Biodiesel have emerged as most sustainable biofuel for automobiles. However, Bioethanol and Biodiesel have some limitations to use as aviation Biofuels as mentioned in Table 2.

The inherent properties of bioethanol and biodiesel have restricted its use as sustainable aviation fuel although they are quite successfully being used in road transport vehicles. An alternative fuel, preferably from renewable sources, and meets all the required fuel specifications, is the preferred option. Therefore, essential criteria for aviation biofuel or Biojet is targeted toward synthetic fuel either hydrocarbon or non-hydrocarbon types meeting essential specifications of fossil-based Jet-A or Aviation Turbine Fuel (ATF) with "DROP-IN" characteristics. Such biofuels are carbon neutral and they require less investment in terms of

refining operation and supply chain infrastructure management. In 2005–2006 the Defence Advanced Research Projects Agency, or DARPA, sponsored projects in the quest for bringing green jet fuel to the U.S. military.[1] They focused on the development of a process that efficiently produces an alternate for petroleum-based military jet fuel (Jet Propellant 8; JP-8) from oil-rich crops produced by either agriculture or aquaculture, and which ultimately can be an affordable alternative to petroleum-derived JP-8 (Roberts 2008).[2] Alternative fuels in aircraft is subject to very specific constraints (safety, logistics, temperature, etc.). Over the short and medium terms, we can only consider drop in solutions, fuels with similar properties to those of kerosene, which do not require drastic changes to be made to equipment architecture and infrastructures, given the degree of investment in air transport (Roberts 2008).

1.3 Renewable Feedstocks

Nevertheless, development of sustainable bio-jet fuel requires sustainable, quantitative, and qualitative supply of renewable feedstocks. Such feedstocks should be available in all the continents of the globe, as it would facilitate smooth supply of fuels to the international airlines. At present, several production options exist—biomass, plant seed oils, and algae being more commonly known feedstocks that are available in South East Asia, EU countries, North and South American countries. Bio-jet fuels produced from plant seed oils have already powered commercial flights in small proportions—amongst them United, Lufthansa, JAL, and several others, all of whom have operated flights with one engine powered by a mix of Jet A and biofuel derived from Jatropha, a nonedible evergreen shrub. British Airways and Solena have partnered to produce a synthetic kerosene product from agricultural and municipal waste that is planned to begin production in 2015. In June 2011, a Gulfstream G450 became the first business jet to cross the Atlantic Ocean using a blend of 50/50 biofuel developed by Honeywell derived from camelina and petroleum-based jet fuel.

The potential feedstocks for production of carbon neutral and cost competitive Bio-jet fuel are classified under three categories:

(a) Lipids—Oil from seeds of camelina, jatropha, rapeseed, karanjia, maize (corn), as well as palm oil, and used cooking oil. Some processes also targeted to utilize animal fats and algal lipids. Lipids from microalgae, oil from seeds of halophytes (salt-tolerant plants), Jojoba wax, Microbial oil produced by yeasts and bacteria.

[1] http://www.greentechmedia.com/articles/read/darpa-gives-logos-196m-for-bio-jet-fuel-6023.

[2] http://www.defenseindustrydaily.com/darpa-solicitation-can-you-replace-jp8-jet-fuelwith-abiofuel-02428/.

(b) Lignocellulosic biomass includes wood, agricultural residues, forest residues. Energy crops including fast-growing trees and grasses such as bamboo, miscanthus, and giant reed.
(c) Sugars.

Most oil plants originate from tropical and subtropical climates. A large share of the world plant oil production comes from countries like Indonesia, Malaysia, China, India, the USA, Argentina, and Brazil with palm, rapeseed, Jatropha, and soybean as the major feedstock oils. Some edible and nonedible oil production with high productivity in the world is given in Table 3.

In spite of sustainable availability potential of plant oils around the globe supply of some of them, particularly edible oils, are scarce in some countries such as India. Other alternate sources are aquatic algae that could be cultivated in offshore or marine water. Microbial lipids such as yeast lipids are also being considered as potential non-plant source of feedstocks.

However, the future of biojet production from oils and fats is about achieving sustainability with volumes and at relatively low cost. To achieve these three major issues the only obvious options are to produce non-food grade oil feedstocks from high yielding sources in places where the very large areas of production space is available, where competition with (or displacement of) existing food production is not an issue, and where adequate water and other inputs are also available. Nevertheless type of fatty acid composition in the oil feedstock play a major role in quality of Bio-jet fuel (Table 4).

In the Indian context, Bio-Jet fuel consumption is around 4.5 Million Tons per annum. Availability of waste land in India is 3 million hector, which can be utilized for Jatropha or pongamia plantation yielding around 9 Million tons per annum of oil for biojet production. In 2012 global biomass energy supply was 10% of total energy supply. Biomass supplies more than 80% of the Biomass energy supply in Nigeria in comparison to 7.5 and 24% in China and India respectively. Situating in tropical region Asia and Africa produce 70% of total biomass supply in the world (Table 5).

Table 3 Oil production per unit area (ha) of different oil plants

Plant	Latin name	Productivity (l/ha/year)
Palm	*Elaeis guineensis*	5698
Coconut	*Cocos nucifera*	2578
Jatropha	*Jatropha curcas*	1812
Castor bean	*Ricinus communis*	1354
Karanja	*Pongamia pinnata*	1250
Rapeseed	*Brassica napus*	1140
Camelina	*Camelina sativa*	500
Cotton	*Gossypium hirsutum*	308
Corn	*Zea mays*	168

http://www.environmentalleader.com/2007/07/06/honeywells-uop-developing-bio-jetfuel-formilitary

Table 4 Role of fatty acid structure on fuel properties

Properties	Short chain	Long chain	Saturated	Unsaturated
Viscosity	Favorable	Unfavorable	Unfavorable	Favorable
Freezing point	Favorable	Unfavorable	Unfavorable	Favorable
Flash point	Desirable	Undesirable	Undesirable	Favorable
Energy content	Low	High	High	Low
Oxidation stability	Acceptable	Acceptable	Better	Unfavorable
Combustion	Unfavorable	Favorable	Better	Unfavorable
NO_X	Increase	Reduced	Reduced	Increase

Table 5 Global primary biomass energy supply in EJ

Year	World	Africa	America	Asia	Europe	Oceania
2000	43.1	10.5	7.33	21.6	3.36	0.26
2005	47.2	12.0	8.19	22.5	4.20	0.27
2010	53.9	13.7	9.64	24.3	5.97	0.22
2012	56.2	14.7	9.73	25.0	6.46	0.26

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2 Bio-jet Fuel Process Technologies

2.1 Hydroprocessed Ester and Fatty Acids (HEFA)

The renewable jet fuel process, developed by UOP LLC Company is a good example of novel technological methods that may be implemented in aviation biofuels production. The process can convert a variety of refined natural oils and fats including edible and nonedible natural oils, tallow, and algal oils. The renewable jet process uses a selective cracking step which reduces the natural oil C_{16}–C_{18} carbon chain lengths to carbon chain lengths in the C_{10}–C_{14} range for jet fuel. The renewable jet process is based on UOP's Ecofining^{TM} process,[3] which is commercially available for the production of green diesel produced from vegetable oils. While the Ecofining unit can produce up to 15% of Bio-SPK jet fuel, as a coproduct with diesel, this new process is designed to maximize the yield of Bio-SPK (Bio-kerosene) to 50–70%.[4] This is achieved by optimizing the catalytic processes of deoxygenation, isomerization, and selective cracking of the hydrocarbons present in natural oils and fats to yield a high quality, ultralow sulfur jet fuel that meets Jet A-1 specifications, including freeze point of −47 °C and flash point of 38 °C (Fig. 2). Coproducts from this new process are diesel and naphtha

[3] http://www.uop.com/hydroprocessing-ecofining/.
[4] See Footnote 1.

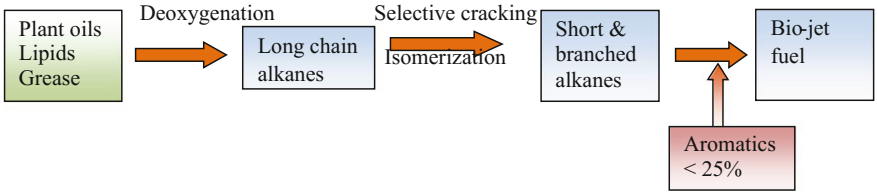


Fig. 2 Ecofining™ process scheme

range fuels. The process can be adjusted to produce a specific freeze point of the Bio-SPK or can alternately be operated in a diesel mode.

Another novel catalytic process was developed by CSIR-Indian Institute of Petroleum Dehradun to convert nonedible vegetable oil such as Jatropha seed oil to biofuels by hydroprocessing for aviation and transportation sectors. A highly selective catalyst was developed and process temperature (400–420 °C) and pressure (60–80 bar) were optimized for hydrotreating, hydrocracking, and hydroisomerization of vegetable oil to Biojet fuel either in a single step or two steps process. The process was successfully demonstrated in a pilot plant processing Jatropha oil 100 kg per day with 99% conversion and 33–40% yield of Biojet fuel and rest liquid products as diesel and gaseous fuel. Better conversion (99%) and maximum yield (40%) of Biojet could be achieved by designing selective catalysts such as mesoporous alumina, silica–alumina, and hierarchical mesoporous zeolites for hydroconversion of seed oil, waste cooking oil, and algal oil (Sinha et al. 2012).

The process has the advantage of using oil with varying FFA content. The process scheme is shown in Fig. 3 and Table 6.

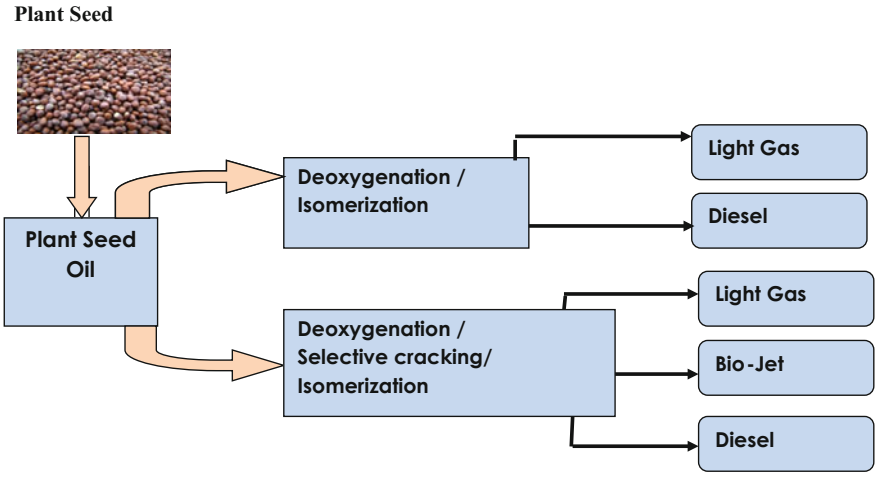


Fig. 3 CSIR-IIP process scheme for aviation and transportation fuel

Table 6 Comparison of properties of biojet produced by CSIR-IIP

Property	Units	Limit	Jet A-1	IIP biojet
Freezing pt.	°C	Max. −47	−52.2	−63
Viscosity (−20 °C)	mm^2/S	8.00	3.72	3.45
Flash pt.	°C	38.0	43	49
Density	kg/m^3	775.84	793	780
Total aromatics	%v/v	Max. 26.5	23	13
Sulfur	%m/m	0.3%	0.2	0.009
Smoke pt.	mm	25.0	26	34
Specific energy	MJ/kg	42.8	42.9	43.5

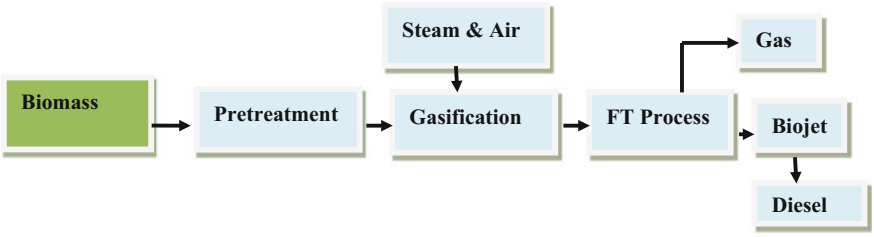


Fig. 4 Synthetic bio-kerosene process scheme

Properties signify that CSIR-IIP process could achieve better aviation fuel specification without any additional aromatics supply as compared to petroleum-based Jet A-1 or Ecofining TM process by tuning of catalyst surface, operating conditions, and pretreatment of feed oil to remove some metal and non-metal inhibitors.

2.2 Synthetic Bio-kerosene Process

The process is basically developed on the concept of Biomass to Liquid (BtL) conversion using feedstocks like lignocellulosic biomass, wood residue, cereal straw, and forest residues to synthetic fuels such as biodiesel, biojet fuel Fig. 4. It is a five-step process consists of (a) pretreatment of biomass (b) gasification or pyrolysis (c) Syn-gas purification (d) Fischer-Tropsch (FT) synthesis (e) Hydroisomerization of FT-wax to Biojet, Biodiesel, and naphtha depending on needs. The kerosene fraction obtained using the BtL process is of very good quality, free from sulfur and other impurities.

2.3 Alcohol Oligomerization Process

Many companies have reported development of processes to produce jet fuel from alcohols, but its economics depend upon the source from which alcohols are produced. Gevo, an American renewable chemicals and biofuels company, has claimed to have successfully produced isobutanol from fermentable sugars derived from cellulosic biomass, and converted this into isobutylene and paraffinic kerosene (jet fuel). Another company, Lanzatech, is also claimed to produce alcohol from industrial waste gases containing "clean" carbon monoxide and converting this alcohol into jet fuel by oligomerization and hydrogenation (Fig. 5).

2.4 Direct Sugar to Hydrocarbons (DSHC)

The Direct Sugar to Hydrocarbons (DSHC) process converts sugar to a pure paraffin molecule that can be blended with conventional jet fuel. The process utilizes an advanced fermentation process to accomplish the conversion. This biological conversion is carried out under aerobic conditions, unlike "traditional" fermentation of sugars to ethanol.

The process in this pathway involves a yeast fermentation process fed by sugarcane (or any other plant sugars including from sugar beet, sweet sorghum, or cellulosic sugars) to produce the unsaturated fermentation product farnesene. This then undergoes another conversion process that results in the hydrogenated and saturated hydrocarbon fernesane. This approved pathway is developed by a collaboration between French petroleum refining and distribution company Total and California-based industrial bioscience company Amyris. However, for its use in commercial aviation this new biojet product is presently only to be used in blends of up to 10% with conventional jet kerosene.

Farnesene is a terpenoid olefin (1,6,10-Dodecatrienes, $C_{15}H_{24}$) biochemically synthesized through mevalonate or isoprenoid pathway present in most of the eukaryotes and higher bacteria. It is insoluble in water and soluble in alcohols. Its Pour point −76 °C and b.p 250 °C, density 0.83 (15 °C). The physic-chemical

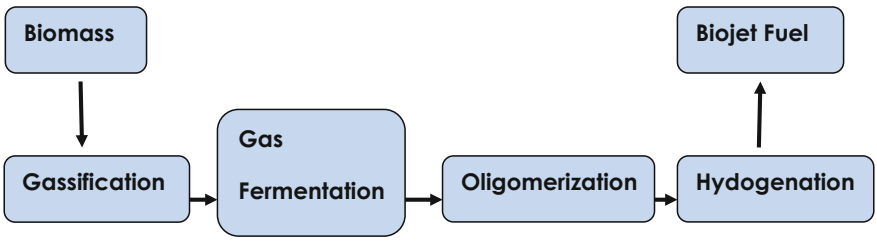
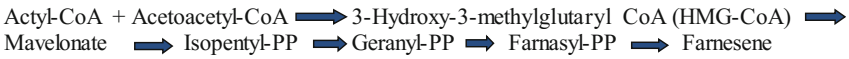


Fig. 5 Alcohol oligomerization to jet fuel (AJT) process scheme

properties of farnesene or its isomers have an advantage over ethanol and butanol as Biofuels since it is non-hygroscopic, very low pour point and density, flash point meets aviation fuel specifications.

2.4.1 Mevalonate Pathway



The major enzymes associated with the terpenoid biosynthetic pathway are (a) acetyl-CoA acetyltransferase, (b) 3-hydroxy-3-methylglutarylcoenzyme A synthase, (c) HMG-CoA reductase, (d) mevalonate kinase, (e) phosphomevalonate kinase, (f) mevalonate pyrophosphate decarboxylase, (g) isopentenyldiphosphate (IPP) isomerase, (h) isoprene synthase, (i) farnesyl pyrophosphate (FPP) synthase, (j) α-farnesene synthase.

The pathway needs 2 mol of NADPH and 3 mol of ATP which are generally available through glycolysis and other pathways in aerobic and anaerobic microorganisms. Recent developments in metabolic engineering and synthetic biology have allowed overproduction of terpenoids based on microbial fermentation rather than plant-based production, resulting in several remarkable breakthroughs, not only in the production of complex natural products, such as precursors of taxol and artemisinin, but also in the production of bulk chemicals and biofuels (Martin et al. 2003; Özaydın et al. 2013, Zhu et al. 2014) (Fig. 6).

2.5 Bio-oil Hydroprocessing to Biojet Fuel

The process is based on hydrotreatment of Bio-Oil produced by Catalytic Fast Pyrolysis of lignocellulosic and wood biomass. Bio-oil can also be coprocessed with Heavy Vacuum Gas oil (HVGO) in hydrotreatment unit of an Oil refinery to

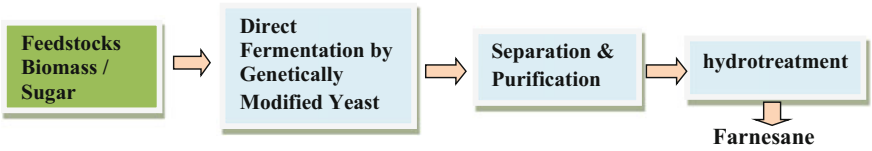


Fig. 6 Direct sugar to biojet (farnesene) process scheme

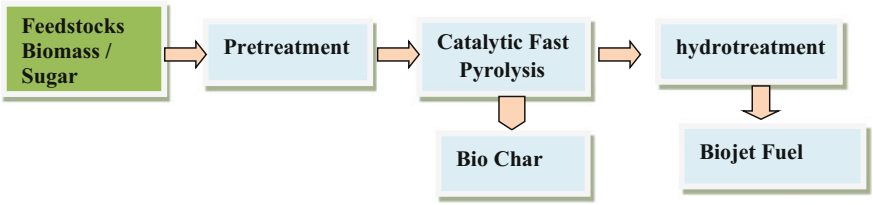


Fig. 7 Hydrotreatment of bio-oil to bio-jet fuel

Table 7 Biojet fuel process development status

Biojet fuel process	Certification	Feedstock type	Feedstock cost	Potential investment
Fischer-Tropsch (FT)	ASTM 2009 max. 50% blend with Jet-A1	Woody and lignocellulosic biomass	low	Very large
Hydroprocessed plant seed oil	ASTM 2011 max. 50% blend with Jet-A1	Plant oils, waste oils from food industry, animal fats, algal oil	High for edible oils but medium with nonedible oil supply	Medium
Alcohol oligomerization to jet fuel (ATJ)	Under process of ASTM certification	Sugars, starches	Medium but restricted in some countries on Food vs. Fuel	Medium
Direct sugar to hydrocarbons (DSHC)	ASTM 2014 max. 10% blend with fossil jet	Sugars, cellulosic materials	medium	Large
Hydrotreated pyrolysis oil	Under process of ASTM certification	Woody and lignocellulosic biomass	medium	Very large

produce Jet-A1 fuel. The process is under developmental stage promoted by few giant companies such UOP, Ensene & BTG to demonstrate the process in a demo plant (Fig. 7).

It has been demonstrated that use of various renewable feedstock such as plant oilseeds, biomass, and sugars could be converted to Bio-jet fuel through different chemical/ catalytic and biocatalytic processes. However, application of such "Biojet Fuel" in commercial flights requires ASTM certification and techno-economic feasibility. Current status of such feasibilities for commercial application is summarized in Table 7.

3 Issues Limiting the Deployment of Biojet Fuels on a Global Scale

The options to deploy cost-effective biojet fuels on a global scale are limited by several issues such as technical constraints, high production costs, price and competing uses for feedstock, production capacity, lack of policy incentives, and the real potential of waste and residues.

3.1 High Cost of Production

Biojet fuel is currently costlier than petro Jet-A1 fuel due to uncertain and poor supply chain of feedstock such Jatropha oil, camellia oil, or Pongamia oil etc. in the international market. Basically cost of biojet fuel is dependent on (a) input cost and composition of feedstock, (b) process technologies, (c) conversion efficiency and product yield, (d) value-added coproducts, (e) process energy efficiency. Biojet fuel produced by HEFA process was projected around US$4.5–5.4/gal which is almost double the current price of petroleum Jet A fuel (NREL Technical Report 2016). It was found that Feedstock and hydrogen represent almost 50–70% of the Biojet fuel price. Therefore, sustainable supply of potential feedstock is the key factor for cost reduction.

3.2 Technology and Plant Capacity

Many processes developed for Biojet fuel are in the pilot or Demo scale. Some of them still require technical maturation. Application of waste/ residues as feedstock more benefit on net greenhouse gas reduction comparing other potential feedstock; however, their availability and supply chain strategy has not yet established globally. High capacity stand-alone production plants need high capital and operating cost. Feasibility of use of Biojet fuel in aviation sector is possible as drop-in fuels in near future. Therefore, such plants should build in the vicinity of petroleum refinery or other biofuel plants.

3.3 Lack of Policy Incentive

Government of several countries has announced incentives on blending of Biodiesel and Bioethanol with petrol or diesel respectively to make parity with the fossil fuels. However, no such action has been initiated for use of Biojet fuel in the

aviation sector. This situation may potentially create global differences in price and availability of feedstock and biojet fuel.

4 Conclusion

Road map for development and application of Biojet fuel as drop-in fuel in the aviation purpose has been laid down. Biojet is considered an important part of the aviation industry's GHG emission reduction strategy. Using it to reach a GHG emissions goal of 2050 emissions at one-half of the 2005 level would involve significant development of standards and regulatory approvals, feedstocks, conversion facilities, transportation infrastructure, and logistics. Initial use of biojet has proceeded in commercial and military applications. The most suitable option for biojet production for petroleum importing countries like India are:

(a) Conversion of low-cost nonedible plant seed oil to biojet by UOP developed Ecofining process or CSIR-IIP developed hydroprocess.
(b) Production of Bio-oil from MSW or biomass by catalytic pyrolysis and hydrotreatment of bio-oil to biojet.
(c) Production of ethanol from sugar cane and convert it to biojet using the ATJ pathway.
(d) Production of sugars from biomass and direct conversion of sugars to hydrocarbons (DSHC) pathway as it has been demonstrated by Amyris in Brazil, producing an aromatic hydrocarbon from sugar that can be blended at up to 10% with jet fuel.

However, there are certain roadblocks in terms of cost, supply chain management of feedstock, establishing techno-economic feasibility, and governmental policy.

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Pretreatment of Lignocellulosic Biomass Toward Biofuel Production

Soumya Sasmal and Kaustubha Mohanty

Abstract To improve the competence of cellulose hydrolysis, pretreatment is required to efficiently break its recalcitrant structure. Pretreatment has been viewed as one of the most expensive processing steps in cellulosic biomass-to-fermentable sugars conversion, and it has great potential for improvement of efficiency and lowering of cost through research and development. Pretreatment is an important cost-driver of lignocellulose conversion to biofuel and an important step prior to enzyme hydrolysis. It disrupts the plant cell wall network and partially separates the major polymer components (lignin, cellulose, and hemicellulose). However, pretreatment of lignocellulosic materials may also result in the release of inhibitors and deactivators of the enzymatic hydrolysis of cellulose. Development of enzyme processes for hydrolysis of cellulose to glucose must reduce inhibition and deactivation effects in order to enhance hydrolysis and reduce enzyme usage. Therefore, great attentions have paid in designing pretreatment technologies to split recalcitrant characteristics of lignocellulose biomass.

Abbreviations

AFEX	Ammonia Fiber Explosion/Expansion
IU	International Unit
IL	Ionic Liquid
LCC	Lignin Carbohydrate Complex
FPU	Filter Paper Unit

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1 Introduction

Lignocellulosic biomass materials are readily available in our surrounding habitats in different forms. Nature produces about 180 billion metric tons of biomass/year, of which about 75% is in the form of carbohydrates such as hexose or pentose sugars. On account of the global annual production of biomass (1×10^{11} tons) as well as the specific energy contents of biomass and crude oil, it has been concluded that in only one decade, Earth's plants can renew in the form of cellulose, hemicellulose, and lignin all of the energy stored as conventional crude oils. As lignocellulosic materials have low nitrogen and sulfur contents, the fuel products (bioethanol, biobutanol, or biodiesel) obtained from this will be environment friendly. The vegetal biomass comes from photosynthesis, and the CO_2 from biomass does not represent a net input to the amount of carbon already making part of the carbon cycle, which displays CO_2-neutrality nature of lignocellulose materials. There have been many attempts by various researchers and government as well as nongovernment initiatives from all over the world to commercialize the production of biofuels such as ethanol, biodiesel with lignocellulose as the biomass feedstock but the complex structure of lignocelluloses acts as a protecting barrier of plants cell wall from attack by potential pathogenic organisms and purveys resistance against mechanical forces. The digestibility of cellulose or other polysaccharides present in lignocellulosic biomasses into monomers or the fermentable sugars is hindered by many chemical, structural, or physiochemical factors. Modern biotechnology research has designed enzymes or the cocktail of several enzymes to hydrolyze raw lignocellulosic material to yield monosaccharides but the process is time-consuming and needs sophisticated supervision as well as operating procedures which makes the process less economically feasible technology. To accelerate the hydrolysis process, scientists are interested to incorporate an additional step, which is known as "pretreatment". Pretreatment process rapidly disintegrates the lignocelluloses to its mother components i.e., lignin, cellulose, and hemicellulose. Therefore, the primary step toward biofuel production from lignocellulosic biomass is pretreatment. This step facilitates enzymatic hydrolysis process by altering the structural features, increasing surface areas and porosity of lignocellulosic biomasses. In general, the basic objectives of any type of pretreatment process are as follows,

1. Production of reactive cellulose fiber, which is suitable for enzymatic hydrolysis for the production of monomers such as glucose, xylose etc.
2. Reduction of byproducts formation during pretreatment process which reduces not only sugar yield but also inhibitory chemicals (e.g., furfural) for further downstream process.
3. Preserve the hemicellulose fraction in biomass.
4. Minimum energy requirement of the entire process.
5. Input of fewer low cost and less hazardous chemicals to initiate process.
6. Reducing the cost of feedstock (lignocellulosic biomass) size reduction.

The pretreatment process can roughly be classified into physical, chemical, and biochemical pretreatment process (Fig. 1).

Cost effective pretreatment of lignocellulosic biomass is a major challenge of lignocellulose-based biorefinery technology (Krishnan et al. 2010). However, there is a sound opportunity for reducing the cost of pretreatment process through extensive research and development for which several pretreatment techniques like physical (milling and grinding), physicochemical (steam explosion/autohydrolysis, hydrothermolysis, and wet oxidation), chemical (alkali, dilute acid, oxidizing agents, and organic solvents), and biological processes have been used for efficient conversion of the structural carbohydrates to fermentable sugars or other value-added chemicals.

Each pretreatment process has their own advantages and disadvantages. Table 1 shows the advantages and disadvantages of different pretreatment processes. Among all the pretreatment processes the chemical pretreatment process results in a rapid and good yield of sugar after enzymatic hydrolysis. The biological pretreatment sounds good but the slower rate of conversion and cost of enzymes does not make the process popular. Beside irradiation, other physical pretreatment processes demand high-energy input to make the pretreatment process successful. The present study focused on two pretreatment processes to achieve high product yields in subsequent enzymatic hydrolysis and fermentation operations. The dilute sulfuric acid pretreatment process and ultrasound assisted lime pretreatment process was conducted and compared accordingly.

In a broad spectrum, the benefits of lignocellulosic biomass materials can be accomplished according to two different philosophies, the headmost is to direct

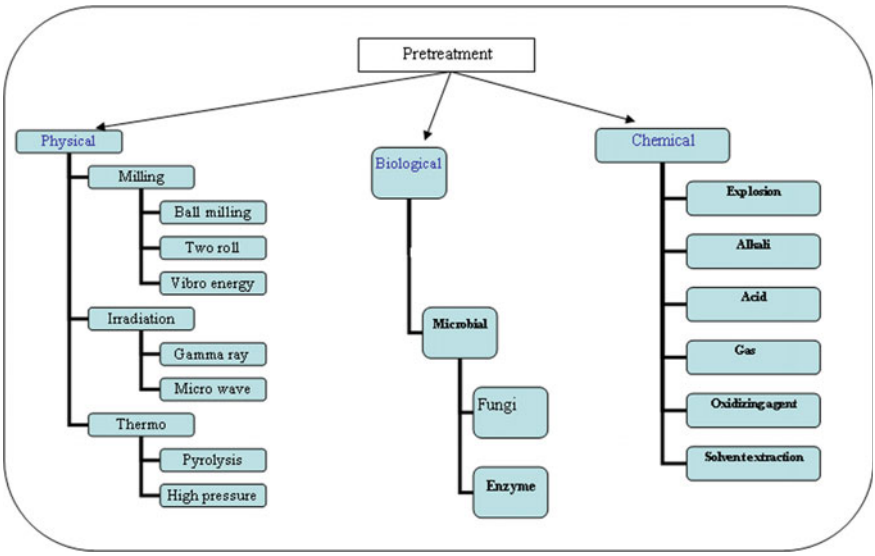

Fig. 1 Different methods of biomass pretreatment

Table 1 Comparison of different pretreatment processes

Pretreatment		Advantages	Disadvantages	References
Chemical pretreatment process	Dilute acid	Operation time is less, High yield of pentose sugar	Acid Recovery and formation of furfural	Chen et al. (2012a, b), Sindhu et al. (2011)
	AFEX	High yield of pentose sugar; No inhibitory compounds	Recovery of ammonia is not effective. Less effective process with increasing lignin content	Speers and Reguera (2012), Tao et al. (2011)
	Lime	No inhibitory compounds	Operation time is more	Jin et al. (2012), Sierra-Ramírez et al. (2011)
	Organosolvosis	High yield of pentose sugar	Solvent recovery is expensive	Zhao et al. (2009), Arato et al. (2005)
Physical pretreatment process	Milling	Operation time is less	The overall yield is poor. Energy requirement is high	Inoue et al. (2008), Craegveld et al. (2008)
	Irradiation	High yield of sugar; No inhibitory compounds	Need special design of equipment and process	Yong et al. (2011), Khan et al. (2006)
	High-pressure	No Inhibitory compounds	Maintaining high-pressure itself is a challenge	Valery et al. (2011)
Biological pretreatment process	Microorganisms	Low energy requirement No production of inhibitory compound Mild operation conditions	Rate of reaction is slow	Bak et al. (2010), Singh and Chen (2008)

utilization of the whole biomass, for example, by combustion, pyrolysis, liquefaction, or gasification. The other mode is known as "fractionation" in which the major components of the lignocellulosic biomass are separated into "fractions" made up of compounds with related properties, followed by separate processing of each fraction for specific purposes. It is expected that operation in biorefineries should follow the basic principles of green chemistry, for example, regarding an efficient utilization of raw materials and avoiding the utilization of wastes instead of performing end-of-pipe waste remediation, enabling the production of end products to fulfill societal needs, and shortening the dependence on fossil resources such as

coal, petroleum etc. In this way, a sustainable industrial and societal development can be achieved by achieving the needs of the present generation without compromising the needs of future generations.

2 Overview of Different Bonds in Lignocellulosic Material

Lignocellulosic biomass can be classified into (1) agricultural wastes that arise mainly from various agricultural cultivations and farming activities, (2) energy crops that are grown especially for biofuel or electricity production, and (3) forestry residues from forest logging areas. Lignocellulosic biomass is composed of different layers of plant cell wall. The chemical composition of the cell walls is dependent on the species of the plants. The principal components of lignocellulosic biomass material are described briefly in Fig. 2.

2.1 Cellulose

Cellulose is the β-1,4-polyacetal of cellobiose (4-O-β-D-glucopyranosyl-D-glucose). Cellulose is more commonly considered as a polymer of glucose because cellobiose consists of two molecules of glucose.

The polymer of cellulose is formed on the basis of two main linkages:

1. The glucosidic linkage is the one that forms the initial polymer chain. More specifically, it is a 1-4 β D-glucosidic bond that connects the glucose units together. The glucosidic bond can also be considered as an ether bond since it is, in fact, the connection of two carbon atoms with an elementary oxygen interfering (Solomon 1988).
2. The hydrogen bond is considered to be responsible for the crystalline fibrous structure of cellulose. The arrangement of the polymer in long straight parallel

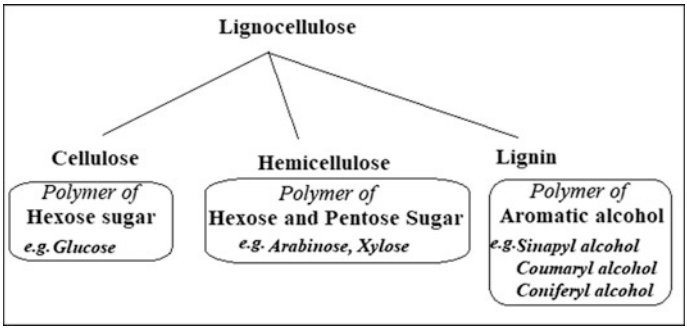

Fig. 2 Schematic diagram of principal components of lignocellulosic biomass

chains together with the fact that the hydroxyl groups are evenly distributed on both sides of the glucose monomer (see 0 and 0), allow the formation of hydrogen bond between two hydroxyl groups of different polymer chains (Faulon et al. 1994). It has been identified that carboxyl groups are also present in cellulose in a fraction of 1 carboxyl per 100 or 1000 monomer units of glucose (Krassig and Schurz 2002), although this does not appear obvious from the main structure of cellulose. As already mentioned, hemicellulose consists of polysaccharides other than cellulose. Its structure reveals that ether type of bonds, such as the fructosic and glucosidic bonds, is the main one that forms its molecule. The main difference with cellulose is that the hydrogen bonds are absent and that there is a significant amount of carboxyl groups. The carboxyl groups can be present as carboxyl or as esters or even as salts in the molecule (Kirk-Otmer 2001).

2.2 Hemicellulose

The most common type of polymers that belongs to the hemicellulose family of polysaccharides is xylan. The molecule of a xylan involves 1→4 linkages of xylopyranosyl units with α-(4-O)-methyl-D-glucuronopyranosyl units attached to anhydroxylose units. The result is a branched polymer chain that is mainly composed of five carbon sugar monomers, xylose, and to a lesser extent six carbon sugar monomers such as glucose.

2.3 Lignin

Lignin is a three-dimensional polymer of phenyl-propanoid units and amorphous phenolic macromolecule that can be found only in the secondary cell wall. It functions as glue, which provides compressive strength to the plant tissue and individual fibers into it. It also imparts stiffness to the cell wall and resistance against insects and pathogens.

Three primary units, namely, guaiacyl (G), sinapyl (S), and p-hydroxyphenyl (H) are the structural unit of lignin. The high level of heterogeneity of lignin is synthesized through the oxidative coupling of 4-hydrophenylpropanoids during secondary cell wall deposition, which results in layers of lignin in different monomers.

There are four main types of bonds identified in the lignocellulose complex. Those are ether type of bonds, ester bonds, carbon-to-carbon bonds, and hydrogen bonds. These four bonds are the main types of bonds that provide linkages within the individual components of lignocellulose (intra-polymer linkages), and connect the different components to form the complex (inter-polymer linkages). The position and bonding function of the latter linkages are summarized as follows.

2.3.1 Ester Bond Linkages

Ester bonds are identified between lignin and polysaccharides as well as within the hemicellulose polymer. In the latter case, it is the acetyl group that forms ester bond with a hydroxyl of the main chain of the polysaccharides. However, with respect to the linkage of lignin with polysaccharides, there is no definite conclusion whether the ester bond lies between lignin and cellulose or lignin and hemicellulose, or between lignin and both cellulose and hemicellulose.

2.3.2 Intra Polymer Linkages

The main types of bonds that connect the building molecules within the lignin polymer are ether bonds and carbon-carbon bonds (Table 2). Ether bonds may appear between allylic and aryl carbon atoms, or between aryl and aryl carbon atoms, or even between two allylic carbon atoms. The total fraction of ether type bonds in the lignin molecule is around 70% of the total bonds between the monomer units. The carbon-to-carbon linkages form the remaining 30% of the total bonds between the units. They can also appear between two aryl carbon atoms or two allylic carbon atoms, or between one aryl and one allylic carbon atom (Kirk-Otmer 2001).

In order to determine the linkages that connect the different polymers of the lignocellulose complex, lignocellulose is broken down and the individual components are separated. However, their separation is commonly achieved by methods that result in alteration of their original structure. As a consequence, the conclusions on the connecting linkages between the polymers are not definite. However, it has been identified that there are hydrogen bonds connecting lignin with cellulose and with hemicellulose, respectively. Furthermore, the existence of covalent bonds between lignin and polysaccharides are identified. More specifically, it is certain that hemicellulose connects to lignin via ester bonds. It is also known that there are ether bonds between lignin and the polysaccharides. It is still not clear though whether the ether bonds are formed between lignin and cellulose, or hemicellulose. Hydrogen bonding between hemicellulose and cellulose is also identified. However, this linkage is not expected to be strong due to the fact that hemicellulose lacks primary alcohol functional group external to the pyranoside ring (Faulon et al. 1994).

From the above discussion on molecular arrangement of lignocellulosic biomass, it is envisaged the need of pretreatment, although it is the most energy-expensive process hence, cost-effective pretreatment of lignocellulosic biomass is a major challenge of lignocellulose-based bioenergy technology. The present discussion is an attempt to put an overview of both comparison as well as critical discussion on existing pretreatment technologies and upcoming promising pretreatment technologies over lignocellulosic biomass.

3 Different Pretreatment Process

3.1 Dilute Acid Pretreatment Process

Acid pretreatment method includes application of dilute or concentrated acids to the lignocellulosic biomass material, which facilitates breaking the rigid lignocellulosic matrix. The most commonly used acid is dilute sulfuric acid (H_2SO_4). The dilute acid pretreatment method may be defined as exposure to high temperature (e.g., 180 °C) during a short period of time; or at a lower temperature (e.g., 120 °C) for longer retention time (30–90 min) to the lignocellulosic materials (Alvira et al. 2010). At present, the dilute sulfuric acid treatment is receiving substantial attention over concentrated sulfuric acid (Kobayashi et al. 2011). Recent articles have reviewed that the dilute sulfuric acid pretreatment process facilitates enzymatic hydrolysis of lignocellulosic biomass (Himmel et al. 2007; Kumar et al. 2009). Dilute sulfuric acid pretreatment process has been successfully applied with different types of lignocellulosic biomasses such as Switch grass (Digman et al. 2010), Corn stover (Xu et al. 2009), and Poplar (Wyman et al. 2009). Olive tree biomass was pretreated with 1.4% H_2SO_4 at 210 °C resulting in 76.5% of hydrolysis yields and cashew apple bagasse pretreated with diluted H_2SO_4 at 121 °C for 15 min yielded ethanol as high as 0.47 g g^{-1} glucose (Cara et al. 2008; Rocha et al. 2009). Dilute acid hydrolysis has many advantages compared with concentrated acids such as less corrosion, shorter reaction times, and higher reaction rate (Huang et al. 2009). Lignin is known as a nuisance material for ethanol makers as it retards enzymatic hydrolysis procedure. Beside lignin, the native crystalline structure (cellulose I) of cellulose is considered to be one of the major factors limiting its potential in terms of cost-competitive lignocellulosic biofuel production (Cheng et al. 2011). Xuebin et al. (2009) used Rapeseed straw as a lignocellulosic material which has 60% carbohydrate. A central composite design of response surface method was used to optimize H_2SO_4 catalyzed hydrothermal pretreatment of rapeseed straw, with respect to acid concentration (0.5–2%), treatment time (5–20 min), and solid content (10–20%) at 180 °C. Enzymatic hydrolysis and fermentation were also measured to evaluate the optimal pretreatment conditions for maximizing ethanol production. The results showed that acid concentration and treatment time was more significant than solid content for optimization of xylose release and cellulose recovery. Pretreatment with 1% sulfuric acid and 20% solid content for 10 min at 180 °C was found to be the most optimal condition for pretreatment of rapeseed straw for ethanol production. This study has demonstrated that hemicellulose in the rapeseed straw can be removed efficiently by H_2SO_4-catalyzed hydrothermal pretreatment at high solid loading (20%). The concentration of inhibitory product like furfural were reported less in this process (Xuebin et al. 2009).

Noureddini and Byun (2010) studied distiller's grain and corn fiber as the biomass for pretreatment process. The total carbohydrate content of distillers' grains and corn fiber were 57.7 ± 2.0 and 77.0 ± 1.0 wt%, respectively. In this

study, dilute sulfuric acid hydrolysis for the conversion of distillers' grains and corn fiber to monomer sugars and the formation of furfural was investigated. The extent of solubilization of biomass beyond monomer sugars was also monitored. Biomass loadings in the range of 5–20 wt% at 5% intervals, acid concentrations in the range of 0.5–1.5 vol% at 0.5% intervals, and temperatures of 120 and 140 °C were studied. The experimental results confirmed an increasing trend in the formation of monomeric sugars as a function of time. The highest yields of monomeric sugars were observed at the lower substrate loadings (5 and 10 wt%), higher concentrations of sulfuric acid (1.0 and 1.5 vol%), and when the temperature was 140 °C [Noureddini and Byun (2010)]. For the majority of the cases under consideration, the most effective period of hydrolysis appeared to be during the initial 20–30 min of the reaction. Formation of furfural during the course of hydrolysis was significantly lower at 120 °C and also lower for the distillers' grains samples compared with the corn fiber samples. The total amount of the solubilized matter during the hydrolysis was significantly higher than the amount of the monomeric sugars. Shi et al. investigated dilute sulfuric acid pretreatment of corn straw and rice straw, and enzymatic hydrolysis of cellulose (Shi et al. 2012). The straw was pretreated at 121 °C with different sulfuric acid concentrations (1, 2, 3, 4, and 5%, vol/vol.) and residence times (30, 60, and 90 min). Pretreatment residence time plays a key role in increasing the glucose concentration comparing to sulfuric acid concentration. Cellulose remaining in the pretreated feedstock was highly digestible by cellulase from *Trichoderma viride*. The result showed that the saccharification yield was 72.38 and 82.84% from corn straw and rice straw respectively. The pretreatment conditions were as follows: acid concentration 2% (vol/vol), temperature 121 °C, and time 60 min. To produce ethanol cost-effectively from herbaceous feedstocks such as corn stover, efficient xylan hydrolysis with a good amount of monomeric xylose yield is required. Dilute acid pretreatment is well-established as one of the pretreatment technologies for xylan hydrolysis; however, the accumulation of salts, production of toxic byproducts, and the release of acetic acid can inhibit enzymatic saccharification and fermentation, which leads to decrease the yield of ethanol. Successful removal of acetyl groups from native corn stover by alkali de-esterification could potentially increase the yield of monomeric xylose from pretreatment and enzymatic hydrolysis, improve cellulose digestibility, and reduce the cytotoxicity of the fermentation broth. Chen et al. (2012a, b), reported that the dilute acid pretreatment process removed a significant amount of acetyl groups from corn stover. Dilute acid pretreatment improved saccharification process of xylan and glucan hydrolysis by 15 and 30% of control samples. In whole slurry enzymatic hydrolysis, a 30% improvement in cellulose digestibility was obtained over the control (Chen et al. 2012a, b).

3.1.1 Theory of Acid Hydrolysis Process

The molecular mechanism of acid-catalyzed hydrolysis of cellulose (cleavage of β-1-4-glycosidic bond) follows the pattern outlined in Fig. 3. The cellulosic

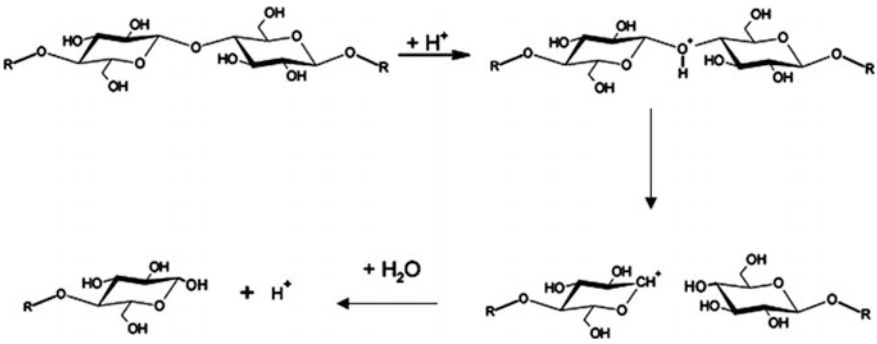

Fig. 3 Mechanism of acid hydrolysis of cellulose

substrate is made up of microcrystalline bundles attached to each other by para-crystalline regions and surrounded by a charged water boundary layer that results from dipole–dipole interactions caused by aligning of water molecules around the polar surface of the cellulose exterior (Roland 1976). Water molecules and H^+ ions (from acid) have to penetrate the cellulose fiber in order to precede the hydrolysis reaction effectively. Otherwise, hydrolysis takes place only on the surface of the cellulose. Acid catalyzes the breakdown of long cellulose chains to form shorter chain oligomers and then to sugar monomers that the acid can degrade. The hydrolysis of cellulose begins with the reaction of acidic proton and oxygen that bonds two glucose units, forming the corresponding conjugated acid. The cleavage of this C–O bond and breakdown of the conjugate acid to the cyclic carbonium ion then takes place, which adopts a half-chair conformation. Free sugar and a proton are liberated after rapid addition of water. The formation of the intermediate carbonium ion takes place more rapidly at the end than in the middle of the polysaccharide chain.

3.2 Pretreatment with Base

Different types of bases such as caustic soda, lime, ammonium hydroxide were also used as pretreatment agent. Alkali pretreatment process can be operated under mild conditions than other pretreatment process besides this most of the alkaline chemicals directly interact on lignin, therefore, the sugar components become unaffected which is the prime target for any biofuel production process. The aforementioned process thus makes alkaline pretreatment process among the front-runner of all other established pretreatment process. The major reactions during alkaline pretreatment process include dissociation of lignin from sugar molecules (cellulose and hemicellulose) by saponification of intermolecular ester bonds. Alkaline pretreatment preserves more amount of pentose sugar than other pretreatment processes which lead to more sugar yield.

3.3 Ultrasound Assisted Lime Pretreatment

Among the various existing pretreatment technologies, lime pretreatment has proven to be a useful pretreatment method because it demands less amount of energy and yields a good amount of fermentable sugar after enzymatic hydrolysis (Xu and Cheng 2011). Moreover, it is a less expensive and nonhazardous chemical agent (Chang et al. 1998). The beauty of lime pretreatment lies in the removal of lignin from biomass without any momentous loss of structural carbohydrate materials e.g., glucose, xylose, arabinose etc. It is also reported that alkaline pretreatments remove acetyl groups from hemicellulose, which improves saccharification by lowering steric hindrance of enzymes (Falls et al. 2011). The sole hitch of this pretreatment process is longer process operation time. Few researchers overcome this drawback by elevating the reaction temperature to curtail operational time (Chang et al. 2001). However, lime can be more effective as a pretreatment agent at a mild temperature as lime is more soluble at a lower temperature, which leads to more alkalinity and may lead to more efficient pretreatment of lignocellulosic materials (Xu et al. 2010).

3.3.1 Theory of Lime Pretreatment

The major effect of alkaline pretreatment is delignification. Alkaline pretreatments successfully increase the lignocelluloses digestibility without the production of furfural and methyl furfural (Harmsen et al. 2010). The alkaline material reacts with biomass in three modes namely,

1. Reaction with lignin
2. Neutralization of organic acids
3. Reaction with resins and waxes of biomass materials

Lignin can be described as three-dimensional macromolecules with high molecular weight in the range of 100 KD. It originates from phenyl-propanoid precursors such as coumaryl, coniferyl, and sinapyl alcohol C_6–C_3 and is present in vascular plants (Sun and Tomkinson 2002). Depolymerization of lignin using alkaline materials (e.g., lime) depends on the cleavage of two types of aryl ether bonds: $C_{aliphatic}$ $OC_{aromatic}$ and $C_{aromatic}$ $OC_{aromatic}$ (ordered from least to most stable). Oxidative agents prominently improve the effects of alkaline pretreatments. In alkaline media (pH > 12) oxygen is reduced through the reaction with phenolic hydroxyl groups to superoxide radical ($-O_2^{\cdot}$). Reactions involved in alkaline oxidative pretreatments are primarily single-electron (radical) reactions. Delignification reactions involve the formation of several different acids produced during the degradation of carbohydrates that introduce hydrophilic groups into the lignin structure (Klinke et al. 2002). Nucleophilic attack also occurs in some extent causing ring opening, which promotes further degradation and solubilization. The schematic diagram (Fig. 4) shows the typical delignification reactions (when OH^- anions involved).

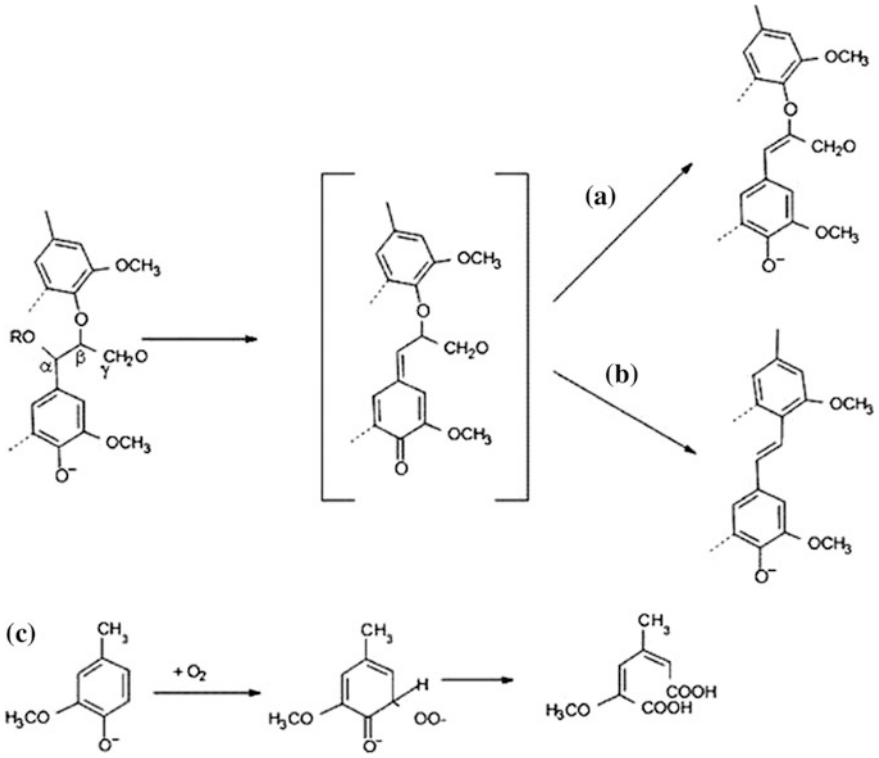

Fig. 4 Lignin degradation reactions in alkaline conditions involving α and β aryl ether linkages

In highly basic environment, the acidic proton of phenols (in lignin) gave phenolate ion. Then the phenolate ion eliminates the ether from benzylic position to produce an intermediate. This intermediate underwent two transformations either regain the aromaticity or produce highly conjugated arylvinyl-phenol after cleavage of CH_2O group (Fig. 3). In presence of molecular oxygen, phenolate moiety (Lignin) oxidized to 1,4-dicarboxylic acid which is soluble in basic solution (Fig. 3). Therefore, lime facilitates delignification steadily in presence of oxygen without any damage of the carbohydrates.

Rabelo et al. (2011) worked on pretreatment of sugarcane bagasse with lime (calcium hydroxide) followed by bioethanol production. Experiments were performed using the bagasse as it comes from an alcohol and sugar factory (non-screened bagasse) and bagasse in the size range from 0.248 to 1.397 mm (screened bagasse) (12–60 mesh). It was observed that the particle size presented influence in the release of fermentable sugars after enzymatic hydrolysis using low loading of cellulase and β-glucosidase (3.5 FPU g^{-1} dry pretreated biomass and 1.0 IU g^{-1} dry pretreated biomass, respectively) (Rabelo et al. 2009). Chang et al. (1998) used lime as a pretreatment agent to enhance the enzymatic digestibility of two common crop residues: bagasse and wheat straw. A systematic study of

pretreatment conditions suggested that for short pretreatment time (1–3 h), required high temperature (85–135 °C) to achieve high sugar yields, whereas, for long pretreatment times (e.g., 24 h), low temperatures (50–65 °C) were effective. The recommended lime loading was 0.1 g $Ca(OH)_2$ g^{-1} dry biomass. Water loading had little effect on the digestibility. Under the recommended conditions, the 3-d reducing sugar yield of the pretreated bagasse increased from 153 to 659 mg Eq. glucose g^{-1} dry biomass, and that of the pretreated wheat straw increased from 65 to 650 mg Eq. glucose g^{-1} dry biomass. A material balance study on bagasse showed that the biomass yield after lime pretreatment was 93.6%. No glucan or xylan was removed from bagasse by the pretreatment, whereas 14% of lignin became solubilized. A lime recovery study showed that 86% of added calcium was removed from the pretreated bagasse by 10 washings and could be recoveredReactions involved in alkaline by carbonating the wash water with CO_2 at pH 9.5 (Chang et al. 1998). Mass et al. (2008) reported an integrated pilot-scale process where lime-treated wheat straw with a high dry-matter content (around 35% by weight) is converted to ethanol via simultaneous saccharification and fermentation by commercial hydrolytic enzymes and bakers' yeast (*Saccharomyces cerevisiae*). After 53 h of incubation, an ethanol concentration of 21.4 g lit^{-1} was detected, corresponding to 48% glucan-to-ethanol conversion of the theoretical maximum. The xylan fraction remained mostly in the soluble oligomeric form (52%) in the fermentation broth (Mass et al. 2008). Cheng et al. (2010) used rice straw as a substrate for alkaline pretreatment. 5 g H_2O g^{-1} straw using sodium hydroxide (NaOH) and compared to pretreatment at 10 g H_2O g^{-1} straw by hydrated lime ($Ca(OH)_2$). The reaction temperature was held constant at 95 °C for lime pretreatment and 55 °C for NaOH pretreatment. The range of delignification was 13.1–27.0% for lime pretreatments and was 8.6–23.1% for NaOH. Treatment at a higher temperature also improved delignification; delignification with water alone ranged from 9.9 to 14.5% for pretreatment at 95 °C, but there was little effect observed at 55 °C. Post-pretreatment washing of biomass was not necessary for subsequent enzymatic hydrolysis. Maximum glucose yield was 176.3 mg g^{-1} dried biomass (48.5% conversion efficiency of total glucose) in lime-pretreated (unwashed biomass) and was 142.3 mg g^{-1} dried biomass (39.2% conversion efficiency of total glucose) in NaOH-pretreated (unwashed biomass) (Cheng et al. 2010).

The carbon-carbon bonds are stable under alkaline conditions, the cleavage of oxygen-carbon bonds are the most significant reaction in the pretreatment at the basic condition. This reaction will take place and is producing phenolic hydroxyl groups from the cleavage of the aryl-alkyl-ether bonds. Several researchers to explain the phenomena of delignification of lignocellulosic biomass materials (Kim and Holtzapple 2006) classified three different stages namely initial, bulk, and residual phases. During the initial delignification stage in alkaline pulping with sodium hydroxide, phenolic α-O-4-linkages in lignin and some non-phenolic ß-O-4-linkages are cleaved. In the bulk stage, the major reaction is the cleavage of non-phenolic ß-O-4-linkages and at the residual delignification stage, carbon–carbon linkages in lignin are cleaved and carbohydrates are degraded.

3.3.2 Theory of Ultrasonication

Ultrasound is sound with a sonic spectrum ranges between 20 and 10 MHz, therefore, its pitch is above human hearing as the human ear can detect up to 16 kHz only. In practice, three ranges of frequencies are reported for three distinct uses of ultrasound (i) high frequency, for diagnostic purposes (2–10 MHz), (ii) low frequency or conventional power ultrasound (20–100 kHz), and medium frequency, or "Sonochemical-effects" ultrasound (300–1000 kHz) (Ince et al. 2001). There are two theory exists to explain the phenomena happened during the ultra-sonication process. Hot spot theory postulates the formation of microbubbles at the site of nucleation when high-frequency sound waves (50–60 kHz) are applied to the liquid. The bubbles generate due to the decrease of boiling point caused by negative pressure and it collapses due to positive pressure created by the same wave. This alternating change of pressure is known as rarefaction. The positive pressure forced the bubble to implode which leads to the formation of local hot spot with temperature and pressure around 5000 K and 500 atm respectively (Thompson and Doraiswamy 1999). Therefore, huge pressure and temperature can be achieved which is required to break the polymeric biomass into their mother components without designing any high-pressure vessel. This method is not only cost-effective but also safe to use.

The electrical theory explained the above phenomena of breaking bonds by means of generating huge electrical charges. The electrical double layer can be formed in any liquid on the surface of the cavitation bubbles at the zone of cavitation. This process is independent of the method of creation of cavitation (Margulis and Margulis 2002).

Application of ultrasound technology exists in food processing technology for depolymerization of biopolymers, emulsification, tanning of vegetables etc. (Sun and Tomkinson 2002). Ultrasonic pretreatment of corn slurry was reported 20-fold reduction in corn size particle, which facilitated 30% more glucose yield at the time of enzymatic hydrolysis (Khanal et al. 2007). Cassava chips were pretreated using the ultrasonic unit, which had a maximum power output of 20 KW and frequency of 20 kHz (Nitayavardhana et al. 2008). Ultrasonic assisted (250 W, 30 min) hydrogen peroxide pretreatment method followed by biological treatment (*Pleurotus ostreatus*) was performed on rice hull yielded 31.8 and 32.2% more total sugar and glucose respectively than that of sole biological treatment (Yua et al. 2009). Considering all the inputs from the literature, it was decided to combine the lime and ultrasound technique for the pretreatment of biomass.

3.4 Ammonia-Based Pretreatment Process

Ammonia is a commodity chemical widely used in the chemical, pharmaceutical, and food industries. It is easily recoverable, noncorrosive, and nontoxic chemical agent and also inexpensive than other pretreatment chemical agent. Its industrial use

and recovery procedures are well-established. Ammonia can be easily recovered and reused because of its high volatility, and it offers versatile processing options (Kim et al. 2013). Aqueous ammonia is used for pretreatment of biomass materials for delignification, without significantly affecting the building block carbohydrate contents in it. It is a very effective pretreatment method especially for substrates that have low lignin contents such as agricultural residues and herbaceous feedstock. Oil palm empty fruit bunches were pretreated by aqueous ammonia soaking for ethanol production was used by Jung et al. (2011). The main effects of ammonia pretreatment methods include removal or modification of lignin, an increase of surface area alone with pore size, and modification of cellulose and hemicellulose structures. In aqueous form, it causes swelling of the biomass, which brings significant morphological changes to the entire biomass morphology. It also affects the degree of crystallinity of the integral biomass, which paves the way to make entire biomass significantly amenable toward enzymatic hydrolysis.

Ammono-lysis is similar to liquid ammonia hydrolysis process in which ammonia ion plays the role of water molecules in hydrolysis process. The ammonia molecule is dissociated producing H^+ and NH_2 species, which then induce breakage of chemical bonds in sugar molecules and lignin. The presence of the lignin-carbohydrate complex in lignocellulosic biomass has been confirmed, and various chemical linkages have been identified between lignin and the carbohydrates present in biomass (Du et al. 2010). The major linkages in biomass include ether (R–O–R) and ester (R–CO–OR) bonds in LCCs, and ether (C–O–C) bonds in lignin polymers. Ammonia molecules promote very effective selective cleavage of these linkages, leading to lignin dissolution and eventually to lignin removal.

The AFEX (ammonia fiber explosion/expansion) process was introduced as a pretreatment method for lignocellulosic biomass, its effect is similar to that of liquid ammonia hydrolysis process except that it utilizes anhydrous liquid ammonia.

In this process, biomass is contacted with liquefied anhydrous ammonia at elevated temperature (60–120 °C). After operation, the pressure is released rapidly. Due to the expansion of the liquid ammonia trapped in the biomass, its structure is disrupted, which is a beneficial pretreatment effect. The ammonia used in the pretreatment is recovered at this stage in the form of a low-pressure gas and reused. The pretreatment conditions of AFEX are moderate for temperature and high for pressure. Because most of it is recovered, net consumption of ammonia is low. During AFEX, neither component dissolution nor weight loss of solids occurs.

AFEX pretreatment has increased glucan and xylan conversions and ethanol yields for a variety of feedstocks including switchgrass, corn stover, bagasse etc.

3.5 Application of Ionic Liquid as Pretreatment Agent

Ionic liquids or more specifically room temperature ionic liquids are the new upcoming potential pretreatment agent for lignocellulosic biomass. Ionic liquids can be defined as those chemical reagents which are liquid at room temperature.

The common characteristic among all the ionic liquids is they are comprised with inorganic anion and an organic cation. Due to their unique hereto-molecular structure, polarity, and low volatility (many ionic liquids can be distilled below 200–300 °C). The researcher claimed that ionic liquids can also act as a very good solvent for lignin and cellulose which facilitates the posttreatment process to obtain valuable chemicals as well as biofuel without acknowledging the harsh effect of traditional acid, alkali method.

Ionic liquids can be classified according to their cations: quaternary ammonium ILs, N-alkyl-pyridinium ILs, N-alkyl-isoquinolinium ILs, and imidazo-lium-based ILs. Liu et al. (2012). Ionic liquids can be used for a number of chemical or biochemical processes, such as process for the production of second-generation biofuels (such as bioethanol or biobutanol) or short-chain organic acids. In these kinds of bioprocesses, lignocellulosic materials are processed to hydrolyze polysaccharides (cellulose and/or hemicelluloses) into monomers i.e., sugars, which are fermented to yield the target products. Presence of strong intermolecular hydrogen bonding of cellulose, makes cellulose almost insoluble to any conventional solvents except carbon disulfide such as N-methyl morpholine-N-oxide etc., but using those abovementioned solvent have some practical usage problems for bulk usages such as toxicity, undesired side chain reaction etc., therefore those solvents are not used in biorefineries. The new researches emphasis on using ionic liquids as a solvent for lignocellulosic biomass. Employing suitable ionic liquids, cellulose can be easily separated which involves the interaction of hydroxyl groups in cellulose with both the cation and anion in IL. The oxygen atoms of OH groups and IL anions together act as strong electron donors (for example, halogen and pseudo-halogen ions) during the dissolution process of lignocellulosic biomass materials, whereas hydrogen atoms of hydroxyl groups and IL cations act as electron acceptors (Wang et al. 2012). The dissolved cellulose can be regenerated again by addition of antisolvents such as water, alcohols, ethers, or ketones. When an anti-solvent is added to the IL–cellulose system, the IL ions form hydrogen bonds with water molecules and are displaced into the aqueous phase, whereas cellulose (which previously interacted with IL) are expelled and reunites its intra- and intermolecular hydrogen bonds and is then precipitated from the mixture.

4 Conclusion

Pretreatment is an inevitable step for lignocellulosic biomass pretreatment irrespective of the final product i.e., bioethanol, biogas or biodiesel. As pretreatment is most energy consuming step research has been going on to low cost, low energy consuming process. The scope of pretreatment is vast and its application also varies depending on the application and quality of biomass. The pretreatment method for biogas production is not the same as the pretreatment process for biodiesel or bioethanol process. The very next step of pretreatment method is saccharification or enzymatic treatment process. Although enzymatic treatment processes are mild in

nature, cost of enzyme is huge. Therefore, recent research aims to reduce the application of enzyme in biomass-based industries. The various modes of pretreatments are described throughout the chapter which shows the recent trends toward pretreatment process.

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Operational Strategies for Enzymatic Hydrolysis in a Biorefinery

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Abstract The [second generation (2G)] biorefinery is gaining wide attention for the production of biofuels such as bioethanol and coproducts such as xylooligosaccharides using lignocellulosic materials as feedstock. However, enzymatic hydrolysis of pretreated lignocellulosic materials to produce fermentable sugars is a complex-step bioethanol production process. In addition, a bottleneck in lignocellulosic biomass conversion to bioethanol is the cost of these enzymes. Thus, one of the most important objectives and challenges in the production of 2G bioethanol is the development of cost-effective processes at large scale. This chapter gives an overview of enzymatic hydrolysis process, the effect of pretreatment on enzymatic hydrolysis, operational strategies, and reactor design and operation as well as the advances achieved in recent years.

Keywords Enzymatic hydrolysis · Biorefinery · Bioethanol · Biomass Lignocellulosic material · Hydrothermal pretreatment

List of Abbreviations

2G	Second generation
LCM	Lignocellulosic material
SHF	Separate hydrolysis and fermentation

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SSF	Simultaneous saccharification and fermentation
CBP	Consolidated bioprocessing
SSSF	Semi-simultaneous saccharification and fermentation
SSCF	Simultaneous saccharification and co-fermentation
HMF	Hydroxymethylfurfural
DP	Degree of polymerization
AFEX™	Ammonia fiber expansion
EGW	*Eucalyptus globulus* wood
DM	Dry matter
WIS	Water-insoluble solids
FPU	Filter paper unit
STR	Stirred-tank reactor
RFA	Renewable fuel association
IEA	International Energy Agency

1 Introduction

Like a classic petroleum refinery, a biorefinery will coproduce biofuels and high value-added chemicals. Cherubini (2010), Ruiz et al. (2013a), and Ruiz et al. (2013b) reported that the biorefinery philosophy encompasses a wide range of technologies able to separate biomass resources (agricultural residues, herbaceous, hardwood, cellulose wastes, corn, sugar cane, and micro- and macroalgae) into their main components (carbohydrates, proteins, extractives, etc.), which can then converted into high added-value compounds, biofuels and chemicals (Ruiz et al. 2013a). According to the definition of IEA Bioenergy "Task 42 Biorefining", biorefining is the sustainable processing of biomass into a spectrum of bio-based products and bioenergy, taking into consideration those bio-based products such as chemicals, materials, human food, and animal feed; and bioenergy such as fuels, power, and/or heat (www.iea-bioenergy.task42-biorefineries.com). An important biofuel obtained thorough in the biorefinery is bioethanol.

Bioethanol is an important fuel alternative for the replacement of gasoline, with global production of 24,570 millions of gallons approximately in 2014 an increase of 6% respect since 2013 (RFA 2015). Baeyens et al. (2015) identified three different generations of bioethanol: (1) first-generation bioethanol production from sugar-based materials or food crops (i.e., sugar cane, beetroot, wheat, barley, rice, sweet sorghum, and corn); (2) second-generation bioethanol production from cellulosic biomass (i.e., agricultural residues such as wheat straw, agave bagasse, sugar cane bagasse, wood waste, corn residues, etc.), and (3) third-generation bioethanol production from aquatic biomass (i.e., micro- and macroalgae); this latest

technology is still in development (Naik et al. 2010; Baeyens et al. 2015). Lignocellulosic materials (LCMs) represent a renewable biomass source that does not compete with food production of first-generation bioethanol (Ruiz et al. 2016; Romaní et al. 2014a). However, obstacles such as production costs, technology, and environmental problems must be overcome for the production of second-generation bioethanol. Wahlström and Suurnäkki (2015) and European Biofuels Technology Platform (www.biofuelstp.eu) identified several demonstration plants (e.g., Inbicon in Kalundborg-Denmark; Abengoa in Salamanca-Spain) and full-scale industrial plants (Chemtex in Crescentino-Italy; Abengoa in Hugoton Kansas-USA; Granbio in Alagoas-Brazil) for 2G bioethanol. In general terms, the process for the production of second-generation bioethanol includes five stages: (1) milling of raw material (size reduction), (2) pretreatment, (3) enzymatic hydrolysis (also called "saccharification"), (4) fermentation, and (5) product separation (distillation). Different operating strategies can be applied for stages 3 and 4: (1) separate hydrolysis and fermentation (SHF), (2) simultaneous saccharification and fermentation (SSF), (3) semi-simultaneous saccharification and fermentation (SSSF), (4) consolidated bioprocessing (CBP), and (5) simultaneous saccharification and co-fermentation (SSCF); these are summarized in Fig. 1a–e. In all these strategies, enzymes play an important role in the production of bioethanol (Geddes et al. 2011; Ruiz et al. 2012a; Gonçalves et al. 2014, 2016; Romaní et al. 2014b, 2016; Liguori et al. 2016). However, enzymatic hydrolysis is one of the most expensive operations, and costs must be lowered substantially to make the cost of cellulosic ethanol competitive with that of fossil fuels or corn ethanol. The cost of cellulase enzymes (2G) must be reduced to less than the current cost of amylases (1G) (Stephen et al. 2011). According to Chen and Qiu (2010), the cost of cellulase enzymes accounts for 30–50% of total costs and thus is the major barrier to low-cost cellulosic bioethanol. Geng et al. (2015) reported that enzymatic hydrolysis is a key process in the biorefinery as the production rate of sugars, conversion yield, and sugar concentration all critically affects the techno-economic feasibility of commercial operations. Macrelli et al. (2012) estimated the cost of 2G ethanol to be 0.97 US$/L and expected the cost to decrease to 0.78 UD$/L. Macrelli et al. (2012) suggested that the cost of ethanol must decrease to 50% of the current cost to be competitive with 1G ethanol and gasoline. Some suggest that this is possible by integrating 1G + 2G bioethanol operations the investment and risks associated with independent 1G or 2G plant (Fig. 2); integration could reduce the cost could to 0.40 UD$/L (Macrelli et al. 2012; Joelsson et al. 2016; Lennartsson et al. 2014). The cost reduction is due to a decrease in energy consumption, production of multiple products, and utilization of pentose (Dias et al. 2012).

The balance of this chapter will focus on enzymatic hydrolysis as a linchpin in the biorefinery.

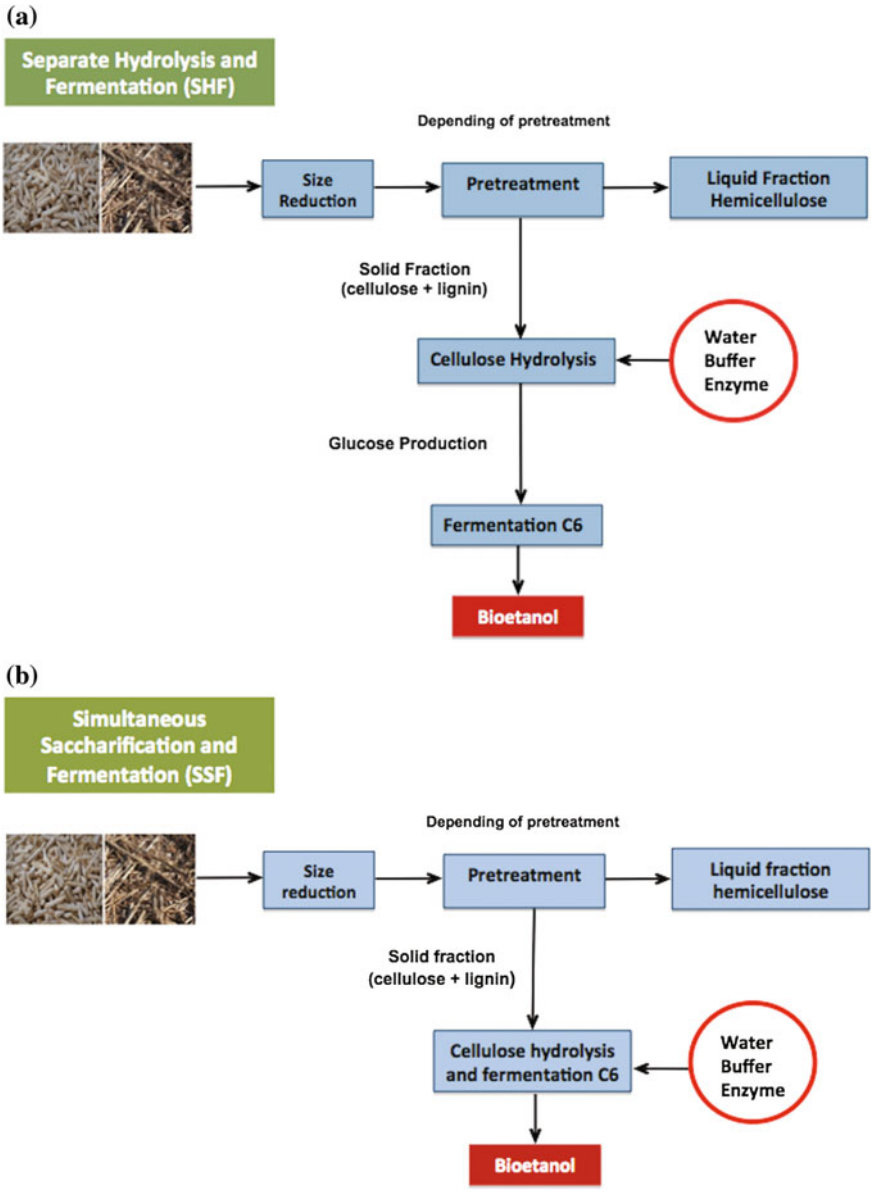
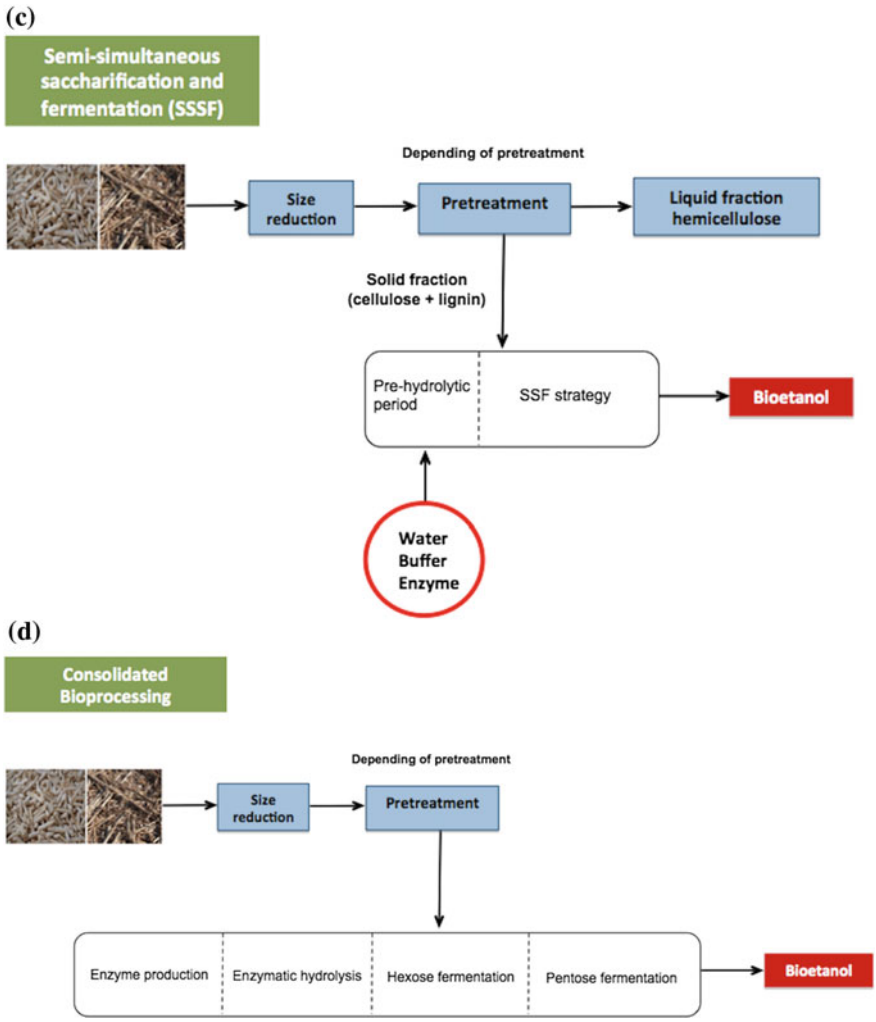


Fig. 1 Operating strategies for bioethanol production: **a** separate hydrolysis and fermentation (SHF), **b** simultaneous saccharification and fermentation (SSF), **c** semi-simultaneous saccharification and fermentation (SSSF), **d** consolidated bioprocessing (CBP), and **e** simultaneous saccharification and co-fermentation (SSCF)



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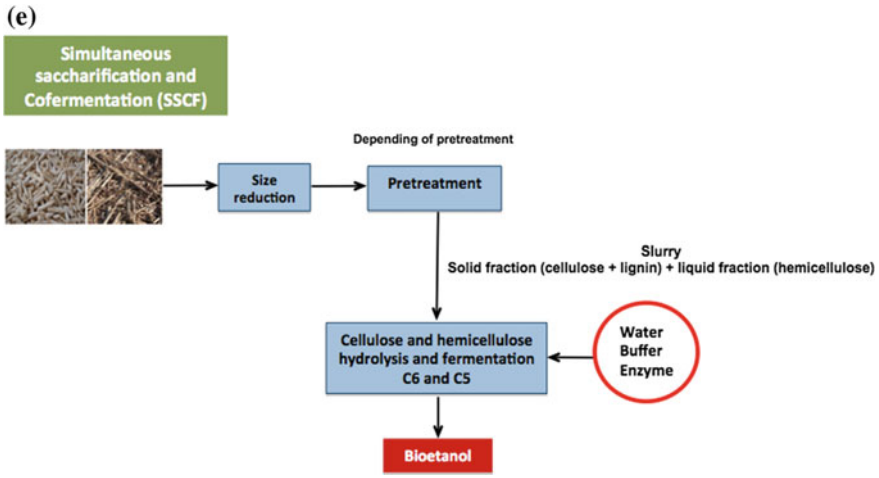

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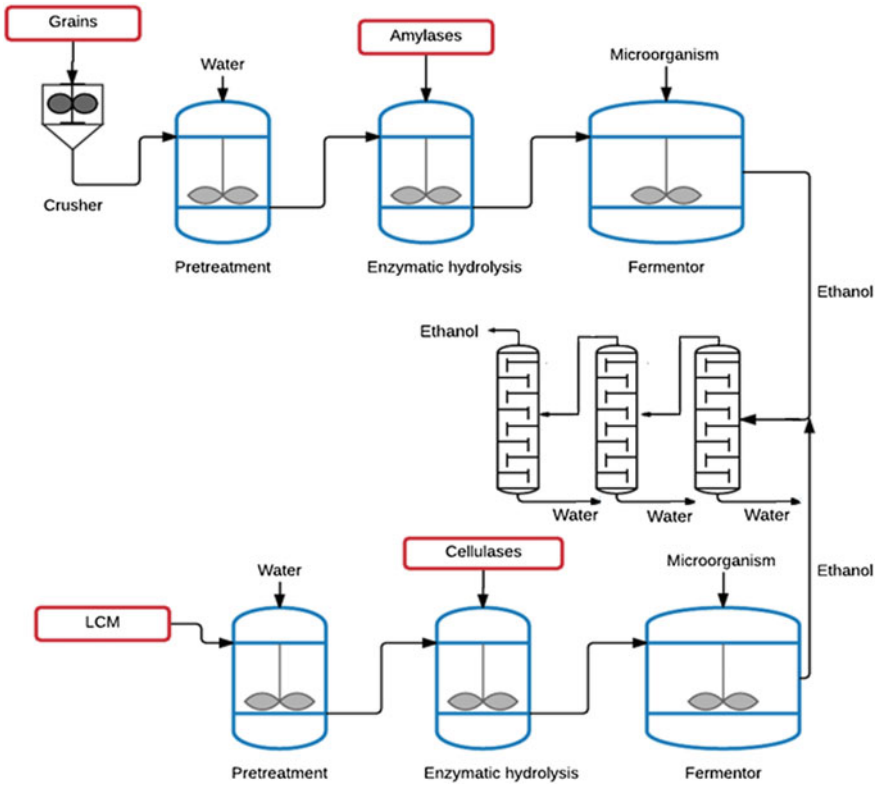

Fig. 2 A simplified scheme of a 1G + 2G biorefinery (Adapted and modified from Macrelli et al. 2012)

2 Hydrolysis of Cellulose

There are three typical technologies for hydrolyzing lignocelluloses into sugars: (1) catalytic (concentrated and dilute acid) hydrolysis at mild temperatures; (2) fast hydrolysis at high temperatures near critical point (350 °C); and (3) enzymatic hydrolysis, showing the advantages and disadvantages of these technologies (Fang 2015; Orozco et al. 2007). The use of acid to catalyze the hydrolysis of lignocellulosic biomass into sugar constituents is a well-known and effective strategy with a long history (Xiang et al. 2004).

Acids reactions can be divided into two types: concentrated and dilute acid hydrolysis. Dilute acid hydrolysis (0.5–3% w/v) requires high temperatures (200–400 °C) to destroy crystalline cellulose. Orozco et al. (2007) reported that the concentrated acid disrupts the hydrogen bonding in the cellulose chain producing an amorphous state. Cellulose depolymerization occurs at temperatures between 202 and 400 °C, producing glucose, and this is followed by the degradation of hexoses to compounds such as hydroxymethylfurfural (HMF) (Harris et al. 1985). Degradation products can inhibit fermentation.

2.1 Enzymatic Hydrolysis of Cellulose

Enzymatic hydrolysis of cellulose, the second stage of lignocellulosic bioethanol production is a heterogeneous reaction system in which cellulases in an aqueous environment react with the insoluble pretreated solids, particularly the structured cellulose, and might be the most complex step in the bioethanol production process due to the effects and interactions between enzyme and substrate (Binod et al. 2010; Ruiz et al. 2012b; Michelin et al. 2015). Figure 3 shows the scheme of enzymatic hydrolysis for bioethanol production using lignocellulosic biomass as feedstock. Enzymatic hydrolysis is carried out under mild conditions of temperature (40–50 °C) and pH (4.5–5.0) (Binod et al. 2010; Taherzadeh and Karimi 2007). Yennamalli et al. (2013) reported that when pH and temperature are not controlled, denaturation and subsequent loss of enzymatic activity occur. Enzymatic hydrolysis is an environmentally friendly process, as it avoids the production of large amounts of wastewater associated with the use of acid or alkaline hydrolysis, and it is not necessary to use corrosion-resistant equipment (Brummer et al. 2014).

Cellulolytic enzymes are divided into three the first classes. The first class is endo1,4-β-D-glucanases (EC 3.2.1.4), which hydrolyze internal β-1,4-glucosidic bonds randomly in the interior of cellulose to produce cellooligosaccharides. In a recent work, Yennamalli et al. (2013) reported that the thermostability of endoglucanases is an important characteristic in 2G bioethanol production. The next class is exo-1,4-β-D-glucanases or cellobiohydrolases (EC 3.2.1.91), which hydrolyze β-1,4-glycosidic bonds of cellulose chain and cleave off cellobiose units from chain ends. The final class is 1,4-β-D-glucosidases (EC 3.2.1.21), which

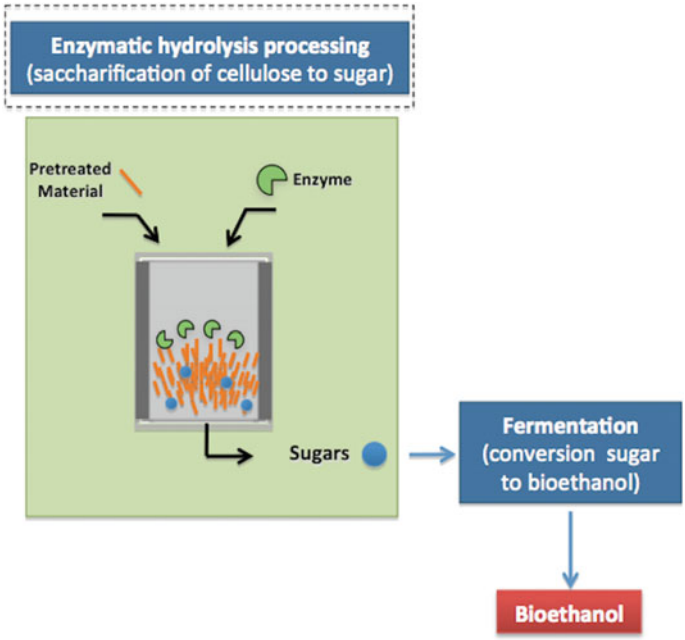

Fig. 3 Enzymatic hydrolysis for bioethanol production using lignocellulosic biomass as feedstock (Adapted and modified from Ruiz 2011)

hydrolyze cellobiose to glucose and cleave glucose units from cellooligosacharides (Ruiz et al. 2012b; Wulfhorst et al. 2015). These enzymes work synergistically to hydrolyze cellulose by creating new accessible sites for each other, removing obstacles and relieving product inhibition (Bansal et al. 2009; Hu et al. 2013; Dutta and Wu 2014).

These reactions can be affected by multiple obstacles such as (1) morphology of pretreated cellulosic residues; (2) fibril and fine structure of cellulose; (3) particle size, surface area, and pore size of cellulose; (4) crystallinity and degree of polymerization (DP) of cellulose; (5) mass transfer phenomena such as adsorption/desorption; (6) the use of high substrate concentrations; and (7) the presence of lignin and hemicellulose (Ramos et al. 1993; Chang and Holtzapple 2000; Öhgren et al. 2007; Iolevich and Morag 2011; Cateto et al. 2011; Khullar et al. 2013; Romaní et al. 2014b; Karimi and Taherzadeh 2016).

Yeh et al. (2010) studied the effect of particle size reduction of microcrystalline cotton cellulose on hydrolysis rate using a media mill. The particle size of cellulose was reduced to submicron scale by media milling, and the crystallinity was also reduced; at this scale, a relatively high glucose yield of 60% was achieved. Cateto et al. (2011) concluded that the determination of cellulose crystallinity and DP contributed to the knowledge of enzyme action in the process of enzymatic hydrolysis, indicating the synergy action between exo- and endo-glucanases in the

enzymatic processing. Öhgren et al. (2007) and Liao et al. (2005) reported that the sequential pretreatment (acid-catalyzed steam followed by ethanol pulping) is an effective process for the extraction of hemicellulose and lignin. They observed an increase in the glucose yield and the effect of hemicellulose and lignin during the enzymatic hydrolysis.

2.1.1 Effect of Pretreatment on Substrate for Enzymatic Hydrolysis

Due to the robust structure of lignocellulosic materials (LCMs), a pretreatment is required to reduce biomass recalcitrance, which is reduced through structural alteration of the cell wall, disruption of the crystalline cellulose structure, increased porosity, and changing chemical composition. This also improves the accessibility of the cellulose component to the action of hydrolytic enzymes (Sun and Cheng 2002; Ruiz et al. 2012b; Pu et al. 2013; Perez-Pimienta et al. 2016).

According to Chandra et al. (2007), Ruiz et al. (2011), and Silveira et al. (2015), a pretreatment should have low capital investment and operating costs, minimize the use of energy, be applicable to a wide range of LCMs, improve the formation of sugars, avoid the degradation or loss of carbohydrates and the formation of inhibitory compounds, and enable the recovery of hemicellulose/lignin and other compounds for further conversion in the biorefinery. Ruiz et al. (2013a, 2015) reported that pretreatment plays an important role in the biorefinery, since it is pretreatment that facilitates the fractionation of LCMs into substrates suitable for conversion into biofuels and high added-value compounds.

Pretreatment can be divided into different categories: (1) physical: milling, grinding and irradiation, sonication, etc.; (2) chemical: dilute acid, alkaline, wet oxidation, organic solvents, ionic liquids, etc.; (3) physicochemical (a combination of both physical and chemical): hydrothermal processing (autohydrolysis) and the heating of this process can be performed by steam, fluidized sand baths, oil baths, electric heating jackets, and microwave radiation; and (4) biological (Ruiz et al. 2013a, 2015; Behera et al. 2014; Aguilar-Reynosa et al. 2017). These pretreatments have been reviewed previously, with operational and economical advantages and disadvantages (Alvira et al. 2010; Conde-Mejía et al. 2012; Galbe and Zacchi 2012; Chaturvedi and Verma 2013; Zheng et al. 2014; Michelin et al. 2015). Table 1 shows the enzymatic hydrolysis yield and the main characteristics of the different pretreatments.

This section gives a summary of the hydrothermal processing (autohydrolysis) as pretreatment with emphasis on the progress achieved in our group (www.biorefinerygroup.com) on enzymatic hydrolysis. Figure 4 shows a schematic of our work on integrated biorefineries using different lignocellulosic biomass as raw material; a variety of applications can be envisioned for the solid and liquid phases produced from hydrothermal pretreatment (Fig. 4). Hydrothermal pretreatment (autohydrolysis) is an environmentally friendly process in which LCM is treated with compressed hot water at high temperatures and pressures for hydrolysis, extraction, and structural modification (Fig. 5). It is based on the selective

Table 1 Enzymatic hydrolysis yield and the main characteristics of different pretreatments (adapted and modified from Limayem and Ricke 2012)

Raw material	Pretreatment	Characteristics	Conditions for enzymatic hydrolysis	Saccharification yield (glucose yield of the theoretical, %)	Author
Corn stover	Dilute acid (H_2SO_4, 0.75, v/v)	1. Does not require thermal energy 2. Effective hydrolysis of hemicellulose with high percent of sugars in the liquid fraction 3. Production of toxic compounds (furans) 4. Requires recovery steps	Cellulase 20 FPU/g dry pretreated solids at 50 °C and pH 4.8	91.42	Zhang et al. (2016a, b)
Wheat straw	Liquid hot water (hydrothermal/autohydrolysis)	1. Solubilization and depolymerization of hemicellulose 2. Do not use chemicals and generates no toxic inhibitors	Celluclast 1.5L and Novozyme 188 (β-glycosidase). Loading of cellulase 40 FPU/g cellulose and β-glucosidase 60 IU/g cellulose, at 50 °C and pH 4.8	90.88	Ruiz et al. (2012a)
Sugarcane bagasse (SB)	Lime (0.1 g $Ca(OH)_2$/g SB	1. High total sugar yield (pentose and hexose) 2. Effective for wood and agricultural residues 3. High pressure and temperature hamper chemical operations 4. Problems with scale-up	Accellerase-1500®, loading of cellulase 15 FPU/g substrate and 75 U/g substrate for β-glucosidase, at 50 °C and pH 4.8	70	Grimaldi et al. (2015)

(continued)

Table 1 (continued)

Raw material	Pretreatment	Characteristics	Conditions for enzymatic hydrolysis	Saccharification yield (glucose yield of the theoretical, %)	Author
Corn stover	Ammonia fiber expansion (AFEX)	1. Effective against the formation inhibitory compounds 2. Not suitable for materials with high lignin content 3. Ammonia recovery 4. No wastewater	Spezyme CP (cellulase), enzyme loading 15 FPU/g cellulase and Novozyme 188 (30 CBU/g cellulose), at 50 °C and pH 4.8	80–85	Bals et al. (2012)
Corn stover	Ammonia recycle percolation	1. Redistribution of lignin 2. Recycling of ammonia 3. Theoretical yield is attained	Spezyme CP (cellulase), enzyme loading 7.5, 15, and 60 FPU/g glucan and 30 CBU of Novozyme 188 (β-glycosidase) at 50 °C and pH 4.8	86–95	Kim et al. (2006)
Rice straw	Catalyzed steam explosion	1. Effective against agricultural residues and hardwood 2. Fractionation of hemicellulose 3. Not effective for softwood	Novozyme Cellic CTec2 (Cellulase), 15 FPU/g at 50 °C and pH 4.8	73	Chen et al. (2013)
Eucalyptus globulus wood	Organosolv	1. High yields with acid combination	Celluclast 1.5L and Novozyme 188 (β-glycosidase). Loading of cellulase 20 FPU/g substrate and	98	Romaní et al. (2013)

(continued)

Table 1 (continued)

Raw material	Pretreatment	Characteristics	Conditions for enzymatic hydrolysis	Saccharification yield (glucose yield of the theoretical, %)	Author
		2. Effective against hardwood and softwood 3. Low concentration of hemicellulosic sugars 4. Production of inhibitory compounds 5. High capital investment	β-glucosidase/cellulase ratio of 5 UI/FPU, at 50 °C and pH 4.85		
Switchgrass	Sulfite (SPORL) pretreatment	1. Effective for high lignin materials as softwood and hardwood 2. Highest pretreatment energy efficiency 3. Minimum production of inhibitory compounds 4. Cost-effective process	Commercial cellulase and β-glucosidase (Novozyme). Loading of cellulase 15 FPU/g cellulose and 30 CBU (β-glucosidase/g cellulose), at 50 °C and pH 4.8	83	Zhang et al. (2013)
Sugarcane bagasse	Ozonolysis pretreatment	1. Fractionation and extraction of lignin from a range of lignocellulosic biomass without production of inhibitory compounds 2. Expensive process	Enzymes NS50013 (cellulase complex) and NS50010 (β-glycosidase). Loading of cellulase 10 FPU/g cellulose and β-glucosidase 10 CBU/g, at 50 °C and pH 4.8	41.79	Travaini et al. (2013)

(continued)

Table 1 (continued)

Raw material	Pretreatment	Characteristics	Conditions for enzymatic hydrolysis	Saccharification yield (glucose yield of the theoretical, %)	Author
Wood pulp waste	Alkaline wet oxidation	1. The combination with oxygen, water, high temperature, and alkali reduces the inhibitory compounds 2. Delignification and solubilization of lignocellulosic material 3. Low hydrolysis of oligomers	Cellulase dosage 35 FPU/g, at 50 °C and pH 4.8	76	Ji et al. (2015)
Wheat straw	Biological pretreatment	1. Environmentally friendly process 2. Low use of energy and chemical 3. Slow conversion	Enzymes mixture of Celluclast 1.5L, NS50013 (cellulase complex), NS50010 (β-glycosidase), and NS50030 (xylanase). Loading of cellulase (15 FPU/g cellulose, 15 IU of xylanase and β-xylosidases/g hemicellulose CBU/g, pH 4.8)	84	López-Abelairas et al. (2013)

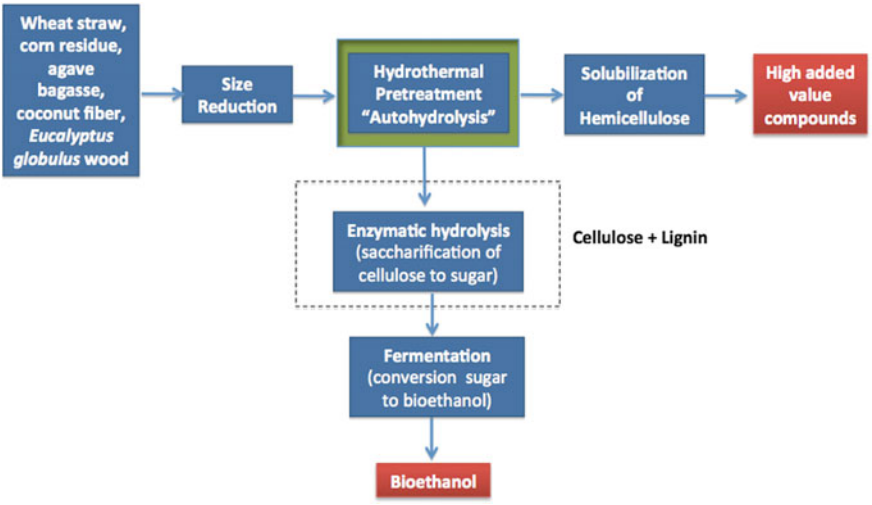

Fig. 4 Scheme of our work according to integrated biorefineries using different lignocellulosic biomass as raw material for bioethanol production (Adapted and modified from Ruiz 2011)

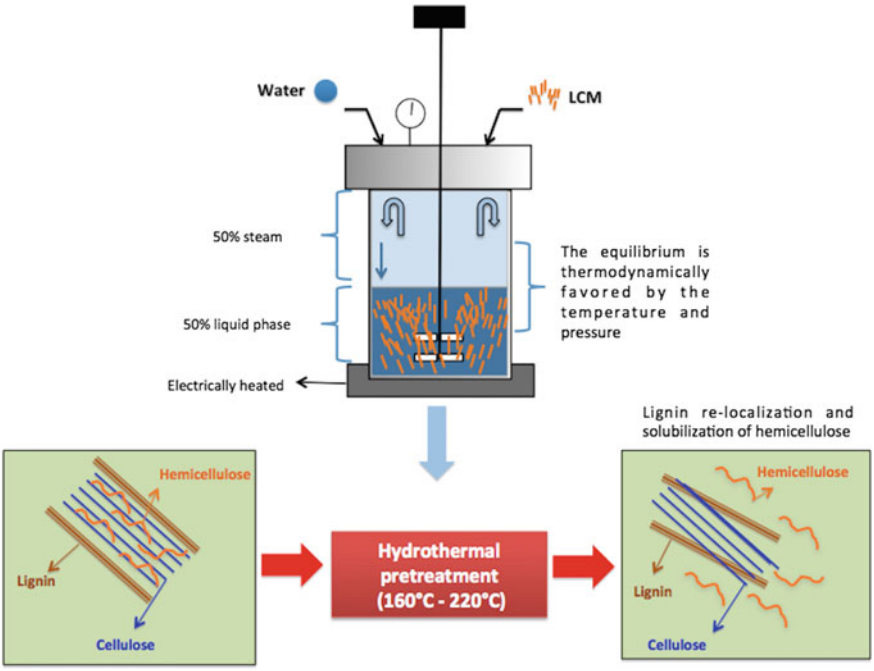

Fig. 5 Hydrothermal pretreatment (autohydrolysis) for lignocellulosic material (Adapted and modified from Ruiz 2011)

solubilization and depolymerization of hemicellulose, which is catalyzed by hydronium ions generated in situ by water autoionization and by acetic acid from acetyl groups (Ruiz et al. 2013a).

Under the biorefinery concept, it is important to obtain products with high added value in order to make the production of bioethanol an economically viable process. One of the main components of lignocellulosic materials is hemicellulose being the second largest polymer present in nature preceded by cellulose. Unlike cellulose, hemicellulose is a heterogeneous polymer composed by pentoses and hexoses (Saha 2003). Hemicellulose compounds can be obtained as oligomer by hydrothermal pretreatment and monomer (xylose) using enzymatic hydrolysis with xylanase enzymes. Xylose can be used in various chemical and biochemical processes for food and pharmaceutical application and converted into xylitol from lignocellulosic materials converting hemicellulose hydrolysates (Gong et al. 2015). Moreover, it is also possible for certain microorganisms to metabolize directly the hemicellulose hydrolysates, reducing steps and costs for the production processes of xylitol (Li et al. 2015; Zhang et al. 2016a, b).

A promising approach for using LCMs is enzymatic hydrolysis of cellulose after hydrothermal pretreatment. Li et al. (2014) reported that lignin acts as a barrier, encapsulating and confining cellulose, negatively impacting the enzymatic hydrolysis process. However, hydrothermal pretreatment causes re-localization of lignin to surface of pretreated solids, thus increasing the accessibility of cellulose (Kristensen et al. 2008). Araya et al. (2015) concluded that during hydrothermal pretreatment, lignin is deposited as droplets on the surface of the cell wall. Pu et al. (2013) reviewed the molecular basis of biomass recalcitrance using hydrothermal pretreatment and suggested that the re-localization of lignin during hydrothermal pretreatment is critical as delignification improved enzymatic hydrolysis of pretreated solids.

Perez-Pimienta et al. (2016) recently compared three pretreatments (AFEX^{TM}, hydrothermal process "autohydrolysis", and ionic liquid) of agave bagasse and concluded that all three pretreatments improved enzymatic hydrolysis; the combined glucose–xylose yields were: 42.5, 39.7, and 26.9 kg per 100 kg of raw material after AFEX^{TM}, ionic liquid, and hydrothermal pretreatment, respectively. Romaní et al. (2014b) studied the effect of hydrothermal hydrolysate on enzymatic hydrolysis by using a hydrothermal pretreatment slurry (hydrolysate and "unwashed pretreated solid") of *Eucalyptus globulus wood* (EGW). They showed that hydrothermal pretreatment improves the enzymatic hydrolysis of unwashed pretreated EGW; however, when 100% hydrolysate was added to the enzymatic hydrolysis process, strong inhibition was reported. During hydrothermal pretreatment, hemicellulose is depolymerized into oligomers and monomers; these oligomers are re-deposited on the surface of the pretreated solid acting and inhibit enzymatic saccharification (Qing and Wyman 2011). Gonçalves et al. (2015) reported a conversion yield of 92% (of the theoretical maximum) after enzymatic hydrolysis of hydrothermally pretreated green coconut shell. Romaní et al. (2014b) optimized the enzymatic hydrolysis of EGW subjected to hydrothermal pretreatment at 201 °C (non-isothermal regimen). Optimal conditions were as follows:

6.5 g/g (liquid to solid ratio), 22.5 FPU/g substrate (cellulase to substrate ratio), and 500 UI (hemicellulase to substrate ratio), obtaining 85.5 g/L of glucose (representing 81.5% of the theoretical maximum). Ruiz et al. (2012b) studied the reaction rate of wheat straw pretreated by hydrothermal processing and organosolv; they reported an initial saccharification rate of 0.47, 0.34, and 0.16 g/(L h) for hydrothermal, organosolv pretreated solids, and untreated wheat straw, respectively.

3 Operational Strategies of Enzymatic Hydrolysis

The enzymatic hydrolysis process has been developed and implemented using different operational strategies and reactor configurations in order to increase the conversion yield (theoretical maximum) and sugar concentration. Past strategies include high-solid loading in batch mode, fed-batch, immobilization of enzymes, membranes, and surfactants, supplementation with hemicellulase, and enzyme recycling.

Cost reduction of 2G bioethanol production requires process intensification through the use of high-solid loadings (15% $\geq$ solids, dry matter; DM). High-solid enzymatic hydrolysis is a complex heterogeneous biphasic (liquid–solid) process carried out with no significant amount of free water present (near-absence of visible liquid water between the biomass particles) (Kristensen et al. 2009; Geng et al. 2015). According to Kristensen et al. (2009) and Modenbach and Nokes (2013), the main advantage of high-solid enzymatic hydrolysis is the high final sugar concentration resulting in high bioethanol concentrations, which reduce separation and purification processing costs, particularly the energy required for distillation. Larsen et al. (2008) mentioned that for an economically viable industrial-scale ethanol distillation process, the starting ethanol concentration should be greater than 4% (w/w). However, some disadvantages of using high-solid loading for enzymatic hydrolysis are as follows: (1) the final sugar yield decreases with increased solid loading, (2) high power consumption and poor mixing due to the viscosity of biomass, (3) adsorption of cellulases on the biomass at high-solid concentrations, and (4) cellobiose inhibition of cellulase (Knutsen and Liberatore 2009; Kristensen et al. 2009; Wang et al. 2011; Modenbach and Nokes 2013). Dunaway et al. (2010) studied the viscosity throughout the course of enzymatic hydrolysis at different high-solid loadings (10–25% w/v) of pretreated corn stover. They concluded that viscosity changes occurred in two phases.

The determination of enzymatic hydrolysis yield is important when high-solid loading is used. Zhu et al. (2011) proposed a mathematical model for calculating sugar yields using high-solid enzymatic hydrolysis. Calculating the sugars yields is challenging due to variation in the liquid density and liquid volume as a result of solid solubilization; therefore, the information needed for sugar yield calculation are as follows: (1) composition of substrate, (2) initial solid loading, (3) initial liquid density, and (4) sugar concentration before and after enzymatic hydrolysis process.

The maximum error in determining the yield of sugar was less than 4%, using Zhu et al. (2011) model.

Lu et al. (2010) studied the influence of high-solid concentration from 10 to 30% (w/w) on enzymatic hydrolysis using corn stover as raw material and steam explosion as pretreatment. At 30% of water insoluble solids (WIS), the concentration of glucose was 103.3 g/L (corresponding to 72.5% cellulose conversion). Romaní et al. (2014b) reported on the effect of adding pretreatment hydrolysate during enzymatic hydrolysis at high-solid loadings using *Eucalyptus globulus wood* pretreated by hydrothermal processing under non-isothermal conditions at 210, 220, and 230 °C. They reported a maximum glucose concentration of 107.49 g/L (74.65% of conversion) without the addition of pretreatment hydrolysate at 25% WIS (4 g/g liquid/solid ratio) and 25 FPU/g of substrate while with the addition of pretreatment hydrolysate on the enzymatic hydrolysis, a maximum glucose concentration of 82.52 g/L of glucose (59.37% of conversion) was achieved at 25% WIS and 16 FPU/g of substrate. They concluded that the pretreatment hydrolysate strongly inhibits enzymatic hydrolysis at high-solid loadings.

Silva et al. (2016) mentioned that an important aspect in the enzymatic hydrolysis is liquefaction; this is to reduce the energy cost required for agitation in systems with high-solid loading. Szijártó et al. (2011) studied the liquefaction on enzyme hydrolysis using purified *Trichoderma reesei* enzymes, and they found that endoglucanases enzymes reduce the viscosity.

Another operational strategy for enzymatic hydrolysis is the enzymatic recirculation, which has emerged as a methodology that could favor the reduction of costs for the bioethanol production. Qi et al. (2011) conducted an investigation for the enzyme adsorption and recycling during enzymatic hydrolysis by performing two different pretreatments with dilute acid and dilute alkali. Recycling by ultrafiltration had the ability to retain β-glucosidase, while the best treatment for recycling enzymes was the alkali. They concluded that the amount of lignin present in the acid treatment affects the enzymatic hydrolysis, adsorption, and recycling of the cellulase. Other parameters to assess for recycling enzymes are the optimal conditions of enzyme desorption; Tu et al. (2009) used the organosolv as pretreatment evaluating different factors such as pH, temperature, ionic strength, and surfactant to maximize the recovery of enzymes and enzymatic activity of the residual substrates. The analysis showed that pH and temperature are the main factors operating in desorption of residual enzyme substrate, and the optimum conditions for pH and temperature were 5.3 and 44.4 °C, respectively. Rodrigues et al. (2015) conducted an enzymatic hydrolysis using Cellic® CTec2 and Celluclast mixed with Novozyme 188 where they employed alkali washes to recover the enzymes present in the residual solids resulting to be effective for the recovery of Celluclast enzyme processes; however, this is only possible with small amounts of cellulose, as for the desorption of Cellic® CTec2, enzyme complex is not affected by the amount of lignin and cellulose. Table 2 shows the enzymatic hydrolysis yields at high-solid loadings.

Another operational strategy to improve enzymatic hydrolysis is fed-batch strategy in which substrates and enzymes are added to the reactor in stages. Geng et al. (2015) used the fed-batch strategy on enzymatic hydrolysis process using

Table 2 Enzymatic hydrolysis at high-solid loadings using different pretreated substrates

Pretreated substrate	Temperature of enzymatic hydrolysis (°C)	Enzyme loading	Solid loading (%, water-insoluble solids)	Saccharification yield (glucose yield of the theoretical, %)	Reference
Agave bagasse (organosolv)	50	15 FPU and 30 CBU β-glucosidase/g substrate	30	91	Caspeta et al. (2014)
Corn stover (dilute acid)	50	5 FPU/g substrate	15	73.2	Geng et al. (2015)
Corn stover—whole slurry hydrolyzate (dilute sulfuric acid)	45	19.2 FPU/g cellulose	10	≅70%	Hodge et al. (2008)
Eastern redcedar (acid bisulfite)	50	46 FPU/g glucan	20	84	Ramachandriya et al. (2013)
Gleditsia saponin (alkaline peroxide)	50	30 FPU cellulase/g cellulose and 45 IU β-glucosidase/g cellulose	20	57.5	Xing et al. (2016)
Gleditsia saponin	50	30 FP/g cellulose	20	74.88	Xing et al. (2015)
Mango stem bark (alkaline pretreatment)	50	15 FPU/g substrate and 30 IU β-glucosidase/g substrate	21	56.3	Nieves et al. (2016)
Olive tree (hydrothermal pretreatment)	50	15 FPU and 35IUU β-glucosidase/g of substrate	20	64	Cara et al. (2007)

pretreated corn stover as substrate and archived a conversion (cellulose to glucose) of 76.8% at 15% WIS. Yang et al. (2010) reported the final glucose concentration of 220 g/L (the final conversion was 60%) from pretreated corn stover using the fed-batch strategy at 12% (w/v) solids loading and 20 FPU/g pretreated solids. Gupta et al. (2012) performed a kinetic study of batch and fed-batch enzymatic hydrolysis at 20% (w/v) of solid loading. Under batch and fed-batch mode, the maximum glucose concentrations ware 80.78 (approx. 40% of conversion) and 127 g/L (65% of conversion), respectively. They concluded that fed-batch mode is an effective strategy for increasing the sugar concentration compared with batch mode.

Cellulase recycling is another promising strategy to reduce enzyme cost (Rodrigues et al. 2014). In a recent work, Haven et al. (2015) studied continuous enzyme recycling (at the demonstration scale with high dry matter content and low enzyme loading for eight days), Inbicon demonstration plant. They were able to reduce the enzyme addition in the fermentation stage.

3.1 Reactor Design and Operation for Enzymatic Hydrolysis

As described in Sect. 2.1.1, a number of factors affect the enzymatic hydrolysis, and all these factors also affect the reactor design and the control strategy. The batch stirred-tank reactor (STR) is most often employed in enzymatic hydrolysis (Fig. 6)

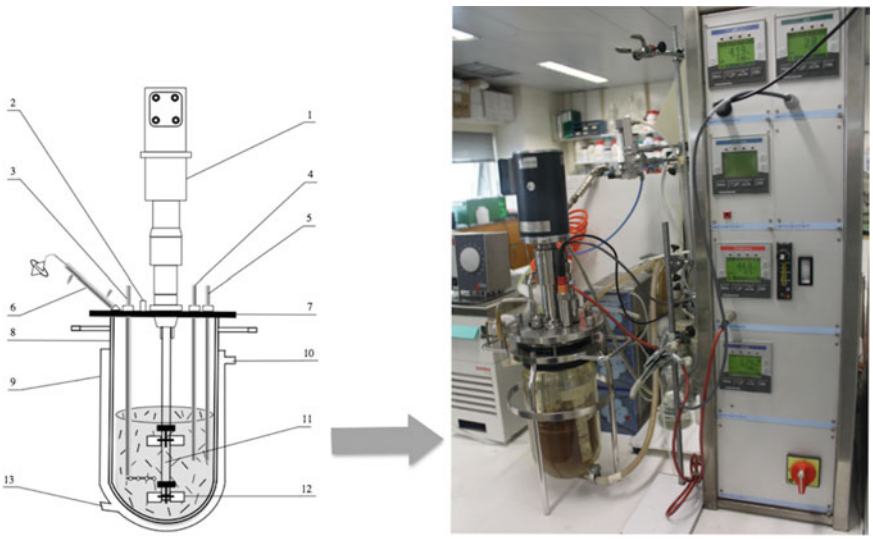

Fig. 6 Diagram of batch stirred-tank reactor (STR) for enzymatic hydrolysis process: (1) motor, (2) sample port, (3) gas disperser, (4) pH meter port, (5) thermometer port, (6) condenser, (7) lid, (8) tank wall, (9), water-bath jacket, (10) water-bath jacket outlet, (11) drive shaft, (12) Rushton flat blade impeller, and (13) water-bath inlet (Adapted and modified from Ruiz 2011)

(Ludwing et al. 2014). However, at high-solid loading, the initial viscosity of pretreated solids is too high and mixing is very difficult. Therefore, this type of reactor requires a large amount of energy (Du et al. 2014). Hodge et al. (2009) used an STR in fed-batch mode with final cumulative solids of 25% (w/w) and pretreated corn stover solids as substrate in the enzymatic hydrolysis. The final cellulose conversion was approximately 80% of the theoretical. Du et al. (2014) studied the effect of mass transfer on the enzymatic hydrolysis of pretreated corn stover at high-solid loading (2, 5, 10, and 20% (w/w)) and different stirring speeds (0, 40, 70, 100, and 130 r/min). With increasing solid concentration (5–15%, w/w), mass transfer limitations were evident. Palmqvist et al. (2011) reported that the impeller speed strongly affected the hydrolysis rate in an STR. Hodge et al. (2009) identified several types of reactor configuration for high-solid loading that reduces the effect of viscosity and mass transfer. Shake flasks, pilot-scale helical ribbon impeller, horizontally mounted paddle impeller, and vertically mounted paddle impeller.

Andrić et al. (2010) noted that a reactor membrane design can minimize product inhibition during enzymatic hydrolysis process by removing glucose, and thus increase the enzymatic hydrolysis reaction rate. Al-Zuhair et al. (2013) proposed a membrane reactor (125 m^3) for enzymatic hydrolysis process to enhance the reaction rate. They reported a conversion hydrolysis yield of 50% using carboxymethylcellulose as substrate.

4 Conclusions

The bioconversion of lignocellulosic biomass in the biorefinery concept is essential for a sustainable future and the production of biofuels such as bioethanol. However, the costs of enzymes and pretreatment are the major barriers to lower the costs in the production of 2G bioethanol. In general terms, this chapter was focused on reviewing the main aspects and strategies for enzymatic hydrolysis process. Depending on the operational conditions (temperature, substrate, pretreatment, enzyme loading, etc.) and strategy (batch, fed batch, high-solid loading, and reactor configuration), enzymatic hydrolysis yield varies. High-solid loading and fed-batch operation are interesting strategies for the production of high concentration sugars and thus higher bioethanol concentrations. Operational strategies are also needed to increase enzymatic hydrolysis yields and reduce the operating costs. Therefore, it is necessary to develop and improve the bioreactors for enzymatic hydrolysis.

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Prospects of Solvent Tolerance in Butanol Fermenting Bacteria

Shuvashish Behera, Nilesh Kumar Sharma and Sachin Kumar

Abstract Butanol tolerance is a critical factor affecting the ability of microorganisms to produce economically viable quantities of butanol through acetone-butanol-ethanol (ABE) fermentation using renewable feedstocks. However, ABE process has certain challenges like maintaining strict anaerobic conditions, slow growth rate of microorganisms, the rapid shift of pH, sensitivity to acetic acid, low butanol titer, solvent tolerance, and product inhibition. Separation of fermentation products through distillation, gas stripping, pervaporation, and adsorption also makes the process costly. Despite their importance at a biofuel platform, a limited number of butanol-tolerant bacteria have been identified so far. This problem can be eradicated through the isolation of solvent tolerating bacteria, development of bacteria through evolutionary engineering, mutation, and genetic engineering with promising product recovery techniques. In the present chapter, an overview of the butanol tolerating microbes, their solvent survival strategies, and the techniques to overcome the problem for a high concentration of butanol have been discussed.

1 Introduction

There is an engineering interest in the biological production of butanol from renewable resources for its application in transportation fuel (Durre 2007; Lee et al. 2008; Honig et al. 2014; Zhang et al. 2016; Hou et al. 2017). Butanol production

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through acetone-butanol-ethanol (ABE) fermentation using microbial species has regained much attention recently. Butanol has certain other applications as a solvent to produce antibiotics, vitamins, hormones, inorganic synthesis, chemical intermediate, processing of paint thinner, and hydraulic and brake fluids (Lee et al. 2008; Garcia et al. 2011; Lehmann and Lütke-Eversloh 2011; Abd-Alla and El-Enany 2012; Zheng et al. 2015).

One of the major bottlenecks of microbial processes is the high cost of the separation of the fermentation products. Product tolerance (especially of butanol) to the fermenting microbes limits its accumulation in the fermentation broth. Hence, butanol production possesses low yield, low titer, low productivity, and high recovery cost. The deleterious action of these solvents is due to its ability to accumulate in the cytoplasmic membrane, tampering with its structure and preventing the cell from performing essential functions, such as dissipation of pH and electrical potential, normal flow of ions, proteins, lipids and endogenous metabolites, and inhibiting membrane protein functions (Pinkart et al. 1996). Ultimately, these actions lead to cell lysis and death. Thus, this problem should be taken into consideration in order to obtain high-yield of butanol via ABE fermentation (Knoshaug and Zhang 2009; Mariano et al. 2011). The diverse degree of success has been made on improving the butanol tolerance level in different microorganisms through modern breeding techniques, mutation, and genetic engineering (Qureshi et al. 2007; Mann et al. 2012; Li et al. 2013; Zheng et al. 2015). However, efforts toward improvement of the tolerance of the strains to butanol using genetic techniques have resulted in limited success to date (Lopez-Contreras et al. 2010; Lutke-Eversloh and Bahl 2011).

The researchers have identified some microbial strains like *Pseudomonas aeruginosa* and *Bacillus cereus* which display outstanding tolerance to organic solvents because of intrinsic long-standing mechanisms and protect themselves from continuous exposure of solvents (Li et al. 1998; Fernandes et al. 2003). The evolution of solvent resistant strains is due to the presence of solvent in the natural growth environment, which propagates resistant microbial variants. The lower tolerance to organic solvents in case of gram-positive bacteria rather than gram-negative bacteria is due to the absence of an outer membrane (Vermue et al. 1993). The current chapter aims to discuss the prospects of butanol-tolerant microorganisms, their mechanism, and further application for the development of a process for the enhancement of high concentration of butanol production.

2 Butanol Production and Challenges

In recent years, new development and interests have been focused on fermentative production of butanol from renewable resources due to increasing environmental pollution and global petroleum shortage and prices (Jang et al. 2012; Jiang et al. 2014). Although, butanol has been known for a long time, being perhaps the oldest product obtained through traditional biotechnology, but the biotechnological processes are always in a hunt to find cheaper substrates or new technology in order to

lower the overall production cost of this bulk product (Kumar and Gayen 2011). There are several alternative cost-effective raw materials such as agricultural wastes and wood residues for an economic butanol production (Qureshi et al. 2010a, b). Acetone-butanol-ethanol (ABE) fermentation is the second largest industrial fermentation after yeast-based ethanol fermentation that is bacterial process to produce acetone, *n*-butanol, and ethanol from carbohydrates present in starch and lignocelluloses (Garcia et al. 2011; Lehmann and Lutke-Eversloh 2011; Abd-Alla and El-Enany 2012; Al-Shorgani et al. 2012; Qureshi et al. 2013; Li et al. 2014; Maiti et al. 2016).

Lignocellulosic biomass needs to be hydrolyzed prior to fermentation using a combination of pretreatment (acid, alkali, or ammonia explosion) and hydrolysis (enzymes: cellulase and xylanase) techniques. 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. Developing co-culture systems and improving cellulolytic and xylanolytic activities might be alternative approaches to utilize cellulose and hemicelluloses efficiently. Pretreatment and hydrolysis are generally performed in two separate reactors due to different and/or adverse conditions. Fermentation of sugars released after saccharification of pretreated biomass is carried out in a separate reactor using required microorganism (Qureshi and Blaschek 2005; Qureshi et al. 2007). The overall process for butanol production using lignocelluloses as the feedstock is shown in Fig. 1.

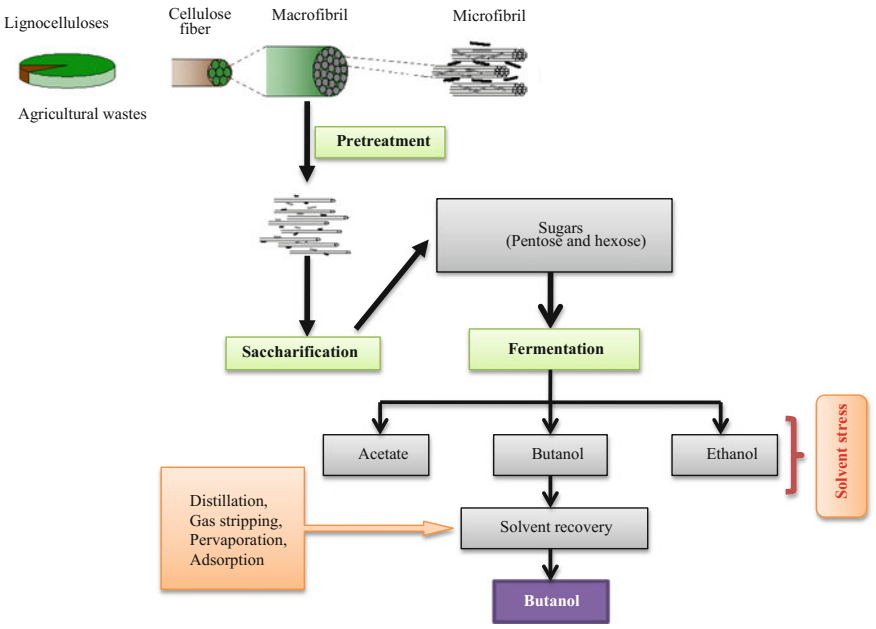

Fig. 1 Butanol production process from lignocellulosic feedstocks and its recovery

There are certain major challenges in butanol production, i.e., anaerobic nature, slow growth rate of microorganisms, availability of compatible feedstocks, rapid shifts of pH, sensitivity to acetic acid and butyric acid, low butanol titer, solvent tolerance, and product inhibition (Li et al. 2010; Liu and Qureshi 2009) (Fig. 2). Several methods like the selection of solvent tolerating bacteria, genetic engineering technique, advanced methods of product separation can be followed to overcome these challenges (Zheng et al. 2009; Fatehi 2013; Li et al. 2014; Cai et al. 2016). The poor butanol tolerance by *Clostridium* strains requires a complicated product removal and recovery process that is expensive to operate on a commercial scale (Ezeji et al. 2007; Merlet et al. 2017; Rochon et al. 2017). Moreover, as the concentration of the product (butanol) in the broth increases, the process encounters to the death of the fermenting microbes. Therefore, a cost-effective product separation method or use of solvent tolerant bacteria is required. In order to develop new tolerant strains, several methods have been studied at the cellular and molecular levels (Liu and Qureshi 2009; Wang et al. 2016; Tanaka et al. 2017). Durre (2011) compared the current technology with the traditional butanol production process

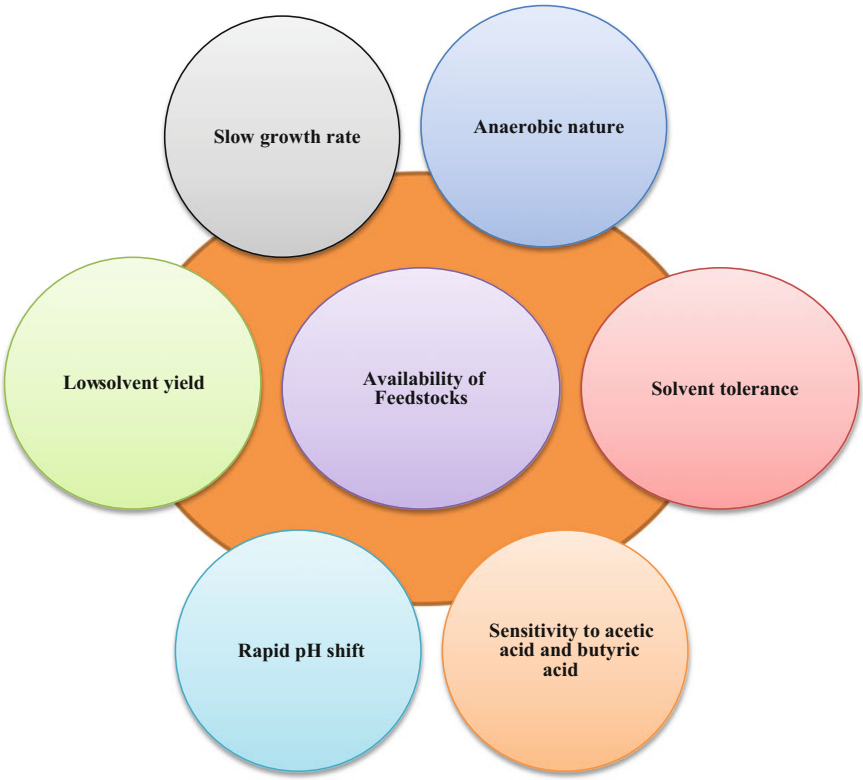

Fig. 2 Challenges in butanol production

and specified some disadvantages like low solvent yield (in the range of 2%), high energy requirement distillation process, the formation of numerous by-products, and culture degeneration.

3 Bacteria Possessing Solvent Survival Strategies

There are different bacterial strains for ABE fermentation, of which many belong to *Clostridium acetobutylicum*, *C. beijerinckii*, *C. saccharoperbutylacetonicum,* and *C. saccharobutylicum* which have been applied for industrial production (Qureshi et al. 2007; Ezeji et al. 2007). ABE fermentation pathway in *Clostridium* species is shown in Fig. 3. A number of different species of butanol-producing clostridia are currently recognized, which mainly differs on the type and ratio of the solvents produced. *C. beijerinckii* (*C. butylicum*) produces solvents in approximately the same ratio as *C. acetobutylicum*, but isopropanol is produced in place of acetone, while *C. aurantibutyricum* produces both acetone and isopropanol in addition to butanol (George and Chen 1983). *C. tetanomorphum* is a newly isolated species which produces almost equimolar amounts of butanol and ethanol but no other solvents (Gottwald et al. 1984). There are very few species which can tolerate up to 2% butanol concentration (Lin and Blaschek 1983). Several classes I, III, and IV genes/proteins have been recently identified in clostridia based on gene expression

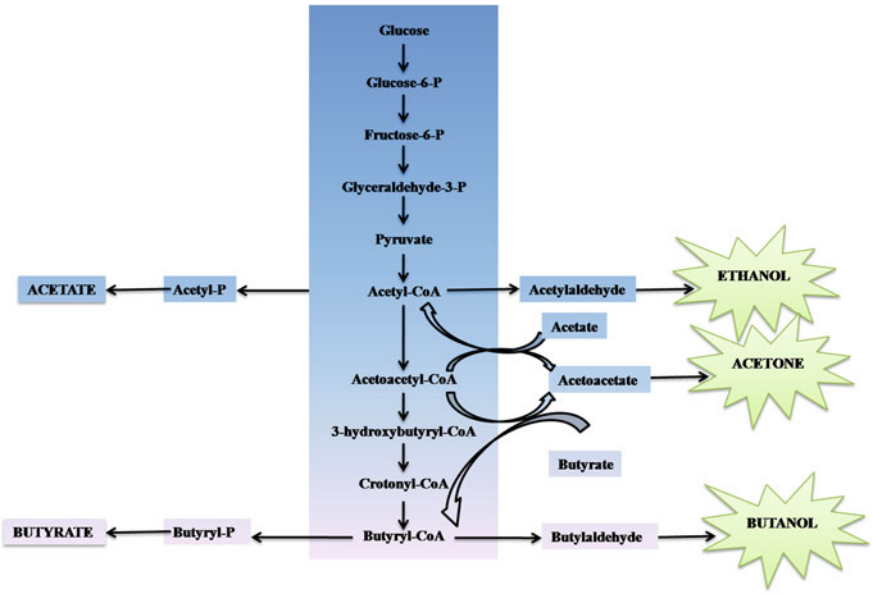

Fig. 3 ABE pathway depicting butanol production in *Clostridia*

patterns in response to chemical (butanol, butyrate, and acetate) stress (Alsaker et al. 2010).

Butanol, as an organic solvent, tends to partition into the cytoplasmic membranes and changes the membrane structures interfering with normal functions. *C. acetobutylicum* cells synthesize increased levels of saturated acyl chains accompanied by decreased unsaturated chains in the presence of butanol (Vollherbst-Schneck et al. 1984). In *Clostridia*, there is a relation between sporulation and solventogenesis. Spore formation starts with the concomitant of solventogenesis, as a result, cells enter a dominant state, where they cannot produce any solvent (Zheng et al. 2009). However, the combined effects of the regulatory genes for both the process of sporulation and solventogenesis are still not clear and needs further investigation to elucidate the relationship.

4 Strain Improvement

To solve the challenges in butanol production, an improved industrial strain with the capability of high butanol tolerance and high conversion efficiency of biomass to butanol is required. Moreover, high butanol titer is being focused through mutation and genetic modifications of *Clostridia* and *non-Clostridia* organisms (e.g., *Escherichia coli*, *Saccharomyces cerevisiae*) in both aerobic and anaerobic fermentation (Woods 1995; Kumar and Gayen 2011; Dunlop 2011; Dunlop et al. 2011; Lo et al. 2013).

4.1 Strain Improvement by Mutation

The isolation of mutant through the induction plays an important role in the selection and improvement of industrially important strains for butanol production. Selection of a competent mutagen is important for designing of mutation protocol. There are certain direct and indirect mutagens which play an important role in the desired microorganism. In direct mutation, mispairing mechanisms occurs, whereas, induction of a post-replication repair system occurs in indirect mutation. The selection procedure in conjunction with efficient mutagens is an important strategy in the mutation process. However, the approach of random mutation and selection is difficult for further improvement of cellular performance due to the complexity associated with the identifying modified gene (Jang et al. 2012). However, these strategies should be developed in near future.

Various methods of treatment for mutagenesis have been employed to increase butanol tolerance in its producers, especially in *C. acetobutylicum* and *C. beijerinckii* (Papoutsakis 2008). *C. beijerinckii* NCIMB 8052 was treated with a mutagen *N*-methyl-*N*9-nitro-*N*-nitrosoguanidine and selectively enriching the mutants on the non-metabolizable glucose analog 2-deoxyglucose, which generated the mutant

strain of *C. beijerinckii* BA101 (Formanek et al. 1997). *C. beijerinckii* BA101 could produce twofold more butanol and acetone than its parent strain and has distinct fermentation characteristics. Syed et al. (2008) developed a novel mutated strain (MEMS-7) through treating the parent organism with *N*-methyl-*N*-nitro-*N*-nitrosoguanidine and ethyl methane sulphonate followed by UV exposure. The developed strain showed 20% more butanol yield than the parent strain. Li et al. (2014) obtained a mutant strain *C. acetobutylicum* SE36 which could withstand 35 g/L of butanol as compared to wild-type strain (20 g/L). The maximum total solvent and butanol concentration were found to be 23.6 and 24.3% higher than that of wild-type strain. In another study, cis-vaccenic acid increased twofold due to mutation in β-ketoacyl-ACP synthases encoding gene *fabF* led to 1.5-fold increase in butanol tolerance (Jeong et al. 2012).

4.2 Strain Improvement by Genetic Engineering

Application of various novel genetic tools and genome sequencing of hyper-butanol-producing Clostridial organism has been proven to enhance the scope of genetic engineering for butanol production (Kumar and Gayen 2011; Zhu et al. 2011; Kim et al. 2013; Lee et al. 2013; Rao et al. 2016). Genetic engineering involves modification of only a limited number of genes. Identification of genes involved in butanol and acetone production in bacteria is the most important prior to genetic modification which can be obtained by metabolic engineering (Kim et al. 2013; Lee et al. 2013). Certain steps were undertaken through suppression of acetoacetate decarboxylase gene (*adc*) to stop acetone production which is a major fermentative product in ABE fermentation. This process enhanced the butanol production from 70 to 80%, while acetone production was reduced to 0.21 g/L (Jiang et al. 2009). The whole genome sequencing of important hyper-butanol-producing bacteria provides the scope of genetic engineering for enhancement of butanol production (Papoutsakis 2008; Kumar and Gayen 2011). Kataoka et al. (2011) engineered *B. subtilis* GRSW2-B1 for bioproduction of butanol. The gene expression could be effectively driven by several promoters with different levels, whereas the highest expression was observed with a xylose promoter. The constructed vector was stably maintained in the transformants, in the presence or absence of butanol stress. Adverse effect of efflux-mediated tetracycline resistance determinant (TetL) to bacterial organic-solvent tolerance property was unexpectedly observed.

Recombinant DNA technology (RDT) is another attractive genetic engineering tool in facilitating the solvent producing ability of microbial strains (Lo et al. 2013). However, this technology in non-Clostridial organisms was unable to improve the yields over native Clostridial bacteria (Huang et al. 2010). The native and genetically modified butanol-tolerant strains are shown in Tables 1 and 2. However, each organism responds differently to different environmental challenges. Developing a bacterial strain with the ability of solvent tolerance is an extensive problem for

Table 1 Native butanol-tolerant strains

Strain	Tolerance capacity (%)	Reference
C. beijerinckii P260	1.95	Qureshi et al. (2007)
C. beijerinckii BA101	1.96	Qureshi and Blaschek (1999)
C. saccharobutylicum Ox29a	0.92	Berezina et al. (2009)
C. saccharobutylicum Ox44a	0.97	Berezina et al. (2009)
Strain CM4A	3.5	Kanno et al. (2013)
Strain SK5A	3.0	Kanno et al. (2013)
Strain GK12	3.0	Kanno et al. (2013)

researchers. Tolerance could be developed through targeting either single gene or cluster of genes, which involved in various action including cell wall synthesis, membrane composition, energy metabolism, and influx/efflux control (Nicolaou et al. 2010; Dunlop 2011; Jin et al. 2014). Moreover, all these actions are independent of each other and their control mechanism also varies. Various energy-dependent transporters play a major role in tolerance of toxic compound (Jin et al. 2014). Likewise, the acridine resistance complex AcrAB also plays an important role in solvent tolerance. As reviewed by Nicolaou et al. (2010), expression of *acrAB* may be controlled by *marR*, *marA*, *soxR*, and *soxS* regulators. The production of AcaA and TolC increases through overexpression of *marA*, *robA,* and *soxS* regulator genes and resulted in cyclohexane tolerance in *E. coli*. Similarly, hexane and cyclohexane tolerance also increases due to overexpression of *man XYZ* operon (sugar transporter gene of the phosphotransferase system) in *E. coli.* (Okochi et al. 2007; Shah et al. 2013; Jin et al. 2014). Further, engineering in mar operon (consisting *marR*, *marA*, and *marB* genes) through mutation in *marR* gene (repressor of mar operon) also increases solvent tolerance (Doukyu et al. 2012; Jin et al. 2014). Similarly, SprABC efflux system in *P. putida* S12 helps in toluene tolerance, which is regulated by *srpR* and *srpS* genes (Kieboom et al. 1998; Nicolaou et al. 2010). Heat-shock proteins (HSPs) also plays an important role in survivability of microorganisms and get up-regulated under stress condition (Brynildsen and Liao 2009; Horinouchi et al. 2010; Rutherford et al. 2010; Jin et al. 2014). On the other hand, overexpression of *groESL* gene (encoding chaperon GroESL) in *C. acetobutylicum* increased growth on butanol stress along with 2.5-fold higher cell metabolism (Tomas et al. 2003; Nicolaou et al. 2010; Dong et al. 2011). Moreover, expression of hsp18 and hsp90 also increased the growth due to overexpression of *groESL* gene (Tomas et al. 2004; Nicolaou et al. 2010). Similarly, expression of *groESL* increased solvent tolerance in *Pseudomonas putida* (Volkers et al. 2006). Also, overexpression of *groESL* gene with its natural promoter in E. *coli* increased 12, 2.8, 3, 4-fold growth in 4% (v/v) ethanol, 0.75% (v/v) *n*-butanol, 1.25% (v/v) 2-butanol, and 20% (v/v) 1,2,4- butanetriol, respectively (Zingaro and Papoutsakis 2012; Jin et al. 2014). Moreover, 10 to 100-fold increased tolerance was observed for ethanol or butanol in *E. coli* when *irrE* gene (global regulator gene of *Deinococcus radiodurans*) was regulated (Chen et al. 2011, Jin et al. 2014). Further, expression of *hsp33* also regulated the solvent tolerance in *B.*

Table 2 Development of butanol-tolerant bacteria using metabolic engineering

Strain	Approach	Result	Reference
C. acetobutylicum ATCC 824	Screening of plasmid-based genomic DNA libraries and microarray analysis	Increased 81% butanol tolerance through serial enrichment	Borden and Papoutsakis (2007)
C. acetobutylicum ATCC 824	Overexpression of *groESL* in a plasmid under the thiolase promoter	Decreased inhibition by butanol, increased solvent titers produced and prolonged metabolism under butanol stress	Tomas et al. (2003)
E. coli JCL17	Alternative genes and competing pathway deletions were evaluated through engineering of the synthetic pathway in *E. coli* for 1-butanol production	Maximum production of 0.55 g/L of butanol using glucose as the substrate	Atsumi et al. (2008)
C. saccharoperbutylacetonicum	Mutation in the *rpsL* gene encoding ribosomal protein S12	Increased butanol production by 1.6-fold (16.5 g/L)	Tanaka et al. (2017)
Synechocystis sp. PCC 6803	Co-overexpression of *slr1037* and *sll0039* genes	Improvement in 133% butanol tolerance	Gao et al. (2017)
C. acetobutylicum ATCC824	Overexpression of g*roESL* and *dnaK* from the extremely radioresistant bacterium *Deinococcus wulumuqiensis* R12	Enhanced tolerance to butanol, furfural, oxidation, and acid in evolved strain 824 (*dnaK* R12), 824 (*groESL* R12) with 49.4%, and 28.7% higher butanol titer, respectively.	Liao et al. (2017)

(continued)

Table 2 (continued)

Strain	Approach	Result	Reference
C. acetobutylicum ATCC824	Overexpression of *groESL* from *C. acetobutylicum* ATCC824	Enhanced tolerance to butanol, furfural, oxidation, and acid in evolved strain 824 (*groESL*824) with 23% higher butanol titer.	Liao et al. (2017)

psychrosaccharolyticus (Kang et al. 2007). Further, in *E. coli*, butanol tolerance increased through overexpression of *focA* gene (Reyes et al. 2011; Jin et al. 2014). Ethanol resistance was increased by overexpression of *murEF* and *murB* genes of mur operon system (involved in cell wall synthesis) (Goodarzi et al. 2010; Nicolaou et al. 2010; Jin et al. 2014). Similarly, another gene imp, which is involved in cell wall synthesis, increased hexane tolerance in *E. coli*. (Jin et al. 2014). Proteomic changes due to butanol stress were studied in *Synechocystis* sp. PCC 6803 and reported 303 proteins regulated differentially out of 1452 total protein, which indicates induction HSPs, efflux system, and compositional changes in cell membrane rather than primary metabolism (Tian et al. 2012). Similarly, Zhu et al. (2013) studied transcriptomic and metabolomic analysis on *Synechocystis* sp. PCC 6803 during butanol exposure and revealed the involvement of genes in stress response including HSPs, oxidative stress-related proteins, and transporters. Moreover, the role of *sll0690* (probable transcription regulator), *slr0947* (OmpR-type DNA-binding response regulator) and *slr1295* (iron transport system substrate-binding protein) in butanol tolerance were also identified through gene knockout and omics analysis (Zhu et al. 2013).

4.3 Strain Improvement by Adaptation

Adaptation or evolutionary engineering has been proven a flawless approach to improve the microorganisms. In general, induction of particular gene occurs through the adaptation process (Behera et al. 2016; Sharma et al. 2016). Further, the adaptation or evolutionary engineering of bacterial cells is much more convenient through certain cycles to increase the solvent tolerant capacity (Kataoka et al. 2011; Liu et al. 2012). The adaptation mechanisms include changes in the content of membrane-embedded proteins, sterols, hopanoids, or carotenoids and changes in the phospholipid composition. The accumulation of solvents in the membrane

bilayer can influence both the membrane lipid-order and the bilayer stability. The permeability of the membrane as well as the activity of membrane-embedded enzymes can be affected by the changes in the physicochemical properties of the membrane (Weber and Bont 1996). These adaptation mechanisms will be considered in view of the effects of organic solvents on the properties and functioning of the membrane. Bacterial solvent tolerance can also be enhanced through modification of medium composition by supplementation of amino acids, sugar, and/or cell-energy-providing nutrients which increase cell-energy supply and thus, increase efflux-pump-dependent solvent tolerance (Ruhl et al. 2009; Wu et al. 2013).

Liu et al. (2012) isolated and found some strains like *Lactobacillus mucosae* strains BR0605-3, BR0605-B15, BR0713-18, BR0713-20, BR0713-30, and BR0713-33, *L. amylovorus* NE-L 0206-19, *Pediococcus parvulus* NE-L 0206-31, *L. crispatus* NE-L 0206-47, and *Weissella confusa* BR0216-18 capable of growth in 3–4% butanol after long-term adaptation. Liu et al. (2014) developed a novel approach called 1-butanol glycerol storage to enhance butanol tolerance and prevent productive degeneration in *C. acetobutylicum* during long-term preservation. Under this condition, *C. acetobutylicum* D64 primarily obtained the cell survival rate of 80% after 12 months. Unexpectedly, these cells still got an enhanced butanol tolerance of 32 g/L which was twofold higher than the wild-type with butanol tolerance of 16 g/L. However, all above studies provide crucial information for future research in the field of genetic engineering towards producing hyper-butanol synthesizing strain. The focus should be on developing more suitable genetic tools. Further research in lipid composition changes, as well as other possible adaptation mechanisms, is therefore required.

5 Future Prospects

In recent decades, the efforts have been made to develop a sustainable process for lignocelluloses based ABE production. Despite the present prospects, the uncertainty is still in the sustainability of lignocellulosic butanol production using solvent tolerant bacteria at industrial scale. Bacteria use a wide variety of mechanisms to tolerate in the presence of high concentrations of toxic organic chemicals, such as aromatic compounds, aliphatic alcohols, and solvents. However, enhancing solvent tolerance is an expensive process from an energetic point of view that involves a wide range of genetic and physiological changes to overcome the solvent damage. Reduced membrane permeabilization, and cell wall biosynthesis may result in dramatic tolerance improvements. Due to complex mechanisms involved in butanol induced stress response, butanol tolerance phenotype is difficult to engineer even in bacteria with well-defined genetic backgrounds. Furthermore, the gene improvements for the development of a single strain through genetic engineering may result in large tolerance improvements. Advances in biotechnology associated with cloning, expression, and mutagenesis as well as directed evolution contribute to

conferring different properties to microorganisms. Therefore, it is recommended to explore more genomic and proteomic information based on the designing of a genome-scale metabolic model, which may be followed by validation through experimental data of fermentation. Further, transcriptome and proteome data set can be built under various conditions, so that identification and targeting the gene in particular pathway can be done.

6 Conclusion

The present chapter describes several challenges in butanol production through overcome the problem of high concentration of solvent in the fermentation broth. Although, solvent tolerance of bacteria and the concomitant release of bio-products from lignocellulose have been greatly improved by new technologies, there are still challenges that need further investigations. These challenges include the development of more efficient pretreatment and production technologies, bioprospecting, and development of stable solvent tolerant, genetically engineered bacteria, improved gene cloning, and sequencing technologies and enhancement of productions based on economies of scale for more efficient and cost-effective conversions of lignocellulosic biomass into solvent production.

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Simultaneous Saccharification and Fermentation of Lignocellulosic Biomass

Avanthi Althuri, Anjani Devi Chintagunta, Knawang Chhunji Sherpa and Rintu Banerjee

Abstract In recent years, with the growing concerns over the depletion of natural resources and food security, researchers are focusing on abundantly available non-food crops such as lignocellulosic biomass as alternative reserves for bioenergy. Since lignocellulosic biomass are a rich source of carbohydrates they can be used to produce various biological products through different fermentation strategies such as separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF) along with consolidated bioprocessing (CBP). Among these, SSF has increased popularity for its cost-effectiveness and high product yield. The major advantages of SSF over SHF are the reduction in end product inhibition during saccharification, use of a single reactor for its operation and utilization of various lignocellulosic substrates under different pretreatment conditions that result in high product yield in short incubation time. However, certain drawbacks exist in SSF such as negotiation with the process parameters mainly temperature and pH; inability to utilize pentoses and low ethanol tolerance of fermenting strains. To overcome these limitations the authors are trying to emphasize a consolidated bioprocessing approach for utilization of pentoses and hexoses for improved bioenergy and other value-added product generation.

Keywords Lignocellulosic biomass · Simultaneous saccharification and fermentation · Biofuels

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1 Introduction

Need for alternative energy and cleaner air is increasing with every passing day due to rapidly growing industrialization, overpopulation, alarming GHG pollution due to the use of private transportation over public and multiple vehicles running from the same household. Understanding the need of the day, various biofuels have been produced with the advent of new technologies aiming at improved yield, low investment, higher biomass conversion efficiency and valorization of the by-products. The production of biofuels with comparable energy density to that of petroleum fuels requires a copious, low cost, and sustainable raw material that could suffice the incessant global demand. Lignocellulosic biomass comprising of recalcitrant lignin (10–25% w/w), crystalline and amorphous celluloses (40–50% w/w) and hemicelluloses (20–30% w/w) as the major entities is abundantly produced annually in the tune of 20×10^{10} tonnes that can serve as the potent raw material for various biofuel generation without disturbing the food-fodder supply chain (Zahid et al. 2014). The conversion of the biomass to value added products can be achieved by understanding the biochemical composition of the biomass, type and quality of the product desired and framing the minimum number of steps to economically recover the product. The fundamental steps in lignocellulosics to biofuel conversion include pretreatment, saccharification, and fermentation. Biomass pretreatment is performed prior to saccharification with the objective to degrade the lignin, reduce the degree of polymerization of holocelluloses, decrease the crystalline cellulose, and increase its amorphous counterpart and improve the yield and productivity of the reducing sugars upon enzymatic hydrolysis (Taherzadeh and Karimi 2007). The different methods of pretreatment such as physical (milling, pyrolysis, and irradiation), chemical (acid, alkali, and ionic liquid), physicochemical (ammonia fibre explosion, acid/alkali treatment-sonication) and enzymatic (laccase, lignin peroxidase, manganese peroxidase) are practiced of which enzymatic pretreatment is of particular interest when both celluloses and hemicelluloses are to be recovered with minimum loss under mild conditions. Saccharification is the hydrolysis of cellulose and hemicellulose polymers to easily fermentable pentoses and hexoses using hemicellulases and cellulases of fungal/bacterial origin. Fermentation of these reducing sugars to value-added deliverables such as bioethanol can be achieved by using wild-type hexose/pentose-fermenting strains or genetically engineered mixed sugar fermenting strains either in free or immobilized state. It is imperative to comprehend that hindrance at any of these unit operations ultimately affects the product thus an integrated approach must be followed to improve ethanol yield. In an attempt to reduce the fermentation time and increase the amount of substrate processed per given volume of hydrolyzing enzymes and fermenting cells different fermentation strategies such as sequential/separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), consolidated bioprocessing (CBP), etc., have been adopted world-wide. The present chapter emphasizes on various aspects of SSF viz. lignocellulosic substrates for SSF, biological agents involved

and the factors effecting the process, different modes of operation for commercialization, constraints in SSF, their mitigation strategies and the major commercial products generated during fermentation in SSF.

1.1 Lignocellulosic Substrates

The first challenging step towards commercial ethanol production is the selection of the raw lignocellulosic biomass and its collection, transportation and storage for large-scale ethanol production. Type of lignocellulosic material chosen decides the time required for pretreatment which depends on the lignin content of the biomass and for those biorefining industries which rely on contract farming for their biomass needs, cost of biomass would be the most influential factor affecting the cost of the entire process. Therefore, a thorough knowledge on available feedstocks in the local regions is essential. The abundantly available lignocellulosic biomass in general can be classified into edible crop varieties such corn, sugarcane, sugar beet, wheat, pineapple, sorghum, etc. These food crops have high holocellulose and low lignin content and thus could be easily hydrolyzed and fermented to ethanol without any pretreatment of the biomass. But it is essential that the feedstock for commercial ethanol production should be sustainable, available and must have nil or barely any consumption as food commodity so as to avoid the food versus fuel controversy. However, the residues from these food crops such as bagasse, rice husks, wheat straw, wheat bran, sugarcane tops, coconut shells, maize cobs, jute sticks, chilly stalk, cotton stalk, etc., considered as fodder/grazable crop residues can be used to produce biofuels. But as these residues are limited to a particular season after harvesting period their availability cannot be ensured throughout the year. Moreover, the agricultural residues are produced in decentralized fashion and thus incur high transportation cost making it uneconomical to use agricultural residues as the primary substrates for biofuel production. Also, these residues vary as a function of regional production, harvesting, processing and storage methods.

Other important feedstocks for biofuel production are forest residues which includes non-harvested biomass or that obtained from commercial hardwood and softwood processing locations, from thinning of forests done as a part of management operations and from dead and dying trees (wood chips, sawdust, dried leaves, tree barks, etc.). These residues can contribute 65% of the biomass energy potential. Several reports have stated their use for bioenergy production at district level through suitable designs of decentralized smaller plants. Nevertheless, the limitations such as the extraction costs, transportation to centralized processing plants make forest fuels expensive.

Therefore, considering these limitations it is appropriate to use the whole plant of non-edible lignocellulosic varieties for fuel production such as *Parthenium* sp., *Miscanthus giganteus,* napier leaves, purple guinea, *Saccharum spontaneum*, switchgrass, silver grass, etc., which either do not have food applications due to the presence of toxic compounds or the same demand as food crops. Example includes

Table 1 Biochemical composition of various lignocellulosic substrates (% dry weight)

S.no.	Lignocellulosic biomass	Cellulose	Hemicellulose	Lignin	References
1	Newspaper	25–40	40–55	18–30	Limayem and Ricke (2012)
2	Switch grass	30–35	40–45	12	
3	Waste papers from chemical pulps	12–20	50–70	6–10	
4	Nut shells	25–30	25–30	30–40	Sun and Cheng (2002)
5	Corn cobs	45	35	15	
6	Coastal Bermuda grass	25	35.7	6.4	
7	Solid cattle manure	1.6–4.7	1.4–3.3	2.7–5.7	
8	Swine waste	6.0	28	NA	
9	Primary wastewater solids	8–15	NA	24–29	
	Sugarcane leaves	36%	28%	20%	Shields and Boopathy (2011)
10	*Lantana camara*	47.25	18.23	19.25	Kuila et al. (2011)
11	*Ricinus communis*	42		19.8	Mukhopadhyay et al. (2011)
12	*Saccharum spontaneum*	38.7	29	17.46	Rajak and Banerjee (2015)

NA Not available

Lantana camara, which contains toxin of the family Lantadene whereas *Ricinus communis* and Jatropha contain ricin and forbol toxin respectively. Thus, non-edible lignocellulosics are gaining more importance due to their less/no competition as food/fodder and sustainable nature. The biochemical composition of various lignocellulosics is summarized in Table 1. Also, a mixture of these lignocellulosics can be employed to ease the laborious process of collection of huge quantity of single type biomass and to run the biorefinery in all seasons with the available lignocellulosic mixtures.

2 Biological Agents

The major biological agents that are involved in saccharification and fermentation process include cellulolytic enzymes that hydrolyze holocelluloses into simple sugars and ethanologenic microorganisms that are involved in ethanol production.

2.1 Cellulase and Xylanase and Their Types

Lignocellulosics are predominantly composed of cellulose, a homopolymer of glucose units linked by β 1-4 glycosidic bonds and hemicellulose which is a

heteropolymer constituting various proportions of monosaccharide units such as D-xylose, L-arabinose, D-glucose, D-mannose, D-galactose, D-glucuronic acid, and D-galacturonic acid. In order to obtain simple sugars for ethanol production, these polymers should be broken down by cellulase and xylanase. These enzymes are produced by numerous microbial sources viz., yeast, bacteria, protozoans, snails, crustaceans, and fungi among which *Trichoderma* sp. is the most prominent fungal source having efficiency to produce both the enzymes at a time. Cellulase and xylanase are classified (Sadhu and Maiti 2013) depending on their mode of action, structural properties, and substrate specificity which are tabulated as in Table 2.

Trichoderma reesei produces two exoglucanases- CBHI and CBHII, seven β- glucosidases-BGI-BGVII and eight endoglucanases- EGI-EGVIII. Cellulase system of *Humicola insolens* is homologous to that of *T. reesei* and contains seven cellulases (CBHI and CBHII, EG-I, II, III, V, VI). The isotypes of exo, endo and β-glucosidases differ from each other in the molecular weight, topology, pH and isoelectric point. For instance, CBHI and CBHII mainly differ in their molecular weight, i.e., 52.2 and 47.2 KDa respectively. BGI and BGII on the other hand differ in their secondary structures, i.e., α and β barrel shaped structures respectively. The optimal pH for different types of cellulases varies with the substrate mostly in the range of 4.2–5.2 and isoelectric point are in the range of 4.5–7.2. Cellulase is an inducible enzyme whose production is controlled by activation and repression mechanisms. In *T. reesei*, genes are coordinately regulated, where cellulolytic enzyme is induced in the presence of cellulose-rich substrates and repressed in the presence of excess glucose. The most probable inducers of *Trichoderma* sp. cellulase system are sophorose and lactose.

A wide range of microorganisms can produce xylanases of which bacteria and fungi are proficient producers. There is a significant difference between the bacterial and fungal xylanases. The bacterial xylanases show low activity than that of the fungal xylanases and additionally they do not undergo post translational modifications. In order to breakdown heteropolymeric xylan, synergistic effect should exist between the xylan degrading enzymes.

Fungal endoxylanases are mostly glycosylated single subunit proteins with molecular weight ranging from 8.5 to 85 kDa and isoelectric point between 4.0 and 10.3. ß-xylosidases may exist as mono, di or tetramer with 26–360 kDa. Arabinofuranosidases mostly exist as monomers, but dimeric, tetrameric, and octameric forms have also been found. The molecular weights are in the range of 53–495 kDa, pI from 3.6 to 9.3 and optimum pH ranges from 2.5 to 6.9.

2.2 Yeasts and Other Microbes for Fermentation

Saccharomyces cerevisiae is most commonly employed strain for the commercial ethanol production. However, ethanol producing bacteria (EPB) like *Zymomonas mobilis* is attracting much attention owing to its faster growth rate, high sugar uptake, high ethanol tolerance up to 16% (v/v) and ability to ferment under low/no

Table 2 Classification of cellulase and xylanase

Enzyme	EC number	Mode of action
Cellulase		
• Endocellulase (Endoglucanse (EG), Endo-1,4-β-glucanse, Carboxymethyl cellulase, β-1,4-endoglucon hydrolase)	EC 3.2.1.4	Random cleavage of cellulose at amorphous sites yielding glucose and cellooligosaccharides
• Exocellulase (cellobiohydrolase (CBH) or glucanase)	EC 3.2.1.91	Releases cellobiose either from reducing or non-reducing ends of cellulose chain by hydrolyzing 1,4-β glycosidic linkages
• Cellobiases (β-D-glucosidase (BG), gluco-hydrolases, cellobiase)	EC 3.2.1.21	Releases glucose from cellobiose and short chain cellooligosaccharides
• Oxidative cellulase	NA	Deploymerize cellulose by radical reaction
• Cellulose phosphorylase	NA	Depolymerize cellulose using phosphates instead of water
Xylanase		
• Endo-β-(1,4)-D-xylanase (β-(1,4)-D-xylan xylanohydrolase)	EC 3.2.1.8	Randomly act on xylan to produce xylooligosaccharides of various chain lengths
(a) Non-arabinose liberating endoxylanases-I		Cannot act on L-arabinosyl initiated branch points at β-(1,4) linkages and produce only xylobiose and xylose by breaking xylooligosaccharides
(b) Non-arabinose liberating endoxylanases-II		Cannot cleave branch points at α-(1,2) and α-(1,3) and produces xylooligosaccharides
(c) Arabinose liberating endoxylanase-I		Produces xylobiose, xylose and arabinose by cleaving xylan at branch point
(d) Arabinose liberating endoxylanases-II		Produces xylooligosaccharides and arabinose by cleaving at branch point
• Exo-β-(1,4)-D-xylanase (β-(1,4)-D-xylan xylohydrolase)	NA	Remove single xylose units from the non-reducing end of the xylan chain
• β-xylosidase (xylobiase)	EC 3.2.1.37	Hydrolyzes xylobiose and xylooligosaccharides
• α-Glucuronidase	NA	Hydrolyzes α-1,2 bond present between the glucuronic acid residues and β-D-xylopyranosyl backbone units found in glucuronoxylan

NA Not available

oxygen conditions. Some natural ethanologenic yeast species such as *Pichia stipitis, Candida shehatae*, *Kluyveromyces marxianus,* and *Pachysolen tannophilius* appeared to have efficiency in utilizing pentoses which can be used to co-ferment along *S. cerevisiae* for complete lignocellulosic ethanol fermentation.

Fermenting pentose sugars present within the saccharified broth is one of the main bottlenecks which restrict the commercialization of ethanol production. In

order to circumvent this problem, Microbial Biotechnology and Downstream Processing laboratory, IIT Kharagpur has isolated a new pentose fermenting strain from the local soil of IIT Kharagpur which could utilize both C5 and C6 sugars. The process was further optimized for SSF of *L. camara* and *R. communis* and the optimized conditions yielded 28.77 and 35.48 g/L ethanol respectively. The result obtained is competitive with the reported literature. Similar attempt was made by Silva et al. (2011) in which *P. stipitis* NRRLY7124 was grown on xylose (90 g/L) as the carbon source under aeration rate of 0.25 vvm at 250 rpm. Under these experimental conditions, the ethanol production was observed to be 26.7 g/L.

Suriyachai and co-workers conducted a study using pretreated rice straw and subjected it to simultaneous saccharification and co-fermentation (SSCF) using *S. cerevisiae* and *Schefferomyces stipitis*. Under optimized SSCF conditions, i.e., 0.31:0.69 cell ratio (*S. cerevisiae*: *S. stipitis*) at 33.1 °C under agitation speed of 116 rpm, the maximum ethanol concentration was found to be 28.6 g/L at 10% biomass concentration in 72 h (Suriyachai et al. 2013). This comparative analysis indicates the efficiency of the newly isolated strain for SSF. The work conducted with this strain revealed that inoculum volume 9–10% (v/v), substrate concentration 18–19% (w/v), incubation time 48 h, 37 °C, inoculum age 48 h are optimum conditions for maximum ethanol yield from substrates like *L. camara* and *R. communis*. As this strain works at mild environmental conditions it has wide scope for industrial ethanol production. However, further improvement in both the ethanol concentration and the process parameters namely, substrate loading and incubation time is vital such that more amount of substrate can be converted to ethanol in shorter incubation time to make it commercially feasible.

In order to compete with the ethanologenic microorganisms, the wild strain of *S. cerevisiae* which can only ferment glucose should be genetically modified so that it can ferment both pentose and hexose sugars for enhanced ethanol production. Employing the microorganisms having both cellulolytic and ethanologenic activity for SSF is another viable alternative for cost effective ethanol production.

2.3 Yeast Growth Studies

2.3.1 Immunofluorescence and FACS Analyses

Immunofluorescence is performed to visualize the cellular features of yeast by conjugating the dyes like fluorescein isothiocyanate and rhodamine B with monospecific antibodies which are raised against yeast structural proteins. Confocal laser beam immunofluorescence microscopy can be used to detect the intracellular localization of proteins in the yeast cell and for its three dimensional ultrastructural information. Fluorescence-activated cell sorting (FACS) helps in studying the cell cycle of yeast and in monitoring changes in organelle biogenesis. Scanning electron

microscopy (SEM) and transmission electron microscopy (TEM) are useful in revealing the surface topology and intracellular fine structures of yeasts (Walker 1998).

2.3.2 Cytometric and Spectrophotometric Analyses

The density of cells in a yeast culture can be determined by direct counting in a haemocytometer chamber and by measuring optical density at 600 nm in spectrophotometer. Wild-type yeast strains with OD_{600} of 1 correspond to $\sim 3 \times 10^7$ cells/mL. Mutations affect the cell size or shape, thereby altering the OD. Some mutant strains exhibit clumpy phenotype resulting in inaccurate density measurements. In such situation, the clumps should be dispersed by mild sonication prior to counting and density measurement. Another method practiced for cytological and physiological studies of the yeast is flow cytometry. It has been developed to determine size, membrane potential, intracellular pH, and levels of cellular components such as DNA, surface receptors, protein, and calcium.

3 Fermentation Strategies

The reducing sugar-rich hydrolyzate of lignocellulosic biomass obtained after enzymatic saccharification is composed of C6 sugars (glucose, mannose, and galactose) and C5 sugars (xylose and arabinose) which theoretically yield 0.51 g ethanol per 1 g glucose/xylose. However, the molecular conversion of glucose to ethanol is slightly higher than xylose (i.e., 1 glucose molecule gives 2 molecules of ethanol and 1 xylose molecule gives 1.67 molecules of ethanol) (Okamoto et al. 2014). To reach the theoretical conversion of the substrate to ethanol the following fermentation strategies can be adopted. The various parameters to be considered for selecting a particular fermentation strategy are incubation time, inoculum volume, temperature, labour involved, and substrate loading into the bioreactor per batch, all of which directly influence the overall ethanol yield.

3.1 Separate Hydrolysis and Fermentation (SHF)

The process of conducting saccharification and fermentation of pretreated lignocellulosic biomass in separate tanks under different reaction conditions is defined as SHF. The holocellulolytic enzymes (cellulases and xylanases) efficiently hydrolyze at 45–50 °C while the generally used fermenting strains produce ethanol between 30 and 37 °C. SHF gives the liberty to conduct both the unit operations under their respective optimum conditions. Based on the substrate used and the microbial source of the enzymes, the reaction conditions for conducting separate hydrolysis

may slightly vary. The major disadvantage in SHF is that the hydrolysis products mainly glucose and its corresponding disaccharide, cellobiose inhibit cellulase action. But the cellulase inhibitory concentration of cellobiose is slightly higher than glucose indicating that glucose has stronger inhibitory effect on cellulase. When cellobiose concentration in the hydrolyzate was 6 g/L the residual cellulase activity was 40% of the initial activity and when glucose concentration was 3 g/L, the residual activity of β-glucosidase unit of cellulase was 25% of the initial activity (Taherzadeh and Karimi 2007). Another constraint in SHF is the hydrolysis of holocelluloses to sugars followed by their separation from saccharified biomass and then separate fermentation of sugars to ethanol which is a two step process that is laborious, additional cost incurring, and time taking. These drawbacks can be avoided by simultaneous conversion of sugars to ethanol within the same reactor.

3.2 Simultaneous Saccharification and Fermentation (SSF)

SSF is the process of conducting saccharification of the pretreated lignocellulosic biomass and the concomitant fermentation of reducing sugars to ethanol in the same fermenting vessel. This phenomenon is practically feasible when the optimum working temperatures of cellulases/xylanases meet as closely as possible to that of fermenting microbial strain because the raw material for fermentation is the end product of saccharification. Thermophilic bacterial and yeast cells such as *C. acidothermophilum* and *K. marxianus* can be used as fermenting strains for conducting SSF without compromising the optimal temperature of hydrolysis.

The major advantage with SSF is that it can be adopted to process any cellulose-rich biomass without the problem of cellulase inhibition by glucose or cellobiose. This is due to the fact that before reaching the inhibitory concentrations of cellobiose/glucose, these sugar molecules are concurrently fermented to high-energy density ethanol molecules. Therefore, SSF improves the ethanol yield in shorter incubation time, reduces the cost of investment and operation as one reactor suffices the work of two thereby cutting down the labour involved in separation of residual biomass from the sugar rich hydrolyzate. Also, it checks the microbial contamination of sugars due to the presence of ethanol in the same vessel (Ohgren et al. 2007). The main drawback in this process is that though hexoses are efficiently converted to ethanol, pentoses are either neglected (when working with only hexose fermenting strain such as *S. cerevisiae*) or separated after pretreatment (such as dilute acid) into separate tank to be fermented to ethanol using a pentose utilizing strain. Genetically engineered mixed sugar utilizing strains can solve this issue and enable fermentation of both the sugar types within the same reactor.

Moreover, the use of lytic polysaccharide monooxygenases (LPMOs), an oxidative metalloenzyme along with cellulase is found to enhance the cellulose degradation in lignocellulosic biomass. LPMO requires molecular oxygen as electron donor for carrying out oxidation of C_1 or C_4 in the scissile β-1,4-glycosidic bonds. When SSF for ethanol production is carried out using this enzyme cocktail

and yeast, the fermenting strain competes with LPMO in the cocktail for the molecular oxygen, thereby creating anoxic environment detrimental to LPMO. Hence the processing strategy has been shifted to SHF instead of SSF where the conditions are more favourable (Cannella and Jorgensen 2014). In another study, Muller et al. (2016) reported that the combination of LPMO-containing cellulase cocktail and fermenting microorganism resulted in maximum lactic acid production under SHF over SSF. These studies indicate that the oxidative and hydrolytic enzyme cocktail works well with SHF rather than SSF strategy.

3.3 Non-isothermal Simultaneous Saccharification and Fermentation (NSSF)

When thermophilic microbial strains are used for SSF as a substitute of *S. cerevisiae* to ferment at 45–50 °C in order to reach the activation energy of cellulase/xylanase, the yield of the main end product viz. ethanol was found to drastically decrease and other by-products such as acetic acid and lactic acid was increased rendering the entire process uneconomical. In an attempt to increase the ethanol yield and overcome the drawbacks of SHF (i.e., cellulase inhibition by hydrolysis products) and SSF (i.e., deviation from optimal temperature), a novel strategy of non-isothermal simultaneous saccharification and fermentation (NSSF) was proposed. This process involves a presaccharification step either in the same or in separate reactor vessel at optimum temperature (50 °C) to maximize the reducing sugar yield. It was reported that cellulase activity increased 2–3 times when the reaction temperature was gradually increased from 30 to 50 °C. The effluent after saccharification comprising of pentoses, hexoses and un-hydrolyzed biomass is pumped to another fermenter vessel or inoculated in the same vessel with microbial cells and maintained at 30–37 °C which is the optimum temperature for metabolism of mesophilic ethanologenic strains. This process was reported to reduce the volume of enzyme needed for hydrolysis by 30–40% by improving the enzyme-substrate kinetics and drastically decreased the fermentation time from 4 days through SSF to 40 h by NSSF (Wu and Lee 1988).

3.4 Simultaneous Saccharification and Co-fermentation (SSCF)

A novel co-fermentation strategy called as SSCF which is the further improvement of SSF has been adopted where the loss of pentose sugars (xylose and arabinose) obtained upon hydrolysis of hemicelluloses is well addressed. The pentose-rich fraction is integrated with hexose stream within the same reactor and fermented using genetically engineered variants of *Z. mobilis, Escherichia coli, S. cerevisiae,*

etc., having genes, enzymes cascades, and sugar transport systems for both hexose and pentose fermentation (Bothast et al. 1999). These strains first metabolize glucose to their primary metabolic product (ethanol) and then use pentose for fermentation. This integrated approach allows maximum utilization of the lignocellulosic biomass and improves the ethanol yield compared to SHF and SSF. Although, genetically engineered strains are used for hexose and pentose sugar utilization, high concentration of glucose can inhibit xylose metabolism making co-fermentation of sugars challenging. This is due to the competition between glucose and xylose for the same transport system to enter into the cell (Meinander and Hahn Hagerdal 1997). Moreover, the affinity of glucose towards the glucose transport system is 200 fold higher than xylose (Kotter and Ciriacy 1993). In order to overcome this problem, continuous mode of co-fermentation can be adopted by adjusting the dilution rate so as to keep glucose concentration in the system below 2.3 g/L for rapid fermentation of both glucose and xylose (Chen 2011).

On the other hand, when co-fermentation of sugars is done using two different strains for hexose and pentose fermentation, compatibility of these strains also needs to be investigated since it may cause end-product inhibition wherein ethanol produced from glucose may inhibit the xylose fermenting strain due to its low ethanol tolerance.

Besides, it is also important to consider that ethanol yield beyond 30 g/L is inhibitory to the cellulase activity (Wyman 1996). In this process the ethanol yield may shoot up beyond the inhibitory concentration as there is an opportunity for mixed sugar utilization depending on the holocellulosic content of the biomass and the reaction conditions. Therefore, continuous stirred tanks reactor systems are ideal in such circumstances where the ethanol produced in the fermentation broth is continuously drawn out of the system. Another mode of operation is fed-batch mode of co-fermentation, where the initial viscosity of the reactants is maintained low to allow easy mixing and avoid mass transfer limitations with in the fermenter thus leading to high ethanol yield (Liu and Chen 2016).

3.5 Direct Microbial Conversion (DMC)/Consolidated Bioprocessing (CBP)

Hydrolysis step of the ethanol fermentation process is the major constraint in ethanol commercialization due to the high cost of the enzymes used for saccharification. Scientists have come up with a probable solution to this expenditure to combine holocellulolytic enzyme production with that of ethanol production rendering the process self-sufficient from start to finish. This approach is advantageous as it reduces the cost for biomass processing and decreases the number of fermenters needed for plant operation as it is a single step system (Mbaneme-Smith and Chinn 2014). The direct microbial conversion of the biomass can be achieved by using genetically engineered strains with excellent cellulolytic and ethanologenic activities. Therefore, an efficient ethanol producing strain can be modified to

express genes for cellulases and xylanases or use the finest holocellulolytic strain and metabolically engineer it as a superbug being able to ferment both hexoses and pentoses (Linger and Darzins 2013). Several anaerobic microbes such as *Clostridium thermocellum*, *Neurospora crassa*, *Paecilomyces* sp. and *Monilia* sp. with cellulolytic activities and high temperature resistance have been reported to have the potential of DMC. Thermophile namely *Caldicellulosiruptor* sp. is an important DMC strain as it can directly hydrolyze the raw lignocellulosic biomass without any delignification step.

The challenge in CBP system is that the microorganisms within the fermenter come across various toxic compounds during biomass pretreatment such as phenolics, furan derivatives, etc. that inhibit their growth and metabolism and ultimately affect the ethanol yield (Hasunuma and Kondo 2012). In this context, detoxification of the hydrolyzate is being used nowadays, but however this additional step adds to the cost of the process on a large scale basis. To avoid this additional step, genetically engineered inhibitor tolerant variants of *S. cerevisiae* have been developed (Larsson et al. 2001).

4 Factors Affecting SSF

There is a tremendous need to select the factors which have significant effect on the ethanol yield. Solid and enzyme loading, yeast concentration, temperature, incubation time, pH and additives have profound influence on ethanol production which is discussed in detail.

4.1 Solid Loading

One of the major factors that can contribute to high ethanol production is optimum solid loading. Increase in solid loading should lead to increased ethanol yield but, in practice, that does not occur and is a challenging task for the scientific community to resolve it. Generally, increase in solid loading increases the reducing sugar concentration up to certain percentage of solid loading and after that it declines. High solid loading results in improper mixing of substrate and enzyme that may lead to limited cellulose conversion to reducing sugars. Moreover, increased viscosity of fermentation broth and mass transfer limitations reduces the efficiency of enzymatic hydrolysis and fermentation. The viscosity problem can be well addressed by adding the substrate gradually rather than adding at once.

4.2 Enzyme Loading

The enzyme loading during SSF should be minimized to reduce the cost of the ethanol production process. The enzyme concentration should be maintained in such a way that it can interact with the maximum substrate available in its vicinity. A decrease in enzyme loading results in less accessibility of enzyme towards the available substrate leading to reduced ethanol yield and subsequently increases the duration of ethanol production. According to techno-economical calculations, 50% reduction of enzyme loading is beneficial if the decrease in the product yield is nearly 6–7% and increase in the residence time is not more than 30% (Sassner et al. 2008).

4.3 Incubation Time

During SSF, the reducing sugar obtained due to hydrolysis of polysaccharides by cellulolytic enzymes is utilized by the ethanologenic microorganisms simultaneously for their growth and ethanol production. The production of ethanol gradually increases and after certain incubation period it declines. The probable reason for the decline in ethanol production is the enzyme inactivation or product inhibition of fermenting strain. Kitagaki et al. (2007) reported that yeast cell has capacity to tolerate ethanol up to certain concentration, above which its growth gets inhibited leading to the damage of the cell. Ethanol-induced yeast cell death occurs due to stress and changes in protein structure, membrane fluidity, mRNA export from the nucleus.

4.4 Temperature

Temperature has immense effect on activity of cellulolytic enzymes, growth and other metabolic activities of yeast cell. The optimum temperature required for the cellulolytic enzymes (50 °C) and yeast (30–37 °C) are different and if SSF is to be performed then there should be a compromise between the two optimal temperatures which may affect the ethanol yield. Ethanol production reduced considerably at high temperature. The plausible reasons might be denaturation of cellulase, shortened exponential phase of the yeast, change in membrane fluidity, increased accumulation of ethanol in the cell, etc. Through employment of thermotolerant yeast strains like *S. uvarum, Fabospora fragilis, Candida brassicae, Candida lusitaniae, and Kluyveromyces marxianus* the temperature of SSF can be maintained closer to that of optimal temperature of cellulolytic enzymes.

4.5 pH

Each and every microorganism possesses a pH range for its growth and activity and deviation from the optimum value results in decrease in their growth and product formation. During SSF, due to high pH, cellulase is destabilized and yeast loses its osmotic balance. The optimum pH for *S. cerevisiae* BY4742 lies in the range of 4–5. The pH lower than 4, prolongs the incubation period for ethanol production and the pH above 5 reduces the ethanol yield substantially. The pH below 4 and above 5 favours the formation of acetic acid and butyric acid respectively (Lin et al. 2012).

4.6 Inoculum Volume

The inoculum volume has impact on the duration of lag phase, specific growth rate, and ethanol production. The higher inoculum loading decreases the lag phase duration. The increase in inoculum volume leads to gradual increase in ethanol yield up to certain extent and after that it does not show a significant increase in ethanol yield. In the industrial perspective, low yeast loading with more ethanol yield is advantageous. The substrate for yeast production also plays a key role in the cost of ethanol production. When yeast is cultured using expensive substrate/medium, higher yeast loading during SSF is not economical. Though volumetric productivity of ethanol is dependent upon yeast loading, the enzymatic hydrolysis is the rate determining factor during SSF.

4.7 Effect of Additives

Supplementing of growth medium of *S. cerevisiae* with additives like yeast extract, peptone, malt extract, and ammonium sulphate enhances the ethanol yield. Supplements also improve the sugar utilization by the yeast cells which might be possible reason for higher ethanol productivity. Yeast extract provides cofactors like biotin and riboflavin to enhance the growth of yeast. Though these supplements enhance the ethanol yield, they cannot be used at industrial scale as they are very expensive. Among the low-price supplements to the yeast medium like sunflower, safflower oil seed meal cakes, wheat mash, groundnut, soy flour, safflower oilseed meal cake resulted in higher ethanol yield. The safflower oil cake is rich in polyunsaturated fats and unsaturated fatty acids which rendered higher ethanol tolerance to yeast (Ding et al. 2009). Also, reducing sugar-rich hydrolyzate obtained after saccharification of lignocellulosic biomass can be used as a cost effective and sustainable substitute for commercial media for the growth of ethanologenic microorganisms.

5 Modes of Fermentation for SSF

The ethanol production with yeast majorly depends on the substrate utilized for its production and mode of fermentation process. Fermentation can be performed majorly by batch, fed-batch, and continuous modes and its selection depends on the kinetic properties of the microorganism and cost of the process.

5.1 Batch Fermentation

Batch fermentation is a traditional and most commonly practiced process for ethanol production due to its low investment cost, easy feedstock management, and its flexibility. During this process, feed constituting substrate, yeast and other nutrients required for ethanol production are charged into the fermenter and after specific incubation time, entire ethanol is recovered. The high sugar concentration imparts substantial osmotic stress on yeast, thus slowing down the rate of fermentation leading to low productivity. The other disadvantages of the process are labour intensive and time consuming as considerable amount of time is wasted in each batch for cleaning, sterilization, inoculum growth, and harvesting. The problem associated with the high sugar loading can be addressed either by fed-batch addition of sugars or by employing a continuous fermentation process in which yeast cells are not subjected to osmotic stress.

5.2 Continuous Fermentation

In the continuous fermentation process, feed is pumped continuously into the fermenter where microorganisms are active. During this process, the addition of feed into the fermenter and removal of fermented broth containing ethanol, biomass, and residual sugars occurs at the same rate which leads to the maintenance of constant liquid volume inside the fermenter. Though the process is less labour intensive, contamination is a serious problem with this process since the system is interrupted several times. Another serious issue with this process is the loss of active yeast cells during removal of fermentation broth. The problem can be addressed by growing yeast at the same rate as that of the dilution rate to avoid washout of the cells or by employing the flocculated yeast or by immobilized yeast cells (Taherzadeh et al. 2001).

The productivity of the process can be improved by reusing the enzymes and inoculum involved in ethanol production. In order to recycle the cells, the concentration of non-yeast insoluble solids should be low and high yeast cell viability should be maintained. The cells can be separated from the medium through centrifugation or sedimentation and reused for the subsequent batches till the cells

tolerate the ethanol concentrations. To improve the fermentability, high ethanol tolerant strain should be employed. The thermotolerant *S.cerevisiae* (IR2-9a) produce significantly high ethanol of 28 g/L from bleached kraft pulp compared to native strain of *S.cerevisiae* (16 g/L) (Edgardo et al. 2008)

5.3 Fed-Batch Fermentation

The fed-batch mode of operation is considered as a combination of both batch and continuous operations and is predominantly practiced in alcohol industries. During this process, yeast inoculum is added initially to a small amount of media and then fresh medium is added continuously at regular intervals without removing the fermented broth. Intermittent pumping of substrate maintains the sugar concentration in the reactor and prevents the osmotic stress on the yeast thereby enhancing ethanol yield. Implementation of cell recycling along with fed-batch cultivation can improve the volumetric productivity of ethanol (Sanchez and Cardona 2008). Besides, it is suitable for dilute acid hydrolyzate fermentation as high concentrations of inhibitors can be avoided during the process (Taherzadeh et al. 2000).

The ethanol productivity can be enhanced by concentrating the cell biomass in the fermenter through immobilization and recirculation of cells. The process can be made economical by improving ethanol productivity using small fermenter and coculture of microorganisms for effective substrate utilization.

6 Major Commercial Products of SSF

Over the last few decades, SSF has shown an increasing trend in bioprocesses and bioproducts and has been attributed for producing primary metabolites that has numerous practical advantages.

6.1 Ethanol

Bioethanol production by SSF from lignocelluloses (energy crops, forest, and agricultural residues) shows prospective advantage over first generation bioethanol from an environmental and sustainable perspective. Though ethanol can be produced by SHF, SSF due to its reduced investment cost, low water requirement and reduction in end-product inhibition of the enzymatic hydrolysis makes it a preferred method. During ethanol production by SSF, there are other co products formed along with it, such as lactic acid, acetic acid. Vincent et al. (2011) reported the production of ethanol using corn stover using *S. cerevisiae* and *E. coli* K011 which showed highest ethanol concentration of 2.29 g/100 g corn stover and 4.79 g/100 g

corn stover respectively. Acetic acid and lactic acid were also monitored with acetic acid production in the range of 0.45 and 0.78 g/100 g corn stover while no lactic acid was detected. The major constraint in the ethanol production process is the separation of ethanol from fermented broth defined as distillation. In order to overcome this constraint various separation technologies have been investigated so as to separate ethanol in best possible way so that ethanol recovery cost is reduced. Due to high energy requirements in distillation, various other separation technologies have been investigated for energy efficiency. Fermentation broth containing low ethanol concentration can be separated by applying pervaporation which economically viable than distillation. It is the process employed for separation of ethanol from the fermented broth through partial vaporization using porous/non-porous membranes.

6.2 Butanol

Butanol, a colourless liquid is miscible with organic solvent and is less hydroscopic and less corrosive than ethanol. It can be used for many purposes such as fuel replacing gasoline and fuel additives, solvents for certain pharmaceutical products and as diluents for brake fluid. Biobutanol was first industrially synthesized during 1912–1914 by Acetone–Butanol–Ethanol (ABE) fermentation of cereal grains and molasses using *Clostridium acetobutylicum* (Jones and wood 1986). Few strains producing high biobutanol yield was also identified such as *Clostridium beijerinckii*, *Clostridium saccharoperbutylacetonicum* and *Clostridium saccharobutylicum* (Keis et al. 2001). Su et al. (2015) compared the efficiency of SHF and SSF by using the hydrolyzate of sugarcane bagasse using *C. beijerinckii* NCIMB 8052 and demonstrated that SSF was able to produce high butanol concentration of 6.4 g/L and total ABE of 11.9 g/L which was comparatively higher than SHF.

7 Scale up, Mass Balance, and Economic Feasibility of SSF

In the present scenario, development of economical technologies for production of biofuels and other value added platform chemicals and biochemicals from lignocelluloses is an important issue among the researchers, private companies and government. Scaling up is a major task and is the basic step in making a process practically applicable on an industrial scale. The different factors that help in the scaling up of the process are the laboratory experiments, derivation of kinetic correlation, mathematical modelling, design and operation of pilot plant. Process engineering tools are very much required along with innovative process configuration that is aimed at reducing energy and developing an ecofriendly technology.

For scaling up of a process, mass balance is very important criteria in processing so as to maximize yield of product and minimize the investment cost. When designing a new process or investigating a present one, mass balance is an important factor that helps in calculating the mass flow rates passing through different physical, chemical or biological processes.

Economic feasibility is an important aspect when adopting a particular process. Ideally the cost of investment must be lower than that of the market price for ethanol which is determined by type and composition of the feedstock, cost of biomass, ethanol plant volumetric capacity, and biomass conversion efficiency. Therefore, it is crucial to evaluate the cost of the process at each unit operation specially the rate limiting step to calculate the process economics and anticipate the commercial feasibility.

The economic feasibility of 2G ethanol from lignocellulosics is primarily influenced by an effective strategy to achieve minimal ethanol selling price (MESP) and second, by the various value added platform chemicals and other biochemicals that can be derived from the residues obtained after ethanol production. The maximum profitability in form of multiple products starting from the same amount of biomass can lead to an integrated bioprocessing approach. Some of the commercially significant deliverables that can be produced from the residues of lignocellulosic ethanol refinery include lactic acid, acetic acid, xylitol, sorbitol, hydroxy methyl furfural, furfurals, alkanes, ethylene glycol, alkenes, glycolic acid, acetone, and ethylene. Apart from these low value-high yield products, high value-low yield lignin degradation products like guaiacols, resins, catechols, syringaldehyde, benzene, quinoline, vanillic acid and vanillin may also be produced (Avanthi et al. 2016). Besides, the biomass obtained after SSF may be used for the production of fuels such as biogas/biomethane and biohydrogen. Carbon dioxide separated from biogas may be used for the growth of microalgae which are the potential lipid source for biodiesel production whereas methane enriched gas has improved calorific value which is pollution free too. The solid residue obtained after these high energy density fuel production can further be fortified with nitrogen, phosphorous, and potassium (NPK) levels using cyanobacteria and used as biomanure to enrich the soil quality of marginal lands (Chintagunta et al. 2015). One such holistic attempt made by Ghosh and co-workers for the generation of fuels and chemicals from sugarcane bagasse where thermophilic yeast, *Kluyveromyces* sp. IIPE453 (MTCC 5314) was used to ferment the hexose stream obtained from the saccharification of sugarcane bagasse to ethanol. The unutilized pentose fraction was used for the thermophilic seed culture preparation and furfural generation whereas the residual solid biomass was subjected to gasification to produce electrical energy. From this integrated study it was found that 1 kg of sugarcane bagasse could yield 366 mL of ethanol, 149 g furfural and 0.30 kW of electrical energy (Ghosh et al. 2015).

8 Conclusion and Perspectives

Rapidly growing global demand for transportation fuels and enormous depletion of fossil fuels necessitates development of an effective biomass to biofuel production technology. Lignocellulosic biomass is renewable, abundantly available resource holding tremendous potential in meeting energy needs and providing environmental benefits. Technical knowledge is needed to design the process and to deal with complexity of biomass for ethanol production. The process of ethanol production includes appropriate pretreatment, saccharification, fermentation, distillation and removal of inhibitory byproducts. The ethanol production can be made economical by praticing SSF, SSCF, PSSF, or CBP and by producing efficient enzymes through biotechnological intervention. Utilizing substrate without or with slight pretreatment, fermenting reducing sugar with co-cultures or with the engineered strain having capability to utilize both pentoses and hexoses can also be implemented to improve the ethanol yield. Adoption of non-polluting, renewable energy sources in combination with strategies such as biodiversity studies, metagenomics, metabolic engineering and systems biology can improve biofuel yield.

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Bioalkanes and Bioalkenes: An Ecofriendly and Alternate Fuel in Bioenergy Research

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Abstract Acute energy crisis and vulnerable climatic conditions are the major factors behind adoption of efficient and ecofriendly fuel source such as bioalkanes/bioalkenes which are produced using biomass as the feedstock while alkanes/alkenes on the contrary are produced through chemical means. In this context, lignocellulosic biomass presents a ray of hope since its usage will make the process ecofriendly, renewable, and sustainable. Against this backdrop, this chapter presents a detailed overview of the possible conversion technologies viz., chemical and microbial to obtain bioalkanes/bioalkenes and also unravels the possible routes through the intervention of genetic engineering approaches in order to make the process more efficient. It also discusses in details the types of hydrocarbons produced by the microorganisms and their physiological roles so that the biosynthetic pathways can be tapped to the maximum of their potential.

Keywords Bioalkanes · Lignocellulosics · Biomass

1 Introduction

The advancement of new technologies for the production of energy and chemicals from renewable resources has encouraged extensive research on biomass valorization (Huber et al. 2006; Corma et al. 2007). Biomass research has now focused more towards the production of innovative candidate fuels over ethanol, diesel, and methane. Target-oriented upgradation of biomass to high-valued fuels such as alkanes and alkenes appear to be an attractive route. These fuels are convenient to

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the present transportation infrastructures such as pipeline systems for liquid fuels and gas stations for gaseous fuels. Biomass such as lignocellulosics consists of a complex heterogeneous mixture of carbohydrate polymers namely cellulose, hemicellulose, and lignin. Biotransformation of these energy rich molecules (cellulose, hemicellulose, and lignin) into liquid hydrocarbon fuels such as bioalkanes and bioalkenes appears to be an innovative approach for advanced biofuels production (Xia et al. 2016). Thus, utilization of lignocellulosic waste such as agricultural and forest residues for fuel production helps to combat the food versus fuel issue while at the same time attaining international energy security along with reduction of greenhouse gas emissions.

Till date the worldwide supply of liquid fuels for transportation heavily depends on petroleum (more than 95%). The production of petroleum and its derivatives such as short/medium chain hydrocarbons has become more expensive and results into political and military conflicts. Its production process is not only costly but also adds large amount of CO_2 in the atmosphere ultimately contributing to global warming. Hong et al. (2013) studied the environmental impact of bio-jet fuels (bio-paraffins 1 (Bio-P1) and bio-jet paraffins 2 (Bio-JP2)) and observed that CO_2 lifecycle can be reduced by 76 and 81% respectively for Bio-P1 and Bio-JP2 respectively with respect to fossil jet fuel. Similar kind of study has also been carried out by Jiménez-Díaz et al. (2016) who reported that emissions due to the utilization of bio-jet fuels lead to lower amounts of greenhouse gas emissions such as SO_x, NO_x, and particulate matter in comparison to conventional jet fuel. Therefore, significant development regarding the raw material selection and processing technologies are indeed necessary, and high-valued fuels produced preferably from the renewable raw biomass will be needed to complement or substitute the present petroleum- and diesel-based fuels. Thus the present chapter discusses the conversion strategies of biomass to bioalkanes/bioalkenes in details with special emphasis on metabolic engineering approaches along with the bottlenecks which hinder their commercial scale production as well as the possible solutions to the impending hurdles.

2 Bioalkanes and Bioalkenes as a Futuristic Source of Fuel

Bioalkanes and bioalkenes are renewable chemicals which have diverse chemical structures and likewise can be used for a number of applications such as fuels, starting compounds for the synthesis of aromatic molecules and as lubricants (Deneyer et al. 2015). The term “bioalkanes and bioalkenes” has a distinct difference with “alkanes and alkenes” in terms of the substrate used for production where biomass is used for production of bioalkanes/bioalkenes while chemical precursors are generally used for producing alkanes/alkenes. Bioalkanes having applications as transportation fuels majorly comprises of hydrocarbons having carbon numbers

ranging from C6 to C15 (Xin et al. 2014) and it encompasses fuels like gasoline, diesel, and jet fuel. Bioalkanes are considered to be superior fuels since its fuel properties are better than its counterparts viz., ethanol and butanol. Energy density of alkanes lies in the range of 42–47 MJ/kg while for others it lies in the range of 27 MJ/kg (ethanol)—36 MJ/kg (iso-butanol) (Wang et al. 2016). A comparison of the physical, chemical, and application aspects of bioalkanes over ethanol and butanol is shown in Table 1 (Wang et al. 2016).

Bioalkanes can mainly be produced using renewable carbohydrate sources such as lignocellulosic biomass and also by lipids. Lignocellulosic biomass majorly comprises of lignin, cellulose, and hemicellulose, all of which can be converted to alkanes through different approaches. Lignin is a phenolic polymer linked through ether and C–C interlinkages and thus decoupling reactions of these molecules will lead to phenolic monomers (C_6–C_{11}) and dimmers (C_{12}–C_{22}). These phenolic molecules can be converted to cyclic alkanes via deoxygenation and hydrogenation. On the contrary, cellulose and hemicellulose, carbohydrate polymers can be converted to alkanes via decoupling of the polymer to yield individual monomers followed by dehydrogenation to form alkanes. Lipid-rich biomass presents alternative source for alkane production since it is mainly composed of triglycerides from which if the glycerol backbone is removed by hydrolysis, β-elimination or hydrogenolysis then a successive hydrogenation and oxygen-removal steps such as hydrodeoxygenation, decarbonylation, and decarboxylation will convert the fatty acids to alkanes (Deneyer et al. 2015; Zhang et al. 2014). Apart from chemical techniques, biological routes viz., microbial production and application of genetic engineering techniques have also been reported for production of bioalkanes/bioalkenes (Jankowski and ZoBell 1944; Schirmer et al. 2010; Stone and ZoBell 1952; Wang et al. 2013; Kageyama et al. 2015; Gehret-Mccarthy et al. 2012).

Biomass-based sustainable fuel production has been gaining increasing attention due to global climatic condition and future energy security. Hydrocarbons, mainly bioalkanes and bioalkenes, are of special interest because of their potential to be utilized as an advanced biofuel (Lennen et al. 2010) and till date only few works have been focused on the bio-based production of bioalkanes or bioalkenes (Schirmer et al. 2010). Microbial production (300 mg/L) of bioalkanes and bioalkenes was reported by using an engineered strain of *E. coli* which contained an alkane biosynthesis operon obtained from cyanobacterial genes encoding for aldehyde decarbonylase and acyl-ACP reductase (Schirmer et al. 2010). Slightly different approach was adopted by Harger et al. (2013) who reported that by expressing FabH2 gene of *Bacillus subtilis* in *E. coli*, 10 folds increase in the production of alkanes was observed. It was also reported that in absence of FabH2 gene, the bioalkanes produced mainly consisted of *n*-alkanes (chain length 13, 15, and 17) while its incorporation into *E. coli* lead to the production of C14 and C16 *n*-alkanes due to the broad specificity of FabH2 for fatty acid initiation in comparison to the native FabH gene of *E. coli*.

Table 1 Comparison of bioalkanes with butanol and ethanol

Properties	Bioalkanes			*n*-butanol	Iso-butanol	Ethanol
	Jet fuel (jet A)	Diesel	Gasoline			
Density (kg/m^3)	775–840	837	770	810	802	788
Energy density (MJ/kg)	42	43	47	33	36	27
Kinematic viscosity (mm^2/s)	3.5 (20 °C)	2.6 (40 °C)	0.0004	3.6 (40 °C)	3.1 (20 °C)	1.2 (40 °C)
Flash point (°C)	40	52–96	−43	26–29	28	16.6
Autoignition temperature (°C)	210	210	257	343	415	363
Application	As jet fuel	As a transportation fuel for heavy duty vehicles	As a transportation fuel for light and medium vehicles	As a transportation fuel for light and medium vehicles	As a transportation fuel for light and medium vehicles	As a transportation fuel for light and medium vehicles

Table 2 List of alkanes found in cyanobacteria with sequenced genome

Cyanobacteria	Alkanes
Synechococcus elongatus PCC7942	$C_{15}H_{32}$ and $C_{17}H_{36}$
Anabaena variabilis ATCC29413	$C_{17}H_{36}$ and $C_{18}H_{38}$
Prochlorococcus marinus CCMP1986	$C_{15}H_{32}$
Cyanothece sp. ATCC51142	$C_{15}H_{32}$
S. elongatus PCC6301	$C_{17}H_{36}$ and $C_{15}H_{32}$
Synechocystis sp. PCC6803	$C_{17}H_{36}$
Nostoc sp. PCC7120	$C_{17}H_{36}$ and $C_{18}H_{38}$
Gloeobacter violaceus	$C_{17}H_{36}$
Cyanothece sp. ATCC51142	$C_{15}H_{32}$
Nostoc punctiforme PCC73102	$C_{17}H_{36}$

Bioalkanes are prevalent in a variety of microorganisms such as in cyanobacteria (Winters et al. 1969; McInnes et al. 1980; Dembitsky and Srebnik 2002) which are phylogenetically uniform, 50 of which have been sequenced completely and the data is available in the scientific domain. Among these, 10 cyanobacterial strains having sequenced genome have the potential to produce bioalkanes (Table 2) (Schirmer et al. 2010). These findings further broaden the research on the production of alkanes.

Advancement in metabolic engineering, systems and synthetic biology have paved a way to engineer the microbes to produce high-valued fuels such as bioalkanes and bioalkenes (Keasling 2010; Nielsen and Keasling 2011). Many factors should be taken care of before developing an organism to produce bioalkanes and bioalkenes such as engine type (compression or spark ignition), quality of combustion or ignition delay, cloud point, toxicity, stability, viscosity, lubricity, energy content, water miscibility, volatility and odor (Lee et al. 2008; Peralta-Yahya and Keasling 2010). The main hurdle in exploiting native hosts for the conversion of feedstocks into advanced high-valued biofuels such as bioalkanes and bioalkenes is to gain control over the endogenous regulation of pathways leading to biofuels production with high yields (Peralta-Yahya and Keasling 2010).

Recently, advances in the field of utilizing different non-edible feedstocks and innovative engineering of metabolic pathways for advanced biofuels production have been witnessed. The viability of these engineered metabolic pathways leading to advanced biofuels production has been revealed by the efficient conversion of CO_2, syngas, switchgrass and algal hydrolysate into free fatty acids, higher alcohols, and biofuels derived from isoprenoid compounds (Peralta-Yahya and Keasling 2010). In the current energy crisis situation, scientific community is eagerly seeking for feedstocks that can produce advanced biofuels cost-effectively with high yield. Genetic engineering of the biofuel producing pathways along with the choice of an appropriate substrate are the major challenges confronted by today's researchers for an optimal production of bioalkanes/bioalkenes.

3 Conventional Routes for the Production of Bioalkanes and Alkenes

In order to accommodate a future transition to renewable carbon sources, development of new process technologies is crucial. Potential biomass feedstocks viz., glycerides, lignocellulosics, platform compounds from biomass such as sugar alcohols, hydoxymethyl furfural, furfurals, levulinic acid, and monofunctional hydrocarbons (Deneyer et al. 2015; Zhang et al. 2014) can satisfy this transition. Bioalkane/bioalkene production is one such futuristic fuel which can be produced using these biomasses. Among these biomasses, non-edible/grazable lignocellulosics have the utmost potential in terms of their rich biochemical composition (15–30% lignin, 35–50% cellulose, and 25–30% hemicellulose) and the capability of eradicating the food, fodder versus fuel controversy. All these three biomolecules comprising the biomass have the potential to be converted to bioalkanes/bioalkenes. Lignin is a biopolymer of phenylpropanoid subunits and has high energy content as compared to holocelluloses. It has phenolic molecules such as *p*-coumaryl, coniferyl, and sinapyl alcohol along with their derivatives. Thus in order to transform lignin and its derivatives to bioalkane, deoxygenation of these molecules has to be conducted. Deoxygenation of lignin is generally carried out by using sulfide (cobalt or nickel-doped molybdenum sulfides) and nonsulfide catalysts (Pt/C, Pd/C, Ni, zeolite-supported nanocatalysts and ionic liquid-stabilized nanocatalysts). Sulfide catalysts are mostly used but they have an innate disadvantage of contaminating the product. Hydrogenolysis of biomass produce monomers (50%) and dimmers (15%) which can be further converted via deoxygenation and hydrogenation to short (C_6–C_9) and mid-range (C_{12}–C_{18}) cyclic alkane products (Zhang et al. 2014). An alternate one step process for producing mid-range alkanes is the coupling of phenolic monomers followed by conversion to bicycloalkanes (C_{12}–C_{18}). In addition to lignin, cellulose and hemicellulose, carbohydrate polymers also have the potential to produce alkanes/alkenes. Cellulose is a homopolymer of D-glucose and hemicellulose is a heteropolymer of C5 and C6 sugars. Hemicellulose is amorphous and has branched structure which makes it more accessible than cellulose. In order to convert these polysaccharides to alkanes, decoupling step is required. Among these two polymers, hemicellulose is more potent owing to is amorphous nature and thus more accessibility. For decoupling of these polymers, acidic pH and high temperature is maintained to convert them to their respective sugar monomers followed by deoxgenation to produce alkanes. Short and linear chain alkanes can be produced by combining the decoupling step along with the dehydration/hydrogenation step while multistep process is needed for branched or longer bioalkanes. During the conversion, initial step is the conversion of sugars into platform molecules such as γ-valerolactone, angelica lactone or hydroxymethyl furfural. Second step is coupling through aldol condensation, radical reaction, oligomerization or alkylation for long chain formation. Final step is the removal of functional group to obtain alkanes with various lengths (C_6–C_{31}), with or without cyclic and/or branched structures (Zhang et al. 2015). A schematic representation of

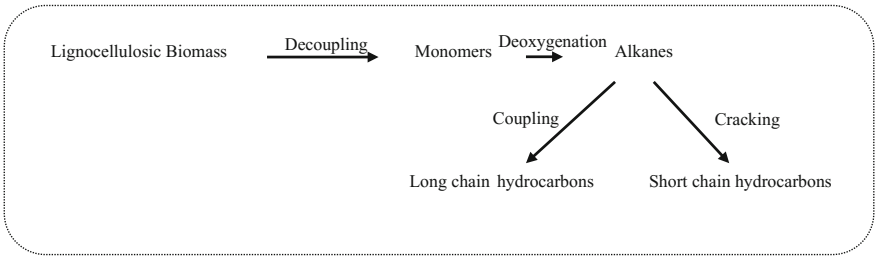

Fig. 1 Strategies for chemical processing of lignocellulosic biomass to alkanes. Cracking reagent and conditions: Catalyst- HZSM-5, 500 °C (Zhang et al. 2015); Coupling reagent and conditions: Catalyst- Pd, 473–523 K (Zhao et al. 2012); Decoupling reagent and conditions: Catalyst-Ru/C, Tungstosilicic acid (TSA) hydrate, water, 483 K (de Beeck et al. 2015)

the chemical processing of lignocellulosic biomass for alkane production is shown in Fig. 1.

4 Biological Route: A Novel Approach for the Production of Bioalkanes and Bioalkenes

The genesis of bioalkane/bioalkene production from biological sources emanated from a simple question about the role of microorganisms in producing fossil fuels and organic substances comprising the sediments. From this point onwards, a plethora of knowledge started arriving on the research arena concerning intracellular and extracellular hydrocarbon content of the microorganisms and a range of studies were conducted on deciphering the biosynthesis of these hydrocarbons within the microbes (Bird and Lynch 1974; Albro 1976; Weete 1976). These studies were highly complemented by the advent of gas/liquid chromatographic techniques which enabled the detection of hydrocarbons with high amount of precision.

4.1 Microbial Synthesis of Bioalkanes and Bioalkenes

Hydrocarbon production by microorganisms can be broadly categorized into internal and external depending upon their site of production. Internal hydrocarbons are widely found in a variety of microorganisms viz., bacteria, fungi and yeast where they serve the physiological role of cell membrane permeability but the yield of accumulation is low (0.005–2.69% in bacteria and 0.01–1.6% in yeast). The first report on the microbial hydrocarbon production dates back to 1944 when Jankowski and ZoBell (1994) reported the production of C_{10}–C_{25} aliphatic hydrocarbons by sulfate reducing bacteria maintained in a growth media containing

seawater along with fatty acids. Stone and ZoBell (1952) reported the production of hydrocarbons (0.03 and 0.25% of the biomass) by *Serratia marinorubrum* and *Vibrio ponticus* respectively when cultured in seawater peptone media. The type of hydrocarbons produced is the signature of the microorganisms as photosynthetic cyanobacteria has a unique ability to produce branched C18 hydrocarbons (7-methylheptadecane and 8-methylheptadecane) in a ratio of 1:1 (Ladygina et al. 2006). Studies have also shown that alkanes (chain length 17–20 carbon atoms) serve the purpose of biomarkers and indicate the contribution of cyanobacteria towards the formation of organic matter in the sediments (Brocks et al. 2003). The distinguishing quality of anaerobic phototrophic bacteria (*Rhodopseudomonas* sp., *Chlorobium* sp. etc.,) is the fact that they produce isoprenoids viz., pristine and phytane. *Clostridium* sp. produces hydrocarbons having a chain length of C_{11}–C_{35} among which the dominant ones are middle chain (C_{18}–C_{27}) or long chain (C_{25}–C_{35}) *n*-alkanes while *Desulfovibrio desulfuricans* produces alkanes of chain length C_{11}–C_{35} (Ladygina et al. 2006). Facultatively anaerobic bacteria of the genus *Vibrio* stores peculiar amount of *n*-heptadecane as 80% of the biomass is constituted by the hydrocarbons (Ladygina et al. 2006). Aerobic bacteria mainly represented by *Micrococcus* and *Sarcina* produces hydrocarbons (C_{23}–C_{30}) which are mainly monounsaturated and have a double bond at the centre of the chain while the chain endings are frequently marked with iso-methyl or anteiso-methyl branches (Ladygina et al. 2006).

Studies were also conducted on yeast accumulating hydrocarbons and it was observed that they accumulated nearly 0.01–1.6% of the cell dry weight under aerobic conditions and if grown anaerobically, *Saccharomyces* sp. has shown to accumulate up to 10.2% hydrocarbon by its dry cell weight (Ladygina et al. 2006). The hydrocarbons produced have a chain length of C_{10}–C_{34} and includes straight chain saturated, unsaturated, and branched hydrocarbons. The effect of carbon source on the type of hydrocarbon produced is significant as middle chain alkanes (C_{16}–C_{19}) are mainly produced by *C. tropicalis* when cultured in glucose, whereas long chain alkanes (C_{22}–C_{25}) are majorly produced when acetate or glycerol are used as the carbon source (Ladygina et al. 2006).

Investigations were also focused towards fungi as the source for hydrocarbon production and it was observed that it produces hydrocarbons majorly in its mycelia and spores and the content differed drastically between these two parts. In mycelia, about 0.06–0.7% of the dry biomass was the hydrocarbon content and mainly consisted of *n*-alkanes having a chain length of C_{15}–C_{36} while spores accumulated about 0.004–0.015% of the dry biomass and contained mainly odd numbered *n*-alkanes (C_{27}, C_{29}, and C_{35}). A summary of some of the important studies conducted in this area have been tabulated in Table 3.

From Table 3, it can be inferred that the yield of intracellular hydrocarbons is less and the only exception to the trend is *V. furnissii* which produces about 60% bioalkanes of its dry weight. Considering the low yield of internal hydrocarbons, their commercial viability as a transportation fuel is questionable considering the costly downstream processing step. Extracellular hydrocarbon production received prime importance owing to the possibility of the process to be commercialized.

Table 3 Intracellular hydrocarbons and their sources

Microorganisms	% of dry biomass	Major hydrocarbon (% of the total hydrocarbons)
Nostoc muscorum, Chlorogloea fritschii	0.025–0.12	*n*-C_{17} (68–90)
Coccochloris elabens	0.05–0.12	$C_{19:1}$ (85–98)
D. desulfuricans	0.8–2.25	*n*-C_{25}–C_{35}
V. furnissii	60.00[a]	*n*-C_{22}, C_{24}
E. coli	0.0035	*n*-C_{16}–C_{18} (22–28)
Bacillus sp.	0.33	*n*-C_{27}–C_{29} (61)
S. lutea	0.40	$C_{29:1}$ (43–79)
Saccharomyces sp.	0.04	*n*-C_{27}–C_{30} (69)
C. tropicalis	0.006–0.013	*n*-C_{16}–C_{19} (50–59)
Aspergillus sp.	0.06–0.70	*n*-C_{27} –C_{30} (47–62)

[a]Includes both intracellular and extracellular hydrocarbon content

Bacteria (*Desulfovibrio* and *Clostridium*) majorly produce extracellular hydrocarbons where the distinguishing feature with its intracellular counterpart is the low chain length, C_{19}–C_{21} and C_{16}–C_{18} respectively in case of *Clostridium pasteurianum* and *Desulfovibrio sulfuricans* (Bagaeva and Zinurova 2004). The exchange of hydrocarbons from the interior to the exterior of the cell through the cell membrane is attributed to the short chain length of the hydrocarbons (Bagaeva and Zinurova 2004). The mode of production was mostly anaerobic in nature along with the presence of essential mineral salts and calcium lactate. Belyaeva et al. 1995 reported the production of short chain aliphatic hydrocarbons (C_{14}–C_{25}) under anaerobic conditions while using mineral salts and calcium lactate as the medium composition.

4.1.1 Metabolic Pathways for Hydrocarbon Production

Cyanobacteria have the capability of producing hydrocarbons through natural process but the yield is low, as only 0.12% of the biomass can be converted to bioalkanes/bioalkenes (Coates et al. 2014; Winters et al. 1969). Thus it can be inferred that through natural route, bioalkane/bioalkene production is not a viable option thus genetic engineering should be adopted for maximizing the yield of bioalkanes. Generally hydrocarbons are produced via fatty acids and the processing involves deformylation and decarboxylation of fatty aldehydes. Some prokaryotes viz., *Feotgalicoccus* sp. synthesize bioalkane through cytochrome P450 mediated decarboxylation of fatty acids. Apart from these steps, alkanes can also be produced by head-to-head condensation of fatty acids, organisms which undergo this procedure include *Micrococcus luteus* ATCC 4698.

Within cyanobacteria two distinct metabolic pathways have been deciphered for the production of hydrocarbons from fatty acids. One pathway involves the

production of bioalkane from fatty acyl-ACPs via its conversion to fatty acyl aldehydes by fatty acyl-ACP reductase (FAAR) which is then converted to an odd chain length saturated alkane by the action of aldehyde deformylating oxygenase. A host of systems viz., plants, eukaryotes, prokaryotes, and humans also contain this catalytic machinery (FAAR). For this reaction to take place, NADPH is required since it provides a hydride for attacking the carbonyl of the fatty acid thioester. A schematic overview of the pathway is shown in Fig. 2 (Coates et al. 2014).

The second pathway is known as the olefin biosynthetic (OLS) pathway which was initially found in *Synechococcus* sp. and *Prochloron didemni*. This pathway was further modified by Mendez-Perez et al. (2011) where an alternate promoter was used to improve the productivity of the process. Gehret-Mccarthy et al. (2012) deciphered the structure of sulfotransferases (STs) in *Moorea producens* and *Synechococcus* sp. and observed that they had a similar structure and both the pathways lead to the production of bioalkanes. This pathway distinctly utilizes fatty acid as the substrate and produces odd numbered hydrocarbons with terminal olefinic bonds. Mendez-Perez et al. (2011) proposed that the OLS pathway includes ATP consuming fatty acyl ACP ligase (FAAL) which is responsible for transferring the fatty acid to OLS acyl carrier protein. The substrate bound to ACP then undergoes elongation by an extension module to add two carbons from malonyl-CoA thus reducing the β keto group. Gehret-McCarthy et al. (2012) observed that the final step of the OLS pathway involves sulfotransferase and thioesterase which transfers sulfonate from 3-phosphoadenosine-5-phosphosulfate to the β-hydroxyacyl substrate. This will activate the intermediate for catalysis using thioesterase (TE) to undergo hydrolysis from the enzyme surface followed by decarboxylation and desulfation to form terminal alkene product. The formation of terminal double bond is a unique structural signature of the OLS pathway compared to the FAAR/ADO pathway. A schematic overview of the OLS pathway is shown in Fig. 3.

Some of the eukaryotic organisms also produce hydrocarbons via fatty acid decarboxylation pathways. Examples of these organisms include *Botryococcus braunii* race A which utilize an aldehyde decarbonylase for the production of bioalkanes, *Gomphonema parvulum* (hormosirene, a pheromone) utilizes

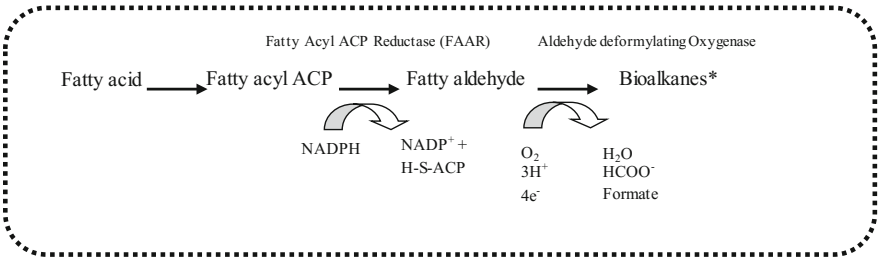

Fig. 2 FAAR/ADO pathway. Asterisk Heptadecane, 8-Heptadecene, 7-Methylheptadecane

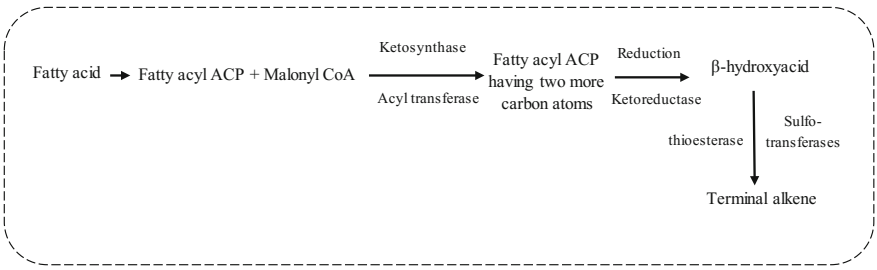

Fig. 3 OLS pathway for the production of terminal alkene

hydroperoxide lyase, lepidoteran insects (7-methyl heptadecane, a pheromone) utilize P450 cytochrome to conduct fatty acid decarbonylase reaction ultimately leading to the formation of bioalkanes. However the biosynthetic pathways in case of eukaryotic organisms are completely different in comparison to the cyanobacterial pathway.

Till now the biosynthetic pathways discussed concerned the intracellular hydrocarbon production. Apart from these pathways, metabolic pathways also exist for the production of extracellular hydrocarbons. The pathway initiates with the formation of acetate and formate from carbon dioxide, followed by their reduction to aldehydes and then to bioalkanes via aldol condensation. A schematic representation of the metabolic pathway is shown in Fig. 4 (Ladygina et al. 2006).

Since the yield of the production of hydrocarbons from microorganisms is not encouraging, therefore no experimental works have been conducted so far on bioconversion of biomass to bioalkanes/bioalkenes. Therefore, sophisticated biotechnological techniques should be explored for improving the yield of bioalkane/bioalkene production.

4.2 Genetic Engineering Approach

The drive for adopting genetic engineering techniques to modify microbes stems from the fact that naturally the yield obtained is insufficient to excite investors for putting their interest in this process. A number of studies have been conducted on *Synechococcus* sp. for improving the yield of bioalkanes/bioalkenes.

As per a study by Wang et al. 2013, genes responsible for biosynthesis of bioalkanes (*sll0208* and *sll0209*) were simultaneously over expressed and the corresponding bioalkane production got improved. Wang et al. (2013) also suggested that *Synechococcus* sp. mutants over expressing two copies of *sll0208* and *sll0209* in both the loci *slr0168* and *slr1556* lead to higher alkane yield in comparison to the over expression of the genes at a single site (*slr0168*). The final yield obtained was 0.44 and 0.5 g/L for wild type and mutant strain (multiple site) respectively. In another approach, genes for alkane biosynthesis in *Synechococcus*

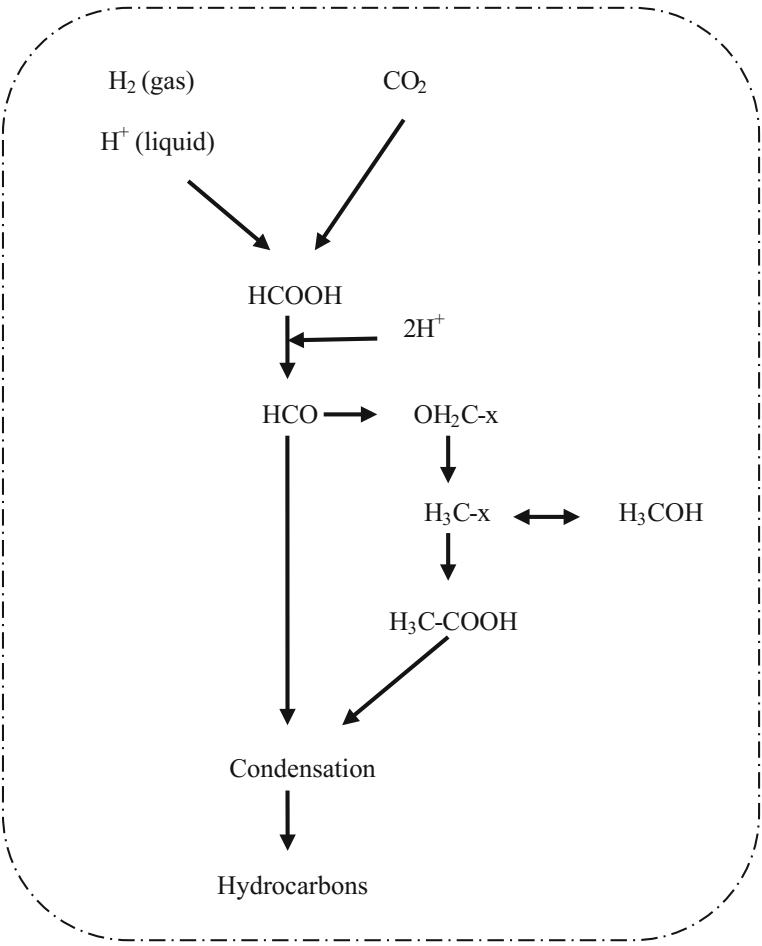

Fig. 4 Biochemical pathway for hydrocarbon synthesis by sulfate reducing bacteria

elongatus PCC7942 (*orf1593* and *orf1594*) and *Nostoc punctiforme* (*npun1710* and *npun1711*) were expressed in *Synechococcus* sp. PCC6803 (Wang et al. 2013; Ladygina et al. 2006). It was observed that the activity of AAR and ADO got increased thus converting more amount of acyl-ACP to alkane/alkene.

Kageyama et al. (2015) studied the improvement in bioalkane production by transforming *Anabena* sp. with the bioalkane synthetic genes from *A. halophytica* and it was observed that the desired transformation lead to enhanced bioalkane production. Buijs et al. (2015) reported that with the deletion of hexadecenal dehydrogenase *Hfd1* along with the expression of the gene responsible for bioalkane synthesis lead to the synthesis of long chain alkanes. Although a number of attempts have been made on improving the efficiency of alkane production through biological route, still the yield of bioalkane production is not encouraging.

This issue can be tackled if the alkane production biosynthetic pathway is introduced into a strain having the capability of producing high quantities of free fatty acids. Some of the techniques developed for improving the free fatty acid production are as follows (Ruffing et al. 2013)

a. **Gene knock out of the free fatty acid recycling enzyme, acyl-ACP synthetase (aas)**: Rationale behind this strategy is to stop the natural mechanism of free fatty acid (FFA) consumption as well as recycling. The mechanism of recycling is mainly done through acyl-ACP synthetase gene (aas). The gene "aas" is homologous to acyl-CoA synthetase and is mainly responsible for the consumption of FFA through β-oxidation pathway. Thus the strategy adopted was to knock out this gene so that both consumption and recycling will be stopped and thus the FFA's will be considered for bioalkane production. Using this strategy, Ruffing et al. (2013) successfully produced >40 mg/L of free fatty acids (FFAs) during the later stages of the stationary phase (after 200 h of incubation) for *Synechococcus elongatus* PCC7942 while the wild strain on the contrary accumulated negligible amount of FFA. Similar study was also conducted by Kaczmarzyk and Fulda (2010) on "aas" deficient *Synechocystis* sp. and *Synechococcus* sp. and observed free fatty acid concentration of 6.4 and 8.4 nmol mL^{-1} OD_{750}^{-1} respectively.
b. **Insertion of thioesterase for releasing fatty acids from the acyl carrier protein**: This strategy was adopted to detach fatty acids from the acyl carrier protein (ACP) so that the FFAs can be accepted as the substrates for bioalkane production. This ability of displacing free fatty acids (FFAs) from ACP is not found innately in *Synechococcus elongatus* PCC7942 thus, thioesterases from *E. coli* and *Chlamydomonas reinhardtii* were introduced into *Synechococcus elongatus* PCC7942 along with the knockout of "aas" gene (Ruffing et al. 2013). After conducting these modifications it was observed that approximately 30 mg/L of free fatty acids (FFAs) was produced in the *S. elongatus* PCC7942 but with no significant improvement in the yield in comparison to "aas" knockout. On the contrary Shin et al. (2016) successfully improved the production of FFAs by expressing signal sequence deficient acyl-CoA thioesterase I (TesA) in *Escherichia coli* cells and the major finding from their work was that the catalytic activity of TesA was more important than its expression for improving the FFA concentration.
c. **Enhancing the activity of RuBisCo**: RuBisCo is responsible for fixing carbon dioxide and is thought to be the rate determining step of photosynthetic organisms considering the fact that it has low affinity for carbon dioxide and also due to its reversible nature. Thus its over expression may lead to improved production of FFAs. However, when it was over expressed along with "aas" knockout and TE expression, no significant improvement was observed. While the expression of nonnative RuBisCO subunits (*rbcLS*) in *Synechococcus* sp. PCC 7002 using *psbAI* promoter lead to greater than three folds increase in FFA production with a concentration of >130 mg/L (Ruffing 2014).

d. **Improving the conversion efficiency of acetyl-CoA to malonyl-CoA**: This step is a key regulator where the flux of carbon can be controlled for either FFA production or energy generation and production of key biomolecules required for cell growth. Thus controlling this step will allow the optimum flow of carbon towards FFA production without compromising the cell growth. This step is controlled by the enzyme acetyl-CoA carboxylase (ACC) and if the gene corresponding to this enzyme is expressed then FFA production might improve. Similar quest was tried where four ACC genes (accBCDA) from *C. reinhardtii* CC-503 were expressed in *S. elongatus* PCC7942 along with "aas" deletion, TE expression and RuBisCO overexpression and it was observed that a mere 20 mg/L (approx) of FFA was produced (Ruffing et al. 2013). Detailed pathway of alkane biosynthesis along with the key step to control has been highlighted in Fig. 5.

After screening all the available techniques for genetic manipulation it can be inferred that if alkane production machinery is successfully inserted into a high FFA producing organism, then there might be a chance for competitive yield of bioalkane/bioalkene for commercial applications. Thus there is an immense potential for metabolic engineers and molecular biologists to work together for engineering a suitable strain by cloning the required gene and its proper expression

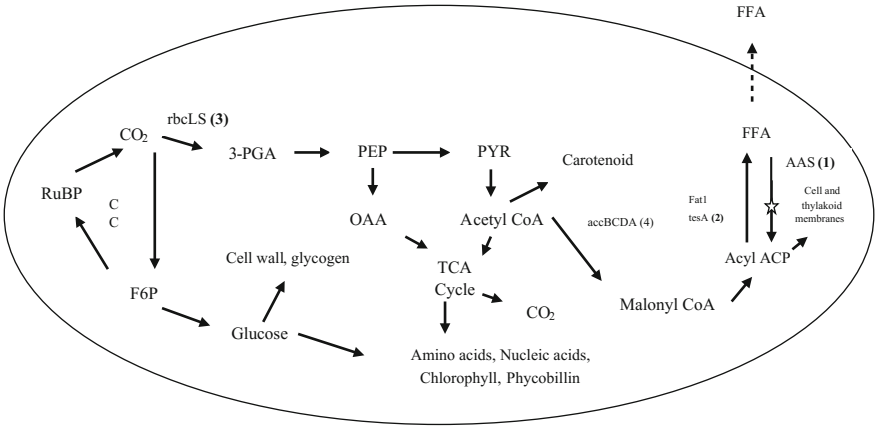

Fig. 5 A pictorial view of the metabolic pathways of *Synechococcus elongatus* PCC7942. 3-PGA: 3-Phosphoglycerate; aas: acyl-ACP synthetase; ACC: Acetyl-CoA carboxylase; accA: ACC subunit, carboxyltransferase α subunit; acein; accC: ACC subunit, biotin carboxylase subunit; accD: ACC subunit, carboxyltransferase β subunit; ACP: Acyl carrier protein; F6P: Fructose-6-Phosphate; *fat1*: acyl-ACP thioesterase from C. reinhardtii CC-503; FFA: Free fatty acid; CC: Calvin cycle; rbc: RuBisCO; rbcL: RuBisCO large subunit; rbcS: RuBisCO small subunit; RuBisCO: ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBP: ribulose-1,5-bisphosphate; TE: thioesterase; tesA: truncated thioesterase from E. coli; TCA: tricarboxylic acid; PEP: phosphoenolpyruvate; OAA: oxaloacetate; PYR: Pyruvate. The digits (1–4) denote the four strategies for improving the FFA production. denote deletion of the corresponding step

for hyperactive yield of bioalkane/bioalkene. Though it is not a very easy task to complete but with continuous efforts and by adopting integrated approach, the purpose of addressing the enigma of finding a potential biofuel can be addressed.

4.3 Application of Novel Enzymes

One of the crucial enzymes involved in alkane production is aldehyde deformylating oxygenase (ADO) since it converts the aldehydes to alkanes in cyanobacteria along with the formation of formic acid. During the reaction, NADPH provides the electrons by ferrodoxin-NADP reductase and ferrodoxin or via phenazine methosulfate. Studies conducted on ADO have suggested that the catalytic turnover of the enzyme is only 3–5 per catalytic site which justifies the low yield of the hydrocarbon production from the microbial source. Even more worrying factor is the inhibition of ADO by hydrogen peroxide which drastically limits its application for an efficiently converting aldehyde to alkane. This issue was tackled ingeniously by Andre et al. (2013) who successfully negated the inhibition caused due to hydrogen peroxide by applying a fusion protein which could encode both catalase and ADO leading to a two folds improvement in the catalytic conversion efficiency of ADO. Catalase was used since it had the capability of converting peroxide to water and oxygen and thus relieving ADO from inhibition.

5 Bottlenecks in the Production of Bioalkanes and Bioalkenes

Biotechnological means of bioalkanes/bioalkenes production is majorly limited by the product toxicity. Alkanes have effect on disorganizing cytoplasmic membrane integrity and its vital functions, such as the loss of ions, metabolites, proteins, and lipids, and the dissipation of the electrical potential and pH gradient. The toxic effect of alkanes (C_9 and C_{10}) have been examined with *S. cerevisiae* using transcriptome analysis by Ling et al. (2013) who suggested that these alkanes influenced various cellular metabolism such as induction of efflux pumps, membrane modification, energy supply, and radical detoxification. Cytosolic alkane toxicity can be overcome by inducing efflux pumps to facilitate alkane secretion from the cell. Heterologous expression of the efflux pumps "SNQ2P and PDR5P" could lower the toxicity of alkanes by reducing intracellular level of alkanes (Ling et al. 2013).

Apart from the product toxicity, limited supply of fatty acid biosynthetic (FAB) precursors and NADPH (cofactors) lowers the yield of alkane production. This problem can be dealt by heterologous expression of $NADP^+$-dependent

glyceraldehyde-3-phosphate dehydrogenase (GAPN) which can enhance the NADPH supply and improve the productivity of fatty acid-derived biofuels.

6 Conclusion

A detailed insight into the research concerning bioalkane/bioalkene synthesis through various means viz., chemical and microbial was conducted in this chapter. From this exercise it can be concluded that a myriad of chemical routes exist which can be efficiently used for bioalkane/bioalkene production. The most effective technique is deoxygenation which can utilize lignin and its derivatives (byproducts of a bioethanol refinery) for bioalkane/bioalkene synthesis. From the review on biotechnological perspective, it can be inferred that a majority of microorganisms have the innate capability of producing hydrocarbons out of which some are produced internally while the other are extracellularly produced. Hydrocarbons produced by the microorganisms serve as the biomarkers which indicate their source of its production. The type of hydrocarbons produced is hugely dependent upon the type of carbon source supplied in the growth media. Naturally the yield of bioalkane/bioalkene production from microorganisms is poor thus limiting its use for commercial applications and hence there is a huge demand for metabolic engineering tools to improve the yield. Studies carried out on metabolic engineering applications for improving bioalkane/bioalkene synthesis have suggested that if the required genetic machinery is expressed in high free fatty acid producing strain then there might be a chance for the commercialization of the process.

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Algal Biorefineries for Biofuels and Other Value-Added Products

Madhulika Shukla and Sachin Kumar

Abstract Biorefining refers to sustainable biomass transformation through different conversion routes and equipment in order to obtain energy, biofuels, and high-value products. A variety of sustainable but low-cost biomass-based industries can be framed using biorefinery concept. Among various feedstocks, algae are also proving its firm candidature for biorefinery processes. This chapter describes the general characteristics of microalgae and their potential to be used as a raw material in the biorefinery process. It also focuses on the products obtained from microalgae, mainly biofuels and different pathways employed in biomass fractionation for other valuable products.

Keywords Biofuels · Biomass processing · Biorefinery
High-value products · Microalgae

1 Introduction

Algae are the photosynthetic organisms belonging to lower classes of the plant kingdom. There are more than 30,000 species inhabiting diverse areas such as ponds, lakes, hot springs, tree barks, snow, etc. (Ramos-Suarez et al. 2015). Their omnipresent nature shows their ability to flourish easily in a wide range of temperature, pH, etc. They can be unicellular or multicellular, solitary, or in colonial form. Algae ranges from few micrometers in cell diameter or some forms grow up to size of several feet such as giant kelps. About 70% of the oxygen on this earth is produced by them. They do not have true roots, stems, leaves, and vascular tissues

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and have simple reproductive structures. These are the primary producers and constitute the base of the food chain. From the physiological and biochemical view, algae are more or less similar to higher plants. Algae possess almost same biochemical pathways. They also have chlorophyll a and almost same carbohydrate and protein end products. Besides, they also conserve varying amount of lipids. Most of the algal species have been analyzed for their chemical composition (Table 1).

The main phylogenetic groups of algae comprise Chlorophyta, Diatoms, Euglenophyta, Dinoflagellata, Chrysophyta, Phaeophyta, and Rhodophyta. Cyanobacteria are traditionally included among algae and exhibit industrial applications. Cyanobacteria are photosynthetic aquatic bacteria and prokaryotic in nature while other algal groups are eukaryotes. Each phylum of algae differs in appearance depending on the pigment they possess. The idea of using algae as a source of food, feed, and energy goes back more than half a century. In the Southeast Asia and Japan, algae are widely consumed as food.

Table 1 Composition of some algae species (% in dry biomass) (Ravindran et al. 2016)

Strain	Proteins	Carbohydrates	Lipids
Anabaena cylindrica	43–56	25–30	4–7
Botryococcus braunii	40	2	33
Chlamydomonas reinhardtii	48	17	21
Chlorella pyrenoidosa	57	26	2
Chlorella vulgaris	41–58	12–17	10–22
Dunaliella bioculata	49	4	8
Dunaliella salina	57	32	6
Dunaliella tertiolecta	29	14	11
Euglena gracilis	39–61	14–18	14–20
Porphyridium cruentum	28–39	40–57	9–14
Prymnesium parvum	28–45	25–33	22–39
Scenedesmus dimorphus	8–18	21–52	16–40
Scenedesmus obliquus	50–56	10–17	12–14
Scenedesmus quadricauda	47	–	1.9
Spirogyra sp.	6–20	33–64	11–21
Spirulina maxima	60–71	13–16	6–7
Spirulina platensis	42–63	8–14	4–11
Synechococcus sp.	63	15	11
Pseudochoricystis ellipsoidea	10.2	34	38
Chlorogloeopsis fritschii	41.8	37.8	8.2
Chlorella emersonii	9.03	37.9	29.3
Chlorella zofingiensis	11.2	11.5	56.7
Chlorella FC2 IITG	10.4	24.5	37.3
Tetraselmis maculata	52	15	3

Fleeting supply of petroleum-based fuels and their contribution to environmental pollution encourage the diversion toward clean and renewable energy. To tackle these issues, environmental policies have been introduced, which favor the research, development, and use of biofuels around the world, mainly in the transportation sector (Demirbas 2008). Biofuels offer many benefits associated with energy security, economic stability, and reducing the environmental impact of greenhouse gases (Popp et al. 2014).

Biofuels derived from the food crops such as sugar cane (Goldemberg et al. 2008), beet (Mortimer et al. 2004), corn (Franceschin et al. 2008), and soy (Olivares-Carrillo and Quesada-Medina 2011) belong to the first generation. Among the various first-generation biofuels, bioethanol and biodiesel are highly investigated till now. Bioethanol production via fermentation of corn and sugar cane (Sarris and Papanikolaou 2016) is in vogue. Similarly, producing biodiesel through transesterification of oilseed feedstocks is also in trend. Availability of the ample raw materials such as sugar and oil is associated with high productivity of these crops. However, their sustainability is questioned by their demand of large land area for farming as well as imposing a threat to the food industry. Moreover, the balance of carbon from bioethanol made from corn can be less favorable than that generated by using fossil fuels (Searchinger et al. 2008).

Biofuels derived from nonfood crops are categorized under second-generation biofuels. It mainly includes lignocellulosic material resulting from crop residue, forestry residue, agro-processing waste, energy plants, etc. (Vohra et al. 2014).

Biofuels that originate from raw materials other than crops specifically microbes and microalgae are termed as third-generation biofuels. Based on current scientific knowledge and the projections of technology, they are considered a viable energy resource without the disadvantages associated with those of the first- and second-generation biofuels. With emerging technologies for biomass processing, the demonstration facilities and pilot plants are being developed; however, these are yet to be commercialized (Goh and Lee 2011).

Microalgae are potential feedstocks for biofuel production (Mata et al. 2010). These are single-celled photosynthetic organisms inhabiting different environments. Algal strains are adaptive to variable temperatures, pH, and nutrient availability. Besides, they can be grown on nonarable land and may not require freshwater. High productivity is easily achieved in warm, tropical, and subtropical regions around the year (Béchet et al. 2016). Their growth rates are 30 times higher than other plants. The biomass availability throughout the year makes a continuous biofuel production system which is similar to the petroleum refineries. Microalgae contribute to sequester atmospheric carbon as it has high photosynthetic efficiency as compared to other plants. The oil productivity of many microalgae exceeds even the best oil-producing crops. In this way, these biofuels are fulfilling the criteria of economic and environmental susceptibility (Schenk et al. 2008).

2 Algae-Based Biorefinery—Promise and Prospective

A sustainable and economic biorefinery system should utilize all the biomass components such as proteins, carbohydrates, and lipids. In similar manner, algal-based biorefinery offers production of wide range of biofuels and chemicals by integrating different improved technologies for biomass processing. The technologies chosen should have a low environmental impact for feasibility of biorefinery working. There are several factors which affect the sustainability of a biorefinery as shown in Fig. 1.

Availability of feedstock is an important factor for running a biorefinery. Microalgae being available throughout the year are interesting feedstock to be fractionated into useful compounds in a biorefinery. Commercial production of algae is achieved by cultivating in open raceways or in translucent containers called photobioreactors. A hybrid approach combining closed-system pre-cultivation with subsequent open system for growing algae is doing well in terms of productivity. Algal culture can be well correlated with the water bioremediation and carbon dioxide mitigation. Industrial wastewater, desalination plant rejects, and flue gases can be utilized for algal growth. The energy products (biodiesel, methane, ethanol, and hydrogen) as well as nonenergy products (nutraceutical, fertilizers, animal feed, bioactive compounds, other bulk chemicals, and polymers) can be obtained from processing functional biomass components. The promising products for different

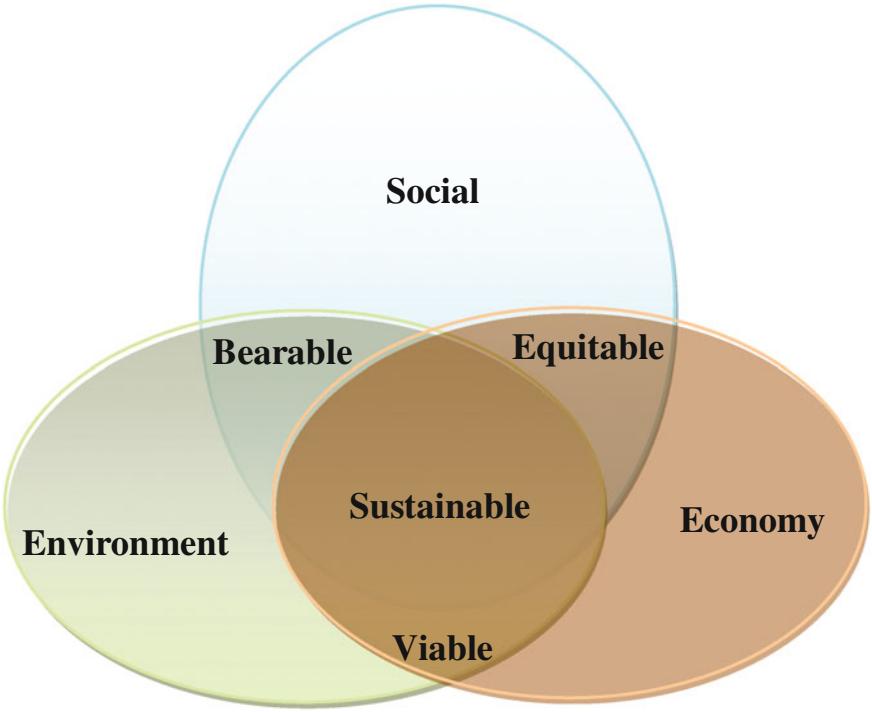


Fig. 1 Important factors imposed the sustainability of a biorefinery

sectors through algal biorefinery are shown in Fig. 2. Algae accumulate bioproducts such as proteins, lipids, carbohydrates, starch, vitamins, and their amount can be modulated considerably with the growth conditions and the type of algal species (Su et al. 2014; Yu et al. 2015). In general, green algae accumulate lipids and starch; diatoms conserve only lipids while golden algae build up lipids and carbohydrates as main reserve material (Ree and Annevelink 2007). Not only the amount but also the composition of lipid also varies with algal strains used. Selective fractionation of lipids from microalgae can be transformed into biodiesel and essential fatty acids (DHA, EPA) or can be used in chemical industries. Apart from lipids, algae also produce proteins, isoprenoids, and polysaccharides. Carbohydrates are processed into bulk chemicals and fuels while proteins are utilized for food and feed (PUFA, vitamins, etc.). A variety of polysaccharides, enzymes, pigments, antibiotics, antioxidant, metabolites, and minerals can also be extracted during the downstream processing of algal biomass. A range of liquid fuels such as biodiesel, bioethanol, biogasoline, biomethanol, biobutanol, biohydrogen, etc., can be obtained. The oxygen produced in photosynthetic growth of algae can be recovered as well.

2.1 *Prospects of Algae Biofuels*

Algae are considered as a promising oleo-feedstock owing to high yield of lipid content. As per literature, potentially around 25–50 ton of oil can be produced per hectare per year for relevant algae species. Among other biochemicals, microalgae also contain neutral lipids (tri-, di-, and monoglycerides free fatty acids), polar lipids (glycolipids, phospholipids), wax esters, sterols, and pigments. The total lipid content in microalgae varies from 1 to 90% of dry weight, depending on species, strain, and growth conditions (Chisti 2007; Li et al. 2008). Microalgae are the

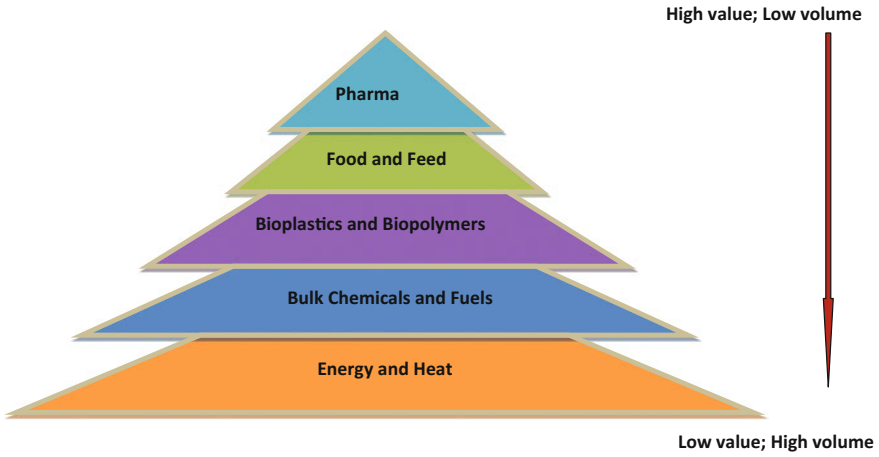


Fig. 2 Sectors promoted through an algal biorefinery

fastest growing photosynthesizing organism capable of completing an entire growing cycle every few days. Up to 50% of algae's weight is comprised of oil, compared with, for example, oil palm which yields just about 20% of its weight in oil. Extensive R&D efforts are going worldwide with a high number of start-up companies developing different options for commercializing algae farming.

Currently, bioethanol and biodiesel are the main focus in the biofuel market. Besides many advantages of algal fuels, cultivation of algae for biofuel industry still seems not profitable in itself because of the high cost involved in biomass production, harvesting, drying, and oil extraction (in case of biodiesel). Research is going on to cut the cost at each step. Recycling of water and nutrients and extraction of coproducts has also been worked out to reduce the biofuel production costs. The coproducts include nutraceuticals, fertilizers, and further utilization of residual algal biomass after oil extraction. Glycerol produced in transesterification reaction is also an important compound for chemical industries.

The algal biomass can be transformed into various biofuels and coproducts through different conversion routes. The processes employed can be biochemical, thermochemical, or chemical ones. The basic processes and their respective products are discussed as under.

2.1.1 Transesterification

The downstream processing of algal biomass involves harvesting and drying followed by lipid extraction to produce biodiesel. Harvesting and drying of algae are energy-intensive processes and account for 30% of total production costs (Grima et al. 2003). Methods for the harvesting of algae include concentration through centrifugation, foam fractionation, flocculation, membrane filtration, and ultrasonic separation. Each method has its own pros and cons. Lipids from the algae are extracted by French press, autoclaving, bead beating, etc., assisted by using organic solvents (Topare et al. 2011; Afify et al. 2010). The extracted oil is converted into fatty acid methyl ester (FAME) through transesterification. The reaction takes place in the presence of alkali/acidic catalyst or some enzymes such as lipases and alcohol (mainly methanol, ethanol). Glycerol is produced as a by-product in this reaction. Direct transesterification of wet algal biomass not only gives a higher yield in less time but also generates less waste (Cao et al. 2013; Hidalgo et al. 2013). The higher degree of unsaturation of microalgal oil makes it more vulnerable to oxidation, which can be reduced by partial catalytic hydrogenation. Before being launched in the market for commercial use, biodiesel must fulfill the criteria as indicated in the standard methods such as ASTM Biodiesel Standard D 6751 (United States) or Standard EN 14,214 (European Union). The biodiesel is fully miscible with the diesel and increases the lubricity. Therefore, when it is used in internal combustion engines with compression ignition, it improves the engine performance. Further, it is biodegradable and nontoxic (Encarnação 2008).

2.1.2 Fermentation

Referring to various biochemical constituents, macroalgae and some microalgae such as *Porphyridium, Chlorella, Dunaliella, Chlamydomonas, Scenedesmus, and Spirulina* contain >50% of the dry weight of starch, cellulose, and glycogen, which can be fermented into ethanol fuel (Chen et al. 2009). *Chlorella* contains about 30–40%of sugars, which greatly increases their usefulness in the production of biofuels (Kamiński et al. 2011). Microalgae can produce a much higher yield of ethanol as compared to other feedstocks (Table 2). The process needs low energy input and is uncomplicated as compared to biodiesel production. Microalgae provide carbohydrates in the form of glucose, starch, and other polysaccharides and proteins that can be used as carbon sources for fermentation by bacteria, yeast, or fungi (Harun et al. 2010). Macroalgae have mannitol, laminarin, fucoidan content, etc., as carbohydrate reserve. The structural carbohydrates, cellulose, and alginates in the cell wall can also be utilized after degrading it into monomers such as glucose and galactose (Nigam and Singh 2011). Algae utilize the CO_2 produced as by-product from the fermentation process in its cultivation, and therefore reduce the global warming. However, the production of bioethanol from microalgae is still under investigation, and this technology has not yet been commercialized (Harun et al. 2010).

Pretreatment of biomass is needed to convert complex carbohydrates entrapped in the cell wall, and then its release prior to fermentation. These may be acid, alkali, hot water pretreatment, etc. However, the acid pretreated biomass is better than other pretreatments (Harun and Danquah 2011). The highest ethanol concentration was found to be 7.20 g/L in *Chorococcum* sp. from 15 g/L biomass at 140 °C using 1% (v/v) sulfuric acid for 30 min. Kumar et al. (2013a) achieved ethanol yield of 0.43 g/g sugar obtained from the residue of red macroalgae after agar extraction. Horn (2000) employed *Pichiaangophorae* for conversion of glucose and mannitol derived from brown macroalgae and obtained an ethanol yield of 0.43 g/g sugar. Therefore, for higher yield of ethanol, mannitol-fermenting microorganisms are required (Ota et al. 2013). The bioethanol production is influenced by acid concentration, temperature, and amount of microalgae.

Table 2 Ethanol yield from different feedstocks (Mussatao et al. 2010)

Source	Ethanol yield (gal/acre)	Ethanol yield (L/ha)
Corn stover	112–150	1050–1400
Wheat	277	2590
Cassava	354	3310
Sweet sorghum	326–435	3050–4070
Corn	370–435	3460–4020
Sugar beet	536–714	5010–6680
Switch grass	1150	10,760
Microalgae	5000–15,000	46,760–140,290

The algal biomass can be used for the production of ABE (acetone, butanol, and ethanol) through the fermentation process. Biobutanol is a very attractive biofuel as it is nonhygroscopic, has a relatively low heat of vaporization, and is less corrosive than ethanol (Kamiński et al. 2011). Butanol has a high calorific value, which is 29.2 MJ/dm^3 (melting point—89.5 °C, boiling point 117.2 °C, flash point 36 °C, and the self-ignition 340 ºC). It is colorless and flammable biofuel which can be easily mixed with the fossil gasoline because of its low vapor pressure. Therefore, it can be used directly in the vehicles without any modification in the existing engine models. All these features enhance its usefulness both as an additive to gasoline, as well as direct to use biofuel.

Biobutanol can be produced by fermentation of sugar-rich algal biomass through ABE fermentation. *Clostridium acetobutylicum* or *C. saccharoperbutylacetonicum* and *C. beijerinckii* are commonly used in ABE fermentation process. The biomass used can be pretreated or the pretreatment step can be skipped. However, this will affect the yield of the product. The pretreated biomass gave higher yield (2.74 g/L of total ABE) as compared to non-pretreated biomass (0.73 g/L of total ABE) from wastewater grown algae (Ellis et al. 2012). In this case, fermentation was done by using *C. saccharoperbutylacetonicum* N1–4, a genetically modified strain. In a pilot study, 0.29 g butanol/g sugar was produced from the hydrolysate of *Ulva lactuca* by fermenting with *C. saccharoperbutylacetonicum* (Potts et al. 2010). Using microalgae biodiesel residues as substrate, *C. acetobutylicum* produced 3.86 g/L of butanol and achieved butanol yield of 0.13 g/g carbohydrate via ABE fermentation (Cheng et al. 2015). More research needed for biobutanol production from microalgal biomass as such fuels have great potential in the biofuels industry.

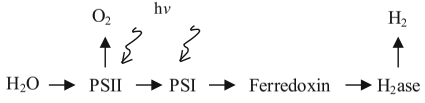
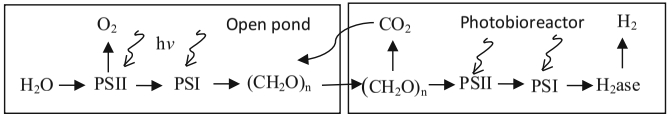
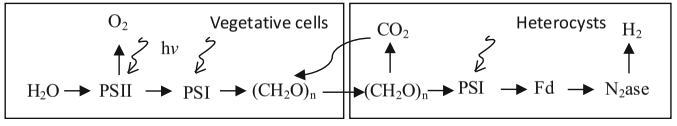
However, the large-scale use of biobutanol is restricted due to low yield and its separation from fermentation broth. Unlike ethanol, distillation is not applicable for butanol separation. The separation and purification are generally performed by adsorption, gas stripping, or using ionic liquids. It can be achieved either through direct application of the liquid in the bioreactor and separation of butanol outside of bioreactor or directing fermentation broth outside the bioreactor and separation of butanol in the membrane contractor.

2.1.3 Biophotolysis/Photofermentation

The fundamental basis of biological hydrogen production is the presence of hydrogen producing enzymes. Among these, the nitrogenase, Fe hydrogenase, and NiFe hydrogenase are commonly known. These enzymes contain metallo-clusters as active sites for catalyzing the reaction. Different routes of hydrogen production from microalgae/cyanobacteria are described in Table 3.

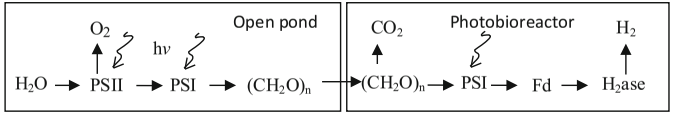
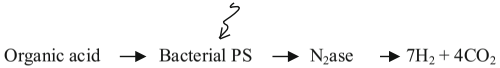
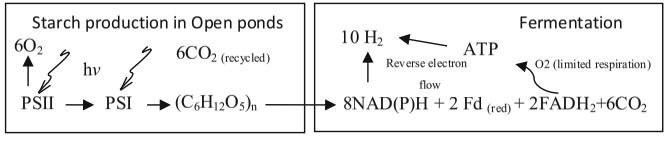
The direct biophotolysis involves splitting of water using solar energy to produce hydrogen in presence of enzyme hydrogenase (Ghirardi et al. 2000). Water splitting also produces oxygen, which inhibits the hydrogenase enzyme implying that hydrogen production is self-limiting (Tamburic et al. 2011). The oxygen should be removed frequently for higher yield and longer duration of hydrogen production.

Table 3 Biohydrogen production through various physiological routes (modified from Hallenbeck and Benemann 2010)

Mechanism	Organism	Reaction	Photons required/mole of H_2	Inferences
Direct biophotolysis	Green algae/cyanobacteria		4	Inhibition of H_2 production by O_2, photobioreactor design, H_2–O_2 mixture
Direct biophotolysis with respiratory O_2 uptake	Green algae/cyanobacteria		9	Production of oxygen, Photobioreactor design
Indirect single-stage biophotolysis	Filamentous heterocystous cyanobacteria		7	Production of H_2–O_2 mixture

(continued)

Table 3 (continued)

Mechanism	Organism	Reaction	Photons required/mole of H_2	Inferences
Indirect two-stage biophotolysis	Microalgae		7	Number of photons required by the PSI in the second stage
Photofermentation	Dissimilation of organic acids present in microalgae/cyanobacteria by photosynthetic bacteria		Not known	Low efficiency of bacteria
Indirect biophotolysis respiration assisted	Microalgae		6	Hypothetical
Hydrogen methane by two-step anaerobic digestion	Microalgae/cyanobacteria	$C_6H_{12}O_6 + (1.2 - 2.4)\ H_2O \rightarrow (1.2 - 2.4)\ H_2 + (2.4 - 2.7)\ CH_4 + 3CO_2$	Not applicable	Limited H_2 yield

To overcome the oxygen hindrance in enzyme activity, algae are first grown in normal conditions for cell growth. The grown cells are then subjected to sulfur-deprived medium, which creates anaerobic conditions. In this way, hydrogen production can be sustained for a week. It is estimated that this two-step process can theoretically yield 198 Kg hydrogen ha^{-1} day^{-1} (Melis and Happe 2001). The use of oxygen absorbers–reversible (hemoglobin) or irreversible (glucose/glucose oxidase, dithionite) (Rosenkranz and Krasna 1984) and endogenous respiration by microalgae could be the possible ways to overcome oxygen sensitivity issues.

In indirect biophotolysis, H_2 and O_2 evolution takes place in separate stages coupled through CO_2 fixation/evolution. The carbohydrate-rich biomass is subjected to anaerobic dark fermentation which produces hydrogen and acetate. The acetate molecules further degrade to produce more hydrogen molecules.

Dark fermentation of starch-rich algal biomass generated in first stage light conversion process also leads to hydrogen production. The majority of microbial hydrogen production is driven by the anaerobic metabolism of pyruvate, formed during the catabolism of various substrates.

The obstacles for commercial hydrogen production by dark fermentation are the low hydrogen production yield and efficiency, which occur because the residual solution obtained during dark fermentation contains large quantities of soluble metabolite products (SMPs), such as acetate and butyrate, which lead to environmental pollution and energy wastage(Cheng et al. 2011). A biphasic process combining dark- and photofermentation can markedly enhance theoretical hydrogen yield from 4 to 12 mol H_2/mol glucose and reduce more than 94.5% of SMP emission (Su et al. 2009, 2010). The actual potential of hydrogen from algae is restricted due to high costs associated with large-scale photobioreactors and by low photochemical efficiencies.

2.1.4 Anaerobic Digestion

Biomass with high water content of about 80–90% is ideal for anaerobic digestion to produce biogas which is mainly composed of 69–75% of methane (Sialve et al. 2009). The energy spent in lipid extraction from algae can be recovered with biogas production. However, the traces of sulfurated amino acid produced in the process count for insignificant proportion of hydrogen sulfide formation which otherwise causes corrosion. Biogas production involves basically three steps to decompose biomass by microbes under strict anaerobic conditions. The process initiates with hydrolysis of proteins, carbohydrates, and lipids into soluble sugars. Lipids are generally hydrolyzed slower as compared to the other compounds. The released sugars are then fermented to volatile fatty acids, alcohol, hydrogen, and carbon dioxide. These fermented products are subjected to methanogenesis to form methane and carbon dioxide (Cantrell et al. 2008). The trace metals found in algae facilitate the methanogenesis, and nutrient released in the slurry can be recycled for further use in next batch of algal cultivation. However, the nitrogen present in microalgae protein releases ammonia, which lowers the pH of the system. Low pH

inhibits the microbial activity and no biogas is produced. A similar inhibitory effect is observed with the presence of sodium ions at concentrations above 0.5 M (Sialve et al. 2009). To balance this carbon/nitrogen ratio, carbon from the waste paper can be used. For this purpose, waste paper and algal biomass can be co-digested in 50:50 ratio to increase methane yield (Yen and Brune 2007). Carbon and nutrient recycling will add up to the sustainability of biogas production process (Stephens et al. 2010).

2.1.5 Gasification

Syngas is primarily composed of H_2, CH_4, CO, and CO_2. It is produced by the gasification of algal biomass at high temperature (800–1000 °C) via the partial oxidation using oxygen and steam. Syngas can be either used as fuel or further processed to synthesize other chemicals such as methanol. Theoretically, thermodynamic efficiency of gasification is reported to be 76 and 92% of the chemical energy, which can be recovered in this process (Calzavara et al. 2005). The effluent released in the processing contains high nutrient levels which can be recycled for algal growth. The gas yield can be increased by varying operating conditions, feeding rate, and suitable catalyst (Guan et al. 2012; Chakinala et al. 2010).

2.1.6 Hydrothermal Liquefaction

Drying of algal biomass and use of solvents elevate the cost of biodiesel production. Therefore, alternative methods have been explored for economic production of biofuels. Hydrothermal liquefaction (HTL) is the method of dissolving organic compounds in subcritical water at high temperature (200–400 °C) to produce biocrude which contains over 1000 compounds (Lam and Lee 2012) and has the same energy content to that of fossil crude (Brown et al. 2010). It comprises light and heavy fractions, along with gaseous, aqueous, and solid by-product fractions.

The organic phase produced by HTL contains oxygen and nitrogen which are removed using catalysts (process known as deoxygenation and denitrogenation). The oxygen and nitrogen are converted to CO_2 and ammonia, respectively. Traces of sulfur are also present in the biocrude, which are converted to hydrogen sulfide. Ammonia is recycled for algal cultivation. This process exhibits a positive net energy balance of 45.3 kJ per kg of biocrude produced. The biocrude is compatible with the existing biorefinery technology. Microalgal liquefaction produces high yields of biocrude, with the maximum reported yield of 64 wt% (Jazrawi et al. 2013; Elliott et al. 2013, 2015). Almost 90% energy can be extracted from the biomass in the form of biocrude. Hydrothermal liquefaction (HTL) process converts different algal strains with high moisture content to high bio-oil yields with lower coke and lower energy consumption in comparison to other methods.

2.1.7 Hydrocracking

Hydrocracking of the algal biomass generates aviation fuel as main product. The other fractions produced are diesel, gasoline, kerosene, naphtha, or light gas fractions. Hydrocracking basically coverts high sulfur compounds into clean-burning fuels having low sulfur content.

Biogasoline is a hydrocarbon containing 6–12 carbon atoms per molecule and can be used in internal combustion (IC) engines. Biogasoline is produced by direct conversion of sugar gasoline. Biogasoline is created by turning sugar directly into gasoline (Ondrey 2010). Virent Energy Systems, Inc is the pioneer in establishing biogasoline demonstration plant in Madison, WI. A technique named bioreforming is introduced, where aqueous phase reforming (APR) is combined with catalytic processing for biogasoline production (http://www.virent.com/technology/bioforming/). The aqueous solution containing a range of polysaccharides, furfurals, organic acids, and carbohydrates including hexose and pentose sugars is converted into hydrocarbons. The aqueous phase reforming step utilizes heterogeneous catalysts at moderate temperatures and pressures to reduce the oxygen content of the carbohydrate feedstock. Some of the reactions in the APR step include (1) reforming to generate hydrogen; (2) dehydrogenation of alcohols/hydrogenation of carbonyls; (3) deoxygenation reactions; (4) hydrogenolysis; and (5) cyclization. The product from the APR step is a mixture of chemical intermediates including alcohols, ketones, acids, furans, paraffins, and other oxygenated hydrocarbons. Once these intermediate compounds are formed, they can undergo further catalytic processing to generate a cost-effective mixture of nonoxygenated hydrocarbons. The chemical intermediates from the APR step are reacted with a catalyst to produce a high-octane gasoline blend stock similar to a petroleum-derived reformate stream. The chemical intermediates from the APR step can also be converted into distillate range hydrocarbon components through a condensation step followed by conventional hydrotreating. The properties of biogasoline are same to that of petroleum-based gasoline; therefore, it can immediately be used as a substitute in conventional gasoline engines.

2.1.8 Pyrolysis

Anaerobic combustion of dry algal biomass between 300 and 500°C under atmospheric pressure produces condensable liquid fraction (pyrolysis oil), gas, and solid material (char) (Lorenzo et al. 2014). The oil can be used directly as a fuel or can be upgraded into other hydrocarbons. Up to 40% (dry wt%) pyrolysis oil can be extracted from biomass (Babich et al. 2011). Pyrolysis oil from microalgae is better from the lignin-based feedstock as it has lower oxygen content, a higher carbon/hydrogen ratio, and greater energy content (Mohan et al. 2011). However, the basic requirement of dry biomass for pyrolysis makes the process energy intensive. The properties of the resulting bio-oil are clearly affected by parameters

such as temperature, reaction time, algae species, algae concentration, reaction atmosphere, and catalysts, in subcritical water reaction conditions.

Biochar is also a by-product of this process which has high calorific value, and thus can be used as biofuel. It can be used as chemical absorbent like activated charcoal or for soil amendment (Brennan et al. 2014; Agarwal et al. 2015). Being extremely porous in nature, it can hold water and water-soluble nutrients very effectively. This also provides a good habitat to microbes. It increases the soil fertility of acidic soils enhancing the agricultural productivity. Chemically, biochar is a stable solid, rich in carbon content ($\sim$90%). It is helpful in sequestering carbon by storing the greenhouse gases in the soil for years.

2.2 *High-Value Products from Algae*

2.2.1 Polyunsaturated Fatty Acids

With changes in human diet and emerging diseases related to poor diet, the polyunsaturated fatty acids especially ω-3 has gained much importance. The lipid content of the microalgal biomass can vary from 1 to 40% by dry weight and can be increased up to 85% in some cases by modulating growing conditions (Cheirsilp and Tolpee 2012; Kim et al. 2013b; da Costa et al. 2016). The algal lipids typically comprise glycerol, sugars or bases, and esterified fatty acids containing from 12 to 22 carbons, and may be both saturated as well as (mono or poly) unsaturated. Among lipids, fatty acids are the major fraction in algae and need to be refined as shown in Fig. 3. The PUFAs ("polyunsaturated fatty acids") range between 25 and 60% (Becker 2004).The PUFAs particularly of $\omega - 3$ and $\omega - 6$ series, EPA (eicosapentaenoic acid), DHA (docosahexaenoic acid), and AA (arachidonic acid) are considered to be pharmacologically important for dietary and therapeutic use against inflammatory diseases (rheumatism and inflammation of the gastrointestinal mucosa) (Mata et al. 2010). The PUFAs have a role in prevention and treatment of a variety of cardiovascular diseases, atherosclerosis, arrhythmia, lowering blood pressure, lowering cholesterol, and triglyceride levels plasma, cancer and are apparently essential in infant nutrition and brain development (Derner et al. 2006). Among the known species of microalgae that have significant amounts of $\omega - 3$ and $\omega - 6$ PUFA, are representatives of Haptophyceae (*Isochrysis spp.*, and *Pavlova lutheri*), Bacillariophyceae (*Phaeodactylum tricornutum, Thalassiosira spp.*, and *Odontella aurita*), Dinophyceae (*Crypthecodinium cohnii*), and Rhodophyceae (*Porphyridium cruentum*), and smaller quantity is found in members of Chlorophyceae (González et al. 2015). Microalgae-derived PUFAs have a very promising market in biotechnology, mainly in the functional food industry (Kovač et al. 2013; Yakoob et al. 2014). The EPA and DHA content from various microalgae have been given in Table 4.

Among the available extraction methods, $\omega - 3$ and $\omega - 6$ are usually extracted by the evaporation method, where a thin film evaporator is used (Zean Consultores

Table 4 EPA and DHA fatty acid contents in microalgal strains

Microalgae	% of total fatty acid			Reference
	EPA/DHA	EPA	DHA	
Pinguiococcus pyrenoidosus	22.03	–	–	Sang et al. (2012)
Thraustochytrium sp.	45.1	–	–	Scott et al. (2011)
Dunaliella salina	–	21.4	–	Bhosale et al. (2010)
Pavlova lutheri	41.5	–	–	Guihéneuf et al. (2009)
Isocrysis galbana	~28.0	–	–	Yago et al. (2011)
Crypthecodinium cohnii	–	–	45	Winwood (2013)
Schizochytrium sp.	–	<2	40	Winwood (2013)
Schizochytrium sp.	–	40	20	Winwood (2013)
Auratiochytrium sp.	25.67	–	–	Kim et al. (2013b)
Auratiochytrium sp.	64.1	–	–	Manikan et al. (2015)
Nannochloropsis gaditana	37.83	–	–	Mitra et al. (2012)
Nannochloropsis oculata	21–23	–	–	Pieber et al. (2012)
Nannochloropsis salina	–	~28	–	Wagenen et al. (2012)
Isocrysis galbana	6–7[a]	–	–	Fradique et al. (2013)

[a]Of dry wt

2013). It can also be made by molecular distillation, which is another alternative. Enzymatic hydrolysis is also promising in terms of saving energy and product selectivity. The chemical extraction methods (chromatographic separation, molecular distillation, etc.) for extraction of $\omega - 3$ PUFAs require high pH. However, high temperature and pH are not suggested as it can partially destroy the structure of the double bonds in all cis $\omega - 3$ PUFAs through natural oxidation, isomerization, or migration (Brudy Technology 2013). In current market, algae-based DHA and EPA oil prices are much higher than that of oil from fish due to the high production cost of algal biomass.

Lipid extraction is not only time taking process but also costly. The organic solvents such as chloroform, n-hexane are commonly used for lipid extraction. A total of 15–28% lipid can be extracted using chloroform/ethanol 1/1 (v/v) (Perretti et al. 2003). In spite of the higher extraction efficiency, their use is not suggestable due to environmental and health risks. Comparatively, SC–CO_2 is a less energy-intensive and cleaner technology for PUFAs-rich oil extraction (Parajó et al. 2008; Nautiyal 2016; Ruiz et al. 2016). Perretti et al. (2003) reported 10.40 g/100 g lipid extraction from photobioreactor grown *Isochrysis galbana* Parke using SC–CO_2. Addition of pretreatment methods increases the oil yield, which makes the process economically viable at commercial scale. In case of *Nannochloropsis gaditana*, higher EPA yield was observed using ultrasonication-assisted extraction (32.5 mg/g) as well as acid digestion (18.9 mg/g) as compared to control (18.2 mg/g) (Abirami et al. 2016). Heterotrophic cultivation also increases EPA and DHA content in microalgae (Winwood 2013; Hamilton et al. 2016).

Production of DHA by the thraustochytrids *Schizochytrium* and the microalgae *Crypthecodinium* was industrialized by Martek Biosciences during the last decade, targeting the high-value product applications in nutraceuticals and infant formula. DSM and Alltech are involved in the development of feed ingredients for the aquaculture sector. DSM is also a leading supplier of vitamins, carotenoids, eubiotics, and feed enzymes to the global feed industry. Alltech Algae (Winchester, KY, USA) has been in full commercial operation since April 2011 and producing two heterotrophic algae, licensed from DSM-Martek. DSM's algal oil DHASCO has been approved for use in infant formula in the European Union (EU) under Commission directive 2006/141/EC. This regulation states that when added, 1% of the total fat content should consist of n − 3 LCPUFA's, and 2% of the fat content should be n − 6 LCPUFA's of which 1% is arachidonic acid (ARA). DSM's Life's DHATM-S algal oil is approved for use as a novel food ingredient in specific food categories and dietary supplements (OJL 144/13, 12.6.2003; OJL 278/56, 23.10.2009). DSM's Life's OmegaTM which is rich in EPA and DHA is also authorized for use under the European Novel Food Regulation (EC) 258/971.

2.2.2 Pigments

Algae comprise different pigments which impart a specific color to them. These pigments are very important medically, are used in food industry as color additives, and have protection ability for harmful solar radiations. They have the ability to prevent degenerative diseases, fight free radicals, and function as anticancer agents and stimulants of the immune system (Custódio et al. 2012; Carvalho et al. 2013). Three main groups of pigments found in microalgal biomass are the chlorophylls, carotenoids, and phycobilins (Cuellar-Bermudez et al. 2015; Miazek et al. 2015). Microalgae-based natural pigments are less vulnerable to sudden extreme changes in temperature. In comparison to synthetic dyes, they are also more tolerant to the presence of ascorbic acid and are effective even if applied in low amounts in food (Skulberg 2004). Rules are implemented for prohibiting the use of synthetic dyes in food flavors; the research in this area encourages the use of microalgal carotenoids in the food industry (Del Campo et al. 2000). The β-carotene from microalgae has a great market and sold as extracts, powders, and as dry biomass. The price of this product varies between $300.00 and US$3000.00 per Kg, according to the product quality and demand (Ben-Amotz 2004). The β-carotene is generally found in a fraction less than 1% of the dry mass but can be enhanced up to 10% by halotolerant species (grow in high salt concentration) such as *Dunaliella* (Abalde et al. 1995).

Carotenoids

Several species of algae can accumulate high concentrations of β-carotene, astaxanthin, or canthaxanthin, which have been widely used as natural dyes and as

antioxidants (Plaza et al. 2010; Fiedor and Burda 2014). All photosynthetic organisms (plants, microalgae, and cyanobacteria) synthesize carotenoids which are fat-soluble pigments rendering yellow, orange, and red colors. The synthesis of these compounds can be manipulated by modifying growth conditions mainly through environmental stress (Lamers et al. 2010; Mulders et al. 2014; Minhas et al. 2016). Carotenoids biosynthesis can also be improved using stimulants, biosynthetic enzymes, acid, -ionone, mevalonic acid, diphenylamine, and other amino acids in the culture medium and adjusting the external conditions of cultivation (Mezzomo et al. 2016).

Most carotenoids are hydrocarbons, containing 40 carbon atoms and two terminal rings (Bell et al. 2000). There are two categories of carotenoids: (a) having linear hydrocarbons chain that can be cyclized at one end or both ends of the molecule (e.g., β-carotene) and (b) the oxygenated derivatives of carotenes such as lutein, violaxanthin, neoxanthin, and zeaxanthin, known as xanthophylls (Botella-Pavía and Rodríguez-Concepción 2006). Each carotenoid is characterized by an electronic absorption spectrum. Due to the coloring properties of carotenoids, they are often used in food, pharmaceutical, cosmetics, and animal feed industries. In addition, they are also used in food fortification because of their possible activity as provitamin A and their biological functions to health benefits, such as strengthening the immune system, reducing the risk of degenerative diseases, antioxidant properties, and anti-obesity/hypolipidemic activities (Mezzomo et al. 2015). The large-scale producers involved in commercialization of carotenoids are shown in Table 5.

The *β*-carotene is a thermolabile orange color pigment, which is sensitive to light and oxygen. This carotenoid provides protection against heart disease and cancer (Willcox et al. 2004; Fiedor and Burda 2014). India has the largest manufacturing industry of *Dunaliella*, where -carotene is used for pharmaceutical purposes. Other major producers are located in Australia, United States, China, Mongolia, and Japan. The small-scale production is found in Mexico, Chile, Cuba, Iran, and Taiwan (Dufoss'e 2006).

The pigment lycopene belongs to the subgroup of nonoxygenated carotenoids, being characterized by a symmetric structure containing 11 conjugated double bonds (Ciriminna et al. 2016). Due to its chemical structure, lycopene stands as one of the best biological suppressors of free radicals (Islamian et al. 2015) and has antiproliferative properties (Kelkel et al. 2011).

The xanthophyll fucoxanthin [(3S,3′S,5R,5′R,6S,6′R,8′R)-3,5′-dihydroxy-8-oxo-6′,7′-didehydro-5,5′,6,6′,7,8-hexahydro-5,6-epoxy-β,β-caroten-3′-yl acetate] is found in numerous classes of microalgae (bacillariophytes, bolidophytes, chrysophytes, silicoflagellates, and pinguiophytes) and brown macroalgae (phaeophytes) (Peng et al. 2011; Gagez et al. 2012). Diatoms exhibit a characteristic golden brown color due to a high amount of fucoxanthin that plays a major role in the light-harvesting complex of photosystems. The diatom *P. tricornutum* has been proposed as a commercial source for fucoxanthin with a production higher than 1.5% dry weight (Kim et al. 2011). A similar amount of fucoxanthin was produced by the haptophyta *Isochrysis* sp. (Crupi et al. 2013). The increasing

Table 5 Market potential of bioactive compounds from algae (modified from Minhas et al. 2016)

Bioactive compound	Organism	Cultivation method	Company	Market (USD million)
Nutraceutical astaxanthin	*Haematococcus pluvialis*	Tubular photobioreactor (two-phase)	AlgaTech (Israel) Blue Biotech (Germany) Fuji Chemicals (Japan) BioReal (Sweden)	200
Biorefinery carotenoids (astaxanthin, lutein), PUFA, lipids, and proteins	*Chromochloris zofingiensis*	Tubular photobioreactor one stage	Blue Biotech, (Germany)	40
Fucoxanthin	*Laminaria japonica*	–	AlgaNova International (China)	<100
Phycocyanin	*Spirulina platensis*	Open ponds, natural lakes	Parry nutraceuticals, SandaKing (Japan) (Photonz Corporate)	50
β-carotene	*Dunaliella salina*	Open raceway	Nutrition & Health (Australia) Betatene (Australia) Western Technology (Australia) Aqua carotene (Australia) Cognis Nutrition and health (Australia) Inner Mongolia Biological Eng. (China) Nature beta technologies (Israel) Cyanotech, Hawaii, USA Tianjin Latai Biotechnology (China) Parry Agro Industries (India)	261 NA

interest for this carotenoid is mainly due to its anti-obesity effect by promoting the oxidation of fatty acids and heat production (Maeda et al. 2005). In addition, fucoxanthin has shown a great antioxidant activity, antidiabetic, anticancer, and anti-photoaging properties (Peng et al. 2011; Kumar et al. 2013b; Kawee-Ai and Kim 2014).

Lutein (3*R*,3′*R*,6′*R*-βε-carotene-3,3′-diol) is a yellow oxycarotenoid or xanthophyll containing two cyclic end groups (one beta and one epsilon-ionone ring). Zeaxanthin (β,β-carotene-3,3′-diol) and lutein are the only carotenoids accumulated in the retina and lens eyes. They help in significant reduction of cataract and age-related macular degeneration (Fujimura et al. 2016). According to the report from financial organizations, the global market of lutein is expected to grow to $309million by 2018 with a compound annual growth rate of 3.6% (http://www.companiesandmarkets.com/Market/Food-and-Drink/Market-Research/The-Global-Market-for-Carotenoids/RPT988273). Lutein is present in nonesterified form in microalgae, while in plants, it is associated with fatty acids which cause saponification during pigment extraction. Microalgae reserve high lutein content from 0.5 to 1.2% dry weight. Ho et al. (2014) reported 4.52 mg/g dw lutein in *Scenedesmus obliquus,* while 7.2 mg/g dw lutein was found in *Chlorococcum citriforme* (Del Campo et al. 2000). Zeaxanthin has been obtained from the cyanobacterium *Synechocystis* sp., *Microcystis aeruginosa* (Lagarde et al. 2000; Chen et al. 2005), *Nannochloropsis oculata* (Chen et al. 2012), and *Chlorella saccharophila* (Singh et al. 2013). Similar to lutein, zeaxanthin possesses antioxidant, anti-inflammatory, and anticancerous properties (Bian et al. 2012; Okuyama et al. 2014).

Violaxanthin (5,6,5′,6′-diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene-3,3′-diol) is a natural xanthophyll pigment with an orange color found in a variety of plants, macro- and microalgae. This product has been isolated from several microalgae including *Dunaliella tertiolecta* (Pasquet et al. 2011a) and *Chlorella ellipsoidea* (Soontornchaiboon et al. 2012). Violaxanthin had antiproliferative, anti-inflammatory, and proapoptotic activity against human cancer cell lines (Talero et al. 2015).

Astaxanthin (3,3′-dihydroxy-β,β-carotene-4,4′-dione) is found in crustaceans and imparts pigmentation when incorporated in salmonids and crustaceans diets (Wathne et al. 1998). Through their application in feed, these pigments serve to intensify the color of egg yolk, chicken skin, fish, and milk. The pigments can absorb light, showing color. Light absorption in particular ultraviolet (UV) and visible spectrum is felicitated by chromatophore showing color. This xanthophyll has 10 times greater antioxidant power than β-carotene and 500 times higher than vitamin E (López et al. 2004). In addition, this pigment has been important in some diseases treatment and prevention, with antitumor properties and protection against free radicals, lipid peroxidation, oxidative damage to LDL cholesterol, oxidation of essential polyunsaturated fatty acids, and the UV light effects on cell membranes and tissues (Hu et al. 2006).

Canthaxanthin is produced by the cyanobacteria *Anabaena variabilis*, *Aphanizomenon flosaquae*, and *Nostoc commune* (Schroeder and Johnson 1995). Lutein (*Chlorella pyrenoidosa*), canthaxanthin (*Dictycoccus cinnabarinus*),

β-carotene (*Dunaliella salina*, *D. tertiolecta*), and astaxanthin (*Haematococcus pluvialis*) (Mezzomo and Ferreira 2016) are also produced for various applications. Extraction of carotenoids is accomplished by using solvents and supercritical fluid extraction. A combination of solvents along with the ultrasonication or microwave treatment is found to be more efficient (Table 6).

Table 6 Extraction methods for carotenoids (modified from Grosso et al. 2015; Mezzomo and Ferreira 2016)

Algae	Extraction method		Carotenoid
Chlorella vulgaris, *Undaria pinnatifida*	Dimethyl ether		Fucoxanthin
Nannochloropsis oculata	CO_2 + EtOH		Total carotenoid content
Haematococcus pluvialis	CO_2, CO_2 + EtOH, and CO_2 + vegetal oils		Astaxanthin
C. vulgaris	CO_2		Canthaxanthin and astaxanthin
C. vulgaris, *D. salina*, *A. maxima*	CO_2		Total carotenoid content, -carotene
Nannochloropsis gaditana	CO_2		Total carotenoid content
Synechococcus sp.	CO_2		Total carotenoid content
Cylindrotheca closterium	Acetone	Microwave assisted	Fucoxanthin and chlorophyll *a*
Dunaliella tertiolecta	Acetone		β,β-Carotene, chlorophyll *a* and chlorophyll *b*
Laminaria japonica	EtOH		Fucoxanthin
Sargassum fusiforme	EtOH		Fucoxanthin
Undaria pinnatifida	MeOH, EtOH, acetone, DMSO and hexane: EtOH, 1:1		Fucoxanthin
Dunaliella salina	MeOH, DMF	Ultrasound assisted	Carotenoids and chlorophylls
Chlorococcum littorale	EtOH 10%	SFE	Violaxanthin, neoxanthin, antheraxanthin, lutein, zeaxanthin, β-carotene, chlorophylls
Dunaliella salina	–	SFE	α-Carotene and β-carotene

(continued)

Table 6 (continued)

Algae	Extraction method		Carotenoid
Nannochloropsis sp.	EtOH 5–20% or CO_2 + EtOH 20% CO_2 + EtOH 20%	SFE	Violaxanthin/neoxanthin, astaxanthin, vaucheriaxanthin, lutein/zeaxanthin, canthaxanthin, β-carotene and chlorophyll *a*
Undaria pinnatifida	–	SFE	Fucoxanthin
Dunaliella salina	Hexane, EtOH, water EtOH	Pressurized liquid extraction	α-Carotene, 13-*cis*-β-carotene, all-*trans*-β-carotene, 15-*cis*-β-carotene, 9-cis-β-carotene
Eisenia bicyclis	EtOH 50%, 100% EtOH 90%	Pressurized liquid extraction	Fucoxanthin

Chlorophyll

Chlorophyll is the pigment capable of harvesting light to carry out photosynthesis in all the plants including algae. It is found in the chloroplasts associated with phospholipids, polypeptides and tocopherols, and a hydrophobic membrane covering. Chemically, it is a porphyrin macrocycle with four pyrrole rings. The minor difference in the chemical structure of chlorophyll changes its absorbance range and properties. Chlorophyll *a* and chlorophyll *b* are the most common forms in microalgae. However, chlorophyll *c* is found in certain marine algae including dinoflagellates. Chlorophyll is used as a coloring agent due to its selective absorbance of light of certain wavelengths and reflecting green color. The main constraint in its usage is the unstability of chlorophyll when removed from its protective environment. Its magnesium ion becomes unstable and is easily displaced by a weak acid. Thus, it is not suitable to be used in foods with low pH value. To overcome this unstability, magnesium ion is replaced by copper ion, which is excreted from the body without any side effect. Some of the chlorophyll has antioxidant and antimutagenic properties and is used as additives in cosmetics and pharmaceutical products (Fiedor and Burda 2014; Hu et al. 2013). Chlorophyll derivatives such as pheophorbide and pheophytin b have always been known as strong antioxidants. It has healing property and cures chronic ulcers, oral sepsis, and proctology.

Pigments are generally extracted using different organic solvents such as acetone, ethanol, methanol, and dimethylformamide (DMF). The solvent enters the cell membrane and dissolves lipids and lipoproteins of chloroplast membrane (Hosikian et al. 2010). It can be achieved through maceration (soaking), percolation, countercurrent extraction, pressurized liquid extraction, and soxhlet. For effective

extraction, various cell disruption methods such as grinding, bead beating, homogenization, ultrasound, or sonication have been suggested (Halim et al. 2013; Biller et al. 2013). Ultrasonication alone, microwave irradiation alone, or combination of both techniques gave excellent extraction efficiencies in terms of yields and time, with a 10-fold reduction in the time needed with conventional methods, and yields increased from 50 to 500% (Cravotto et al. 2008). Besides cell disruption, the storage conditions of the filtered microalgae prior to the analysis, the organic solvents used, the duration of the extraction, and the number of extraction steps employed in the analysis also affect the pigment yield (Macías-Sánchez et al. 2009). Excess light, oxygen/air, high temperatures, and acidic or alkali conditions also affect the pigment yield. The limitation of using organic solvent is their bulk amount and risk of thermal denaturation or transformation of pigment (Pasquet et al. 2011b). Hot water extraction and steam distillation increase the yield but also denature the pigment (Manzan et al. 2003). Use of enzymes (xylanases, pectinases, or cellulases) enhances the extractability in case of macroalgae and unfrustuated microalgae (Deniaud et al. 2003; Gerken et al. 2013). To overcome the chemical transformation of pigments, samples can be frozen (−80 °C, liquid nitrogen), freeze-dried, dessicated, or stored in water vapor saturated atmospheres (Esteban et al. 2009). The use of supercritical carbon dioxide (SCF-CO_2) or combination of solid-phase extraction with SCF–CO_2 to extract pigments from microalgae is an efficient but costly process (Guedes et al. 2013). A comparison of different combinations of extraction processes with cell disruption methods and pigment recovery is given in Table 7.

The fractionation and purification of pigment (chlorophyll) and its derivatives are done by paper chromatography, thin layer chromatography, and high-performance liquid chromatography (HPLC). Organic adsorbents such as sucrose and cellulose were found to be the most efficient stationary phases for use in two-dimensional thin layer chromatography (Jeffery 1968). HPLC is most appropriate because it requires even less sample for analysis, is faster, more precise, highly sensitive, and features automatic detection system (Wright and Shearer 1984). Reverse phase HPLC is preferred to normal phase as the latter does not separate polar compounds, and its polar stationary phase promotes pigment degradation (Wright and Shearer 1984). Fluorescence detection is more sensitive and more selective than absorbance detectors for chlorophyll analysis (Jeffery et al. 1997).

Because of the high revenue from the pigments, it can be integrated with biodiesel extraction so as to reduce the cost and making the system feasible.

2.2.3 Protein

The growing market for protein supplements seeks high quality and safe alternatives of protein sources. The algal proteins are safe for human consumption as they are proven to be nontoxic (Chamorro 1980). Although most of the algal species contain variable amounts of proteins, only a few are used for commercial

Table 7 Comparison between different extraction methods for pigments, modified from (Hosikian et al. 2010)

Algae species	Solvent	Cell disruption	Results
Phytoplankton	MeOH (90%), EtOH (90%), EtOH (100%), DMF	In all cases	DMF was superior and cell lysis improved extraction in all cases
Dunaliella Salina	DMF, MeOH	Ultrasound	DMF was found to be more efficient methanol
Scenedesmus quadricauda, Selenastrum capricornutum, Microcystis aeruginosa	EtOH (95%), MeOH, acetone (90%)	Homogenization, sonication, boiling, homogenization, sonication, boiling	(1) Methanol and ethanol were better than acetone (2) Boiling the algae (in methanol or 95% ethanol) and extraction time 24 h resulted in complete extraction of pigment without any degradation
Stichococcus, Chlorella	Acetone, DMF	Grinding, ultrasound, bead beating	(1) DMF was most efficient solvent (2) DMF does not require cell disruption (3) Freeze drying before analysis aids extraction
Selenastrum obliquus	MeOH, acetone	Probe sonication, bath sonication, tissue grinding (mortar and pestle)	(1) Under sonication, methanol removed three times more pigment than acetone (2) Under tissue grinding, methanol removed 20% more than acetone
Botryococcus braunii	Supercritical CO_2	CO_2 rapid depressurization	Carotenoids and chlorophyll recovery increased by 2.4- and 2.2-fold, respectively
Ulva lacuata, *Ulva reticulate*, *Caulerpa scalpeliformis*, *Kappaphycus alvarezii*	EtOH Ethyl acetate Acetone	Grinding (mortar and pestle) homogenization	Ethyl acetate showed higher extraction as compared to ethanol and acetone
Scenedesmus obliquus, *Cryptomonas erosa*, *Cyclotella meneghiniana*, *Microcystis aeruginosa*, *Staurastrum paradoxum*	ultrasonication	Quartz sand Freeze drying	Over 90% extraction achieved

applications as a food substitute. A large number of nutritional and toxicological evaluations demonstrated the ability of algal biomass as a valuable dietary supplement or replacement of conventional sources of protein (soybean meal, fish meal, rice bran, etc.). Despite high protein content, they are not much appealing, due to their dark green color, light fishy smell, and consistency of powdered biomass. However, the use of microalgae as animal feed is more recent. As compared to the other conventional protein sources, microalgae production cost is so high which restricts its use in health foods.

2.3 Different Routes of Algal Biorefinery

Olguin (2012) highlighted that the biorefinery strategy offers new opportunities for a cost-effective and competitive production of biofuels along with nonfuel compounds. The author studied an integrated system where the production of biogas, biodiesel, hydrogen, and other valuable products (e.g., PUFAs, phycocyanin, and fish feed) could be possible. The different conversion routes for biofuels production from algal biorefinery are shown in Fig. 4.

2.3.1 Electricity–Ethanol Production

To meet the increasing power demand, microalgae can be utilized for electricity generation in conjunction with the ethanol production. The CO_2 released from the ethanol plant and its combustion in the engines can be utilized for algal cultivation. Thus, combustion of algae-based biofuel does not release extra CO_2 than was consumed in algal biomass production. The combined production of bioethanol and bioelectricity from microalgae is promising economically. The O_2 released in photosynthesis process during algal growth can also be utilized at cathodes in microbial fuel cells. It is estimated that every ton of CO_2 absorbed releases approximately 500 kg of O_2 (González et al. 2015).

2.3.2 Biodiesel–Biohydrogen Production

In most of the algal biorefineries, the main concept is to produce biodiesel first. Then, the leftover biomass will be suitable for another conversion processes to obtain a variety of products. A few possible ways are worked out by researchers. Microalgae can be utilized for biodiesel, biohydrogen, and carotenoids extraction in a biorefinery. Nobre et al. (2013) used *Nannochloropsis* microalga in biorefinery and fractionated the biomass after extracting lipids for biodiesel production into carotenoids and fatty acids, mainly EPA for food and feed industry. The compounds were recovered by supercritical extraction using CO_2 and ethanol. To utilize biomass totally, the leftover biomass can be fermented by hydrogen-producing

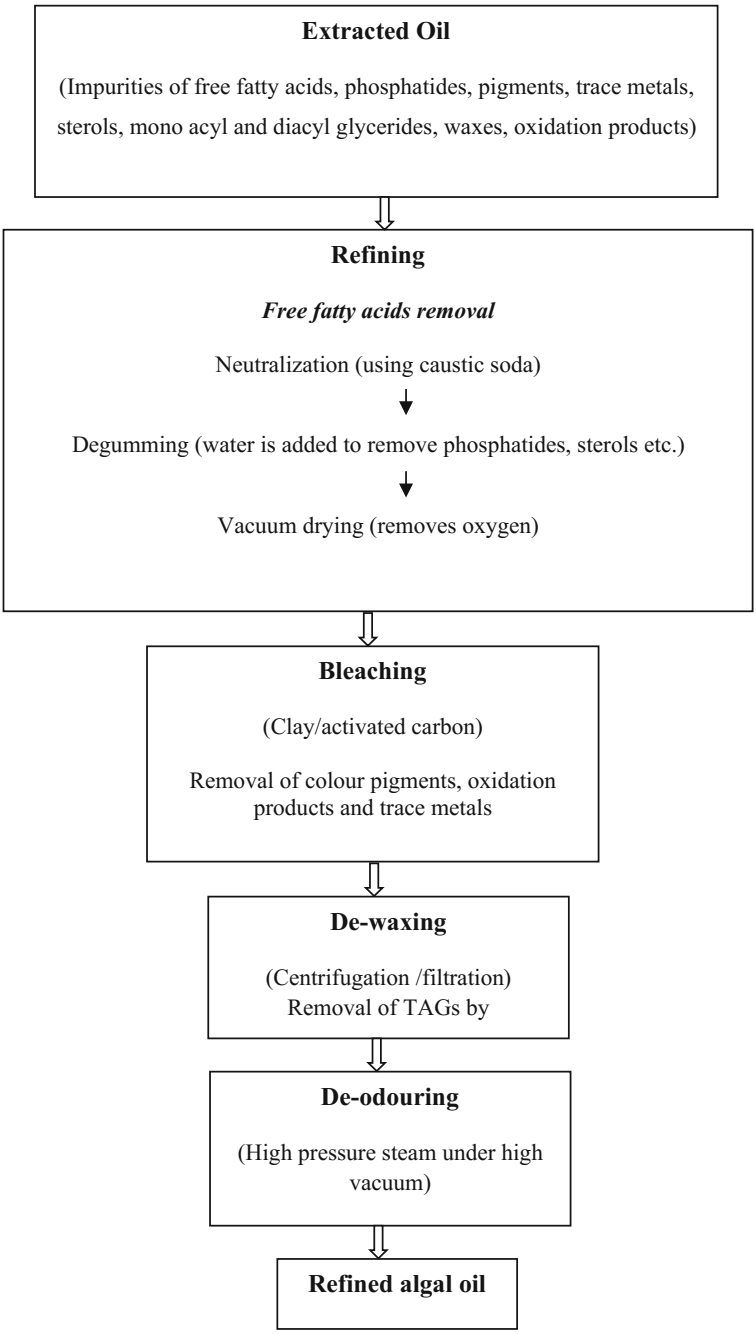


Fig. 3 Flowchart of oil refining

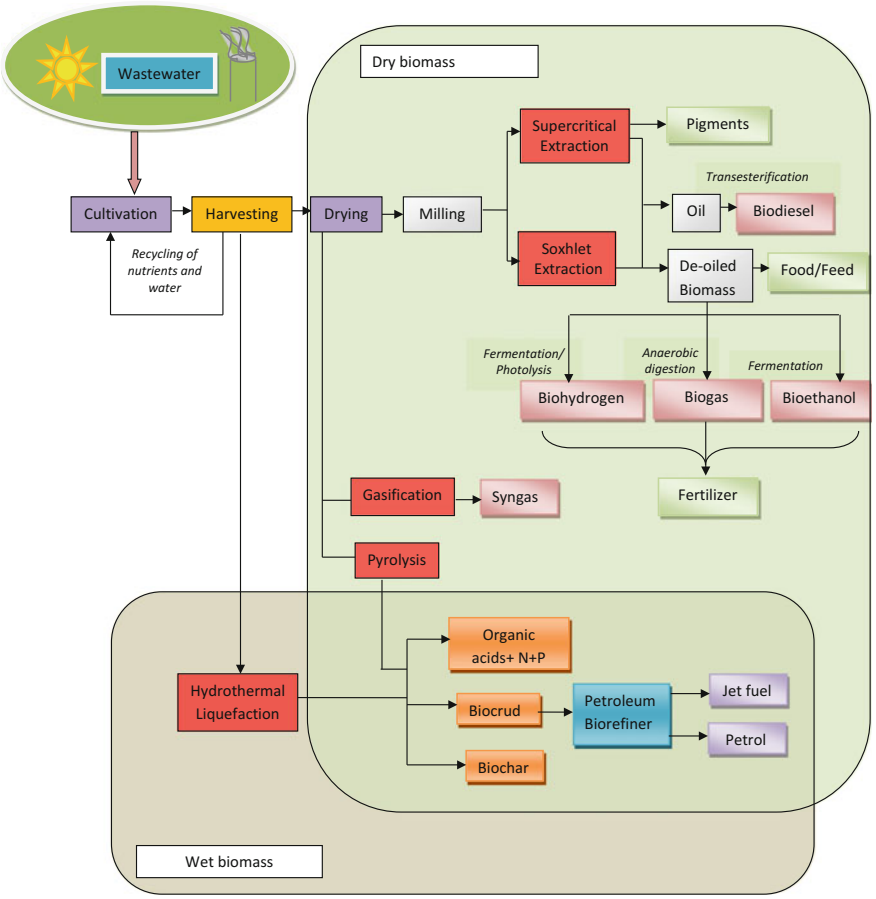


Fig. 4 Schematic representation of algal biorefinery for production of biofuels and value-added products

bacteria such as *Enterobacter aerogenes* to produce hydrogen through dark fermentation. The maximum yield was reported to be 60.6 mL H_2/g dw. Once the maximum biohydrogen was produced photoautotrophically, the same algal biomass can be fermented in dark conditions to get higher yields (Marques et al. 2011; Ferreira et al. 2013).

2.3.3 Biodiesel–Biomethane Production

Another possible way of algal biomass utilization is the production of biodiesel followed by conversion of de-oiled cake into biomethane through anaerobic digestion. This biorefinery concept was used by Sialve et al. (2009) on *D. salina*

while Collet et al. (2011) used *Chlorella vulgaris* for the same process. Algal biomass can also be processed for ethanol production after lipid extraction. However, with *Dunaliella*, 50% methane yield was obtained in 18 days, which was comparatively more than *C. vulgaris*. Collet et al. (2011) also performed the life cycle analysis and demonstrated that methane if produced from algal biomass is not good in comparison to the other algae-derived products such as biodiesel due to depletion, ionizing radiation, human toxicity, and global warming. However, algal methane is a much better option in terms of acidification and eutrophication.

Another way is the simultaneous production of biodiesel and methane in a biorefinery concept (Ehimen et al. 2011). Biodiesel was produced by direct transesterification of the *Chlorella* biomass followed by the biogas production through anaerobic digestion. They reported a maximum methane composition of 69% (v/v) with a specific yield of 0.308 m^3 CH_4/Kg VS at a temperature of 40 °C and a C/N mass ratio of 8.53. However, in this work, the biodiesel yield was not provided.

2.3.4 Biodiesel–Bioethanol Production

When lipid is extracted for biodiesel purpose, the residual biomass can be saccharified using enzymes or chemicals. The extracted sugar is fermented using suitable microorganisms. Kim et al. (2013a) tried similar extraction on *D.tertiolecta* without any pretreatment and fermented into bioethanol. The reported yield was 0.14 g ethanol/g residual biomass and 0.44 g ethanol/g glucose. This is a good example of process integration for economically feasible microalgal biorefinery.

Another possible way to incorporate biodiesel and bioethanol production was reported by Powel and Hill (2009). They integrated photosynthetic *C. vulgaris* (at cathode) that captured CO_2 emitted by yeast in fermentor for bioethanol production. Thus, fermentor containing yeast acted as anode chamber, and a column photobioreactor with *C. vulgaris* acted as cathode chamber constituting a microbial fuel cell. The power generated from this MFC unit is utilized in the bioethanol plant facility, and algal biomass grown in photobioreactor is utilized for biodiesel production. The remaining biomass after oil extraction can also be used in animal feed supplement.

2.3.5 Biohydrogen–Pigments Production

Focusing on the sugar-rich algae, biohydrogen can be produced in conjunction with pigments. Pacheco et al. (2015) analyzed the life cycle of *Spirogyra* and concluded that it is essential to increase the sugar content for increased hydrogen yield. For cost-effective harvesting and dewatering of tiny microalgal cells, electrocoagulation and solar drying can be done. Reducing centrifugation in the processing steps can save up to 90% of energy. Pigments being a high-value product offer a great scope for biorefinery. However, its extraction demands high energy (up to 60% of the total

energy). In order to reduce the cost of pigment extraction, other methods besides acetone solvents can be chosen. Nobre et al. (2013) reported a maximum H_2 yield of 60.6 mL/g dw through dark fermentation after pigment extraction from *Nannochloropsis* sp. The maximum yield was found to be 1.34 ± 0.02 and 1.47 ± 0.10 mg total pigments/g dry biomass (cold extraction with acetone) with 70% pigments (mainly carotenoids) using SCO_2.

2.3.6 Electricity and Pigments Production

The *C. vulgaris* biorefinery approach as studied by Gouveia (2014) offers simultaneous production of bioelectricity and added-value pigments, with possible wastewater treatment. The photosynthetic algal microbial fuel cell (PAMFC) was used where the microalga *C. vulgaris* were present in the cathode compartment. The direct correlation between light intensity on PAMFC and carotenogenesis was also observed. The maximum power produced in this system was 62.7 mWm^{-2} with a light intensity of 96 $\mu Em^{-2}s^{-1}$.

2.3.7 Biodiesel and Pigments Production

Campenni et al. (2013) designed a biorefinery where *Chlorell pritothecoides* was cultivated autotrophically to produce lipids and carotenoids. The growth medium was nitrogen deprived with added salt (20 g/l NaCl) solution. The canthaxanthin (23.3%), echinenone (14.7%), free astaxanthin (7.1%), and lutein/zeaxanthin (4.1%) were found with the total carotenoid content of 0.8% (w/w), which have applications in food industry. Furthermore, the total lipid content reported was 43.4% (w/w), with a fatty acid composition of C18:1 (33.6%), C16:0 (23.3%), C18:2 (11.5%), and C18:3 (less than 12%) fulfilling the biodiesel EN 14214 quality specifications (2008) and can be used for the biofuel (biodiesel) industry. The leftover biomass still containing sugar can be used for bioethanol or hydrogen production, thus taking full benefit of the biomass composition.

2.3.8 Biohydrogen and Biogas Production

The production of biohydrogen and the consequent biogas (methane) production by anaerobic fermentation of the residue of *Chlorella reinhardtii* biomass was achieved by Mussgnug et al. (2010). Fermentation of the green alga *Chlamydomonas reinhardtii* was efficient with a production of 587 ml (±8.8 SE) biogas/g volatile solids. The authors reported that the biogas production increased by 123% using the biomass after the hydrogen production cycle instead of using the fresh biomass. The authors attributed these results to the storage compounds, such as starch and lipids with a high fermentative potential which is the key in the microalgae-based integrated process and could be used for more value-added

applications. Wirth et al. (2015) worked on a mixed culture of *Chlamydomonas* sp. and *Scenedesmus* sp. in a biorefinery manner. The maximum yield of 1.91 mlH_2/L was obtained after 4 days. The methane content of biogas was 61% (220 ml/g TS).

3 Conclusion

Microalgae have simple growth requirements with a high potential for energy and bioactive compounds production. The economic sustainability can be strengthened by incorporating technical advancement and huge biomass supply. CO_2 sequestration, wastewater remediation, and extraction of high-value products provide a great scope for profitable algal market. The microalgae biorefinery opens enormous opportunities for entrepreneurs, researchers, and technicians. Their collective efforts will surely make the algae business a success across the globe.

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Biodiesel—Technical Viability for India

S. Sakthivel, S. Suresh and N. Selvaraju

Abstract The aim of the article is to investigate the different technologies for the production of biodiesel, which includes process description, pre-treatment of raw feedstock, esterification/transesterification and product purification/standardization. Further, technology comparison has been carried out for homogeneous acid/alkaline catalysis process, heterogeneous acid/alkaline catalysis process, supercritical methanol process and enzymatic (biochemical) process. In a technology viewpoint, the homogeneous (acid/alkaline) catalyst is mostly used for commercial production of biodiesel from batch and continuous processes. In India, sodium-based catalyst (e.g. NaOH) is preferred for a small-scale commercial production as it is inexpensive and easy to handle in transportation and storage. Presently, Indian commercial producers are using a two-stage homogenous process. The market price of biodiesel is Rs. 55 (±2), excluding tax. This price is a little high in the market due to the cost of feedstocks which makes up to 80–90% of the total production cost, operating cost and the capital cost in the view of business experts. The market price is almost near to the petroleum diesel price (approximately Rs. 60). However, in using biodiesel, there are enormous benefits towards the environment-friendly factors such as fewer emissions of CO, SO_2, NO_x and less particulate matter.

Keywords Feedstocks · Biodiesel · Technology assessment · Commercial view Product purification and Standards

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1 Introduction

Bioenergy is obtained from various biological resources, such as Oil crops, Sugarcane, Wood waste, Agricultural wastes, Grasses, Sawdust, Aquatic plants, algae, animal waste and municipal solid waste. The various prime processes employed to extract the bioenergy from these sources include Thermochemical (combustion, gasification, pyrolysis and supercritical), Biochemical (fermentation, anaerobic digestion, aerobic conversion and enzymatic processes) and Chemical processes (esterification, pulping, catalytic processes, methanization and hydrogenation) (Sengutpa and Pike 2012). The derivative of biomass is classified into two main categories: (1) Biofuel and (2) Organic Products, as shown in Fig. 1.

Around the world, there are many potential feedstocks identified for biodiesel production. The availability of wide range of potential feedstocks offers more flexibility in the process and helps in accommodating the local demands. However, the production of biodiesel from the edible oil such as sunflower, mustard, rapeseed, soybeans, palm oil, canola, safflower, corn, coconut and peanut, which are considered as the first generation of feedstock, is not suggested for commercial scale production due to depletion of the food supply. According to the Indian national biofuel policy, usage of edible oils for biodiesel production is forbidden (or) not recommended due to social issues and economic view. The government recommends that biodiesel should only be produced from non-edible oils as second-generation feedstocks, such as non-food crops, biomass sources, algae, waste vegetable oils and fats, forestry residues etc. The second-generation

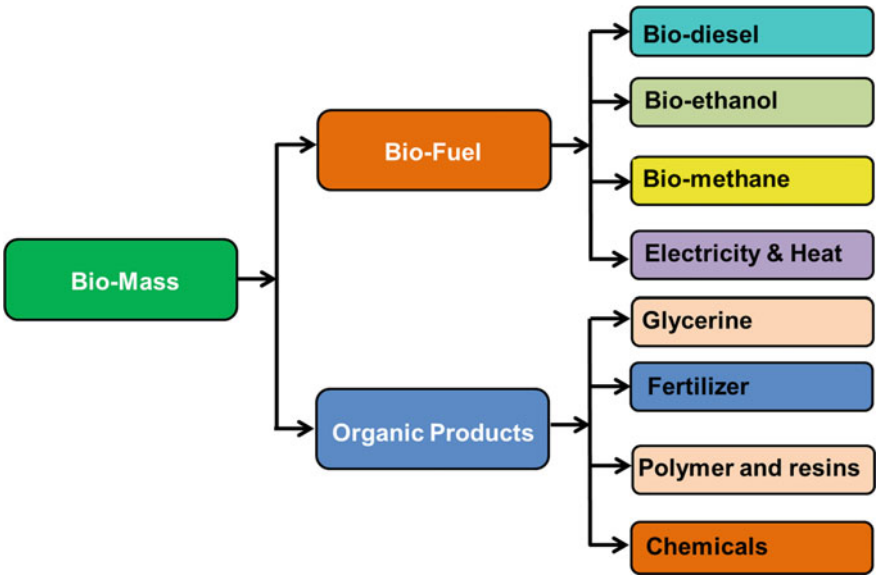

Fig. 1 Derivatives of biomass

feedstocks can be an encouraging parameter for the sustainable production of biodiesel (Atabani et al. 2013) with affordability and with greater environmental benefits. The non-edible plants can be grown in harsh and marginal lands which require less maintenance, less soil fertility and less water as opposed to the arable lands used for farming edible vegetable oils (Bhuiyaa et al. 2016).

In India, many varieties of oil bearing seeds/crops are available. For example, *Jatropha curcas* (Jatropha), *Pongamia pinnata* (karanja), *Madhuca indica* (mahua), *Azadirachta indica* (neem), *Shorea robusta* (Sal), *Scheleichera oleosa* (Kusum) *Salvadora oleoides* (Pilu), *Citrullus colocynthis* (Tumba), Simarouba (*Simarouba glauca*), Jojoba (*Simmondsia chinensis*), Cheura (*Diploknema butyracea*), Kokum (*Garcinia indica*), wild Apricot (*Prunus armeniaca*), wild Walnut (*Aleurites moluccana*), Kusum (*Schleichera oleosa*), Tung (*Vernicia fordii*), *Ricinus communis* (Castor), *Argemone mexicana* L (Mexican prickly poppy), *Cerbera odollam* (sea mango), *Pinus roxburghii* (Lucky bean tree), Mukorossi (Soapnut), *Hevea brasiliensis* (Rubber tree), *Calophyllum inophyllum* (Polanga), *Melia azedarach* (Syringa or Persian lilac), *Schisandra chinensis* (Jojoba), *Thevetia peruviana* (Yellow oleander), etc. These crops can be grown in a piece of wasteland and under varied agro-climatic conditions (Kumar and Sharma 2011; Dhyani et al. 2015). Other categories of feedstocks are microalgae and waste/used cooking oils, which are primarily used by academic/industrial researchers at a laboratory level. In particular, the microalgae are considered as one of the potential feedstocks for biodiesel production, as it has high oil content than the other traditional crops.

Although many non-edible feedstocks have been identified thus far, there remains a primary concern. The availability of feedstocks in the domestic market would largely decide the economic viability of the process, especially in developing countries. In India, the major feedstocks for the production of biodiesel have been identified as Jatropha, Karanj, Neem and Mahua which contain more than 30 wt% (wt) oil in their seeds (Padhi and Singh 2011).

1.1 Process of Biodiesel Production

The following three key sequential steps are primarily involved in the production of biodiesel process (1) Pre-treatment, (2) Esterification/Transesterification reaction and (3) Product purification and Standardization.

1.1.1 Pre-treatment of Feedstock

The oil feedstock will have a lot of undesirable compounds, such as water, pigments, colloidal mater, extraction residues, particles and other impurities like gums, waxes, etc. These impurities must be removed prior to transesterification

reaction as it interferes with the reaction pathway and reduces the process efficiency/yield. Many methods/techniques are suggested for the removal of these impurities. The gum can be removed by adding phosphoric acid or citric acid (Mitchell 2011). The particulate matter and colloidal matter can be removed by filtration. Mostly, filters like baghouse filter, pressure leaf filter and cartridge filter are used. The filter selection depends mainly on factors such as the type of the particulate matter, size or cake discharge, filter unit size in combination with the plant capacity, space requirement, batch or continuous process conditions and cost. Free Fatty Acid (FFA) content is one of the most process hindering compounds for a biodiesel production. The FFA could reduce the quality, yield and the product separation efficiency (i.e. separation of ester, glycerine and wash water) due to the formation of soap. The FFA content can be reduced to a desired level (<1%) by using an acid catalyst process (i.e. called as esterification reaction) before transesterification. It could also be achieved by integration of the two-stage processes, i.e. esterification and transesterification reaction, as it would be economically viable for treating oil having higher FFA content.

Similarly, the water/moisture content (>0.1 wt%) in the feedstock would reduce the conversion of triglycerides to biodiesel. Besides, the presence of water/moisture causes more negative impact than the presence of FFA in oil. Thus, it is suggested that the water content should be removed prior to the transesterification reaction, by using a suitable preheating method (Ma and Hanna 1999; Atadashi et al. 2012).

1.1.2 Esterification/Transesterification Reaction

Transesterification is a chemical reaction of triglycerides/oil with short-chain alcohol (e.g. methanol or ethanol) in the presence of a catalyst. The resultant products of this reaction are biodiesel (called as ester) and glycerol as by-product, as shown in Fig. 2. The transesterification reaction is reversible and excess alcohol (1:6 mol ratio of oil and methanol) is used to shift the reaction to the product side. The alcohols that are commonly employed for transesterification are methanol, ethanol, propanol, butanol and amyl alcohol. These are primary and secondary monohydric alcohols having 1–8 carbon atoms. Among them, methanol and ethanol are usually preferred due to their low cost and favourable physical and chemical properties compared to that of other alcohols. Most of the academic and industrial researchers are using methanol which is a polar and a short-chain alcohol that quickly reacts with triglyceride. Esterification is the conversion of FFA into ester in the presence of an acid catalyst. Transesterification/esterification reaction can be carried out in a batch mode or in a continuous mode. The yield of biodiesel mainly depends on the feedstock quality (e.g. water content, FFA, etc.) process conditions (temperature, pressure, batch/continuous mode) and operating process parameters (ratio of oil/methanol), catalyst type and their amount, residence time, etc.

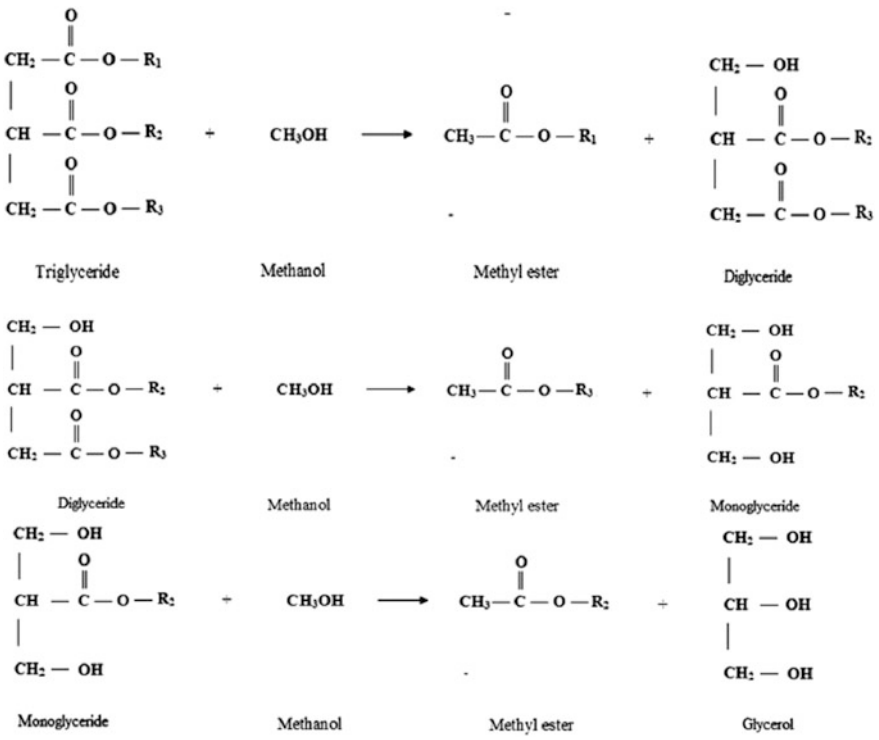

Fig. 2 Transesterification reaction

1.1.3 Product Purification and Standardization

The quality of biodiesel depends on several factors, including the quality of feedstock, composition of fatty acid in the parent vegetable oil, process operating conditions and handling and storage of biodiesel (Bankovic-Ilic et al. 2012).

Typically, the product mixture contains an ester, glycerol as by-product, excess alcohol, traces of un-converted oil (i.e. mono-, di and triglycerides), un-converted FFA, catalyst metals and water content. The product is to be brought to desired standard level (Indian Standard: IS 15607, European standard: EN14214, American standard: ASTM D6751) for the purpose of diesel engine function. Conventionally, the separation and purification processes are carried out by the following steps.

1. Biodiesel and glycerol are commonly separated by using gravitational or centrifugal filtration, as the density of biodiesel ($\sim$0.88 g/cm^3) is lower than the density of glycerol ($\sim$1.26 g/cm^3). However, recently several other techniques which employ membrane/ion exchange resins/solid adsorbents (e.g. magnesium silicate) are suggested for the separation of glycerol from the product of biodiesel (Saleh et al. 2010).

2. Excess/residual methanol is typically recovered by distillation and it can be reused.
3. The FAME (Fatty Acid Methyl Ester) is separated from product mixture by vacuum distillation method. This separation and purification step leads to increase in operating costs for producing the biodiesel.
4. Exchange resin (Masato and Hidaka 2013) and Deep Eutectic Solvents (DES) (Hui min et al. 2015) techniques are used to remove the trace of catalyst contaminant/inorganic salts from the product mixture. In a small-scale production, the glycerin and catalyst contaminant removal is achieved simply by 'water wash'. However, it leads to a range of issues (Wal et al. 2011): (a) Water wash creates a large amount of wastewater that must be treated subsequently, which can increase the processing time and also the operating cost of the plant as it involves additional units of operations, such as drying, multiple washes and water-biodiesel separation (b) Deionized water should be used to avoid further product contamination (c) The final product of biodiesel would have traces of water content which will bring down the standard level desired and (d) It also leads to formation of emulsion if biodiesel has a high content of soap which would eventually effect on the loss of yield significantly.

2 Technologies for Biodiesel Production

The technologies such as pyrolysis (thermal cracking), micro-emulsification, dilution and transesterification are used for the production of biodiesel (Ruhul et al. 2015). This section focuses on the transesterification process only. The biodiesel production by transesterification of vegetable oils with alcohol can be carried out by using the following processes (Martin and Grossmann 2012) and the details are given in Fig. 3.

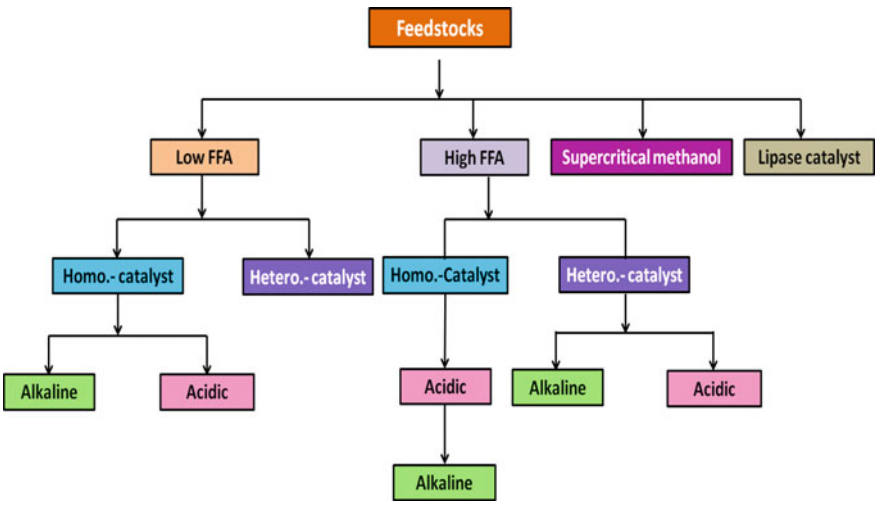

Fig. 3 Various processes of biodiesel production

(i) Homogeneous acid/alkaline catalysis process
(ii) Heterogeneous acid/alkaline catalysis process
(iii) Supercritical methanol process
(iv) Enzymatic (biochemical) process.

2.1 Homogeneous Catalysis Process

In the homogeneous alkali transesterification process, catalysts, such as sodium hydroxide (NaOH) (Ranganathan and Sampath 2011), potassium hydroxide (KOH) (Meher et al. 2006), sodium methoxide ($NaOCH_3$) (Zeng et al. 2014), sodium ethoxide ($NaOCH_2CH_3$) (Lima et al. 2006) or potassium methoxide ($KOCH_3$) were used along with alcohol (preferably methanol/ethanol). When the feedstock has higher free fatty acid (FFA), it was found that usage of alkaline catalyst leads to the formation of soap, which in turn reduces the yield of biodiesel in the transesterification reaction and inherently results in poor separation of biodiesel from the by-product of glycerol.

The selection of suitable catalysts is mainly based on higher kinetic reaction rates, stability, reusability, regeneration and product yield. (Singh et al. 2006) studied and reported the performance of various catalysts like NaOH, KOH, $KOCH_3$ and $NaOCH_3$ for the production of biodiesel via transesterification between crude canola oil and methanol. They reported that potassium (KOH)-based catalysts were found giving better yields than the sodium-based (NaOH) catalysts which is attributed to the fact that the dissolution rate of KOH is extremely faster than NaOH, in methanol. Consequently, the rate of reaction and the subsequent yield are high. Similarly, methoxide catalysts were found giving higher yields than the corresponding hydroxide catalysts. The order of catalysts on the basis of better yield are illustrated as $KOCH_3 > NaOCH_3 > KOH > NaOH$.

In India, sodium-based catalyst (i.e., NaOH) is preferred for a small-scale commercial production as it is inexpensive and easy to handle in transportation and storage. Further, a few more reasons for using NaOH for commercial purposes, are given below:

(a) The required quantity of KOH in transesterification reaction is higher when compared to NaOH to obtain the same yield. It is mainly attributed to the difference in the density and molar ratios between the two catalysts. The market price of KOH is about two and a half (2.5) times more expensive than NaOH.
(b) At very high temperatures, methanol boils very violently when it contacts with KOH. However, the same is not true for NaOH. This might be due to the fact that NaOH takes a longer time to dissolve in methanol.

However, the alkaline catalyst process results in increased operating costs due to water washing of the product and cost of the catalyst recovery and regeneration. Hence, the alkaline catalyst is typically useful for conversion of edible oil/used

cooking oil (i.e., less than 1% of FFA content) or low FFA content oil. To overcome the problems in using alkaline catalyst, transesterification process was investigated under an acidic system using homogeneous catalysts. In the homogeneous acidic transesterification process, sulphuric acid (H_2SO_4) (Wang et al. 2006), hydrochloric acid (HCl) (Matthew et al. 2008), H_3PO_4 and BF_3 are used as catalysts. The acid catalyst could simultaneously carry out esterification of FFAs and transesterification of triglycerides, which is of great interest for biodiesel production. In general, esterification reactions are faster than transesterification. It was found that the acid catalysts are better substitutes for base catalysts since they do not show measurable susceptibility to FFAs and can catalyze esterification and transesterification simultaneously. Generally, there are a few common issues in both the processes of alkaline/acid homogeneous catalyst such as

(a) Sensitive to FFA and water content
(b) Formation of soap with high FFA
(c) Recovery and regeneration of catalyst
(d) Formation of salts during the catalyst neutralization
(e) Generation of wastewater in large quantity for product recovery.

Many researchers (Berrios et al. 2010) have studied on the integration of two-step processes like esterification (as a pre-treatment of FFA) and transesterification process and to minimize the soap formation and maximize the product yield. In the first step of esterification, FFA reacts with methanol in the presence of an acid catalyst to form biodiesel (which is also called as fatty acid methyl ester, FAME) and water. Further or next step of transesterification, triglycerides react with methanol in the presence of an alkaline catalyst to form biodiesel and glycerol. The schematic diagram of the homogeneous process is shown in Fig. 4.

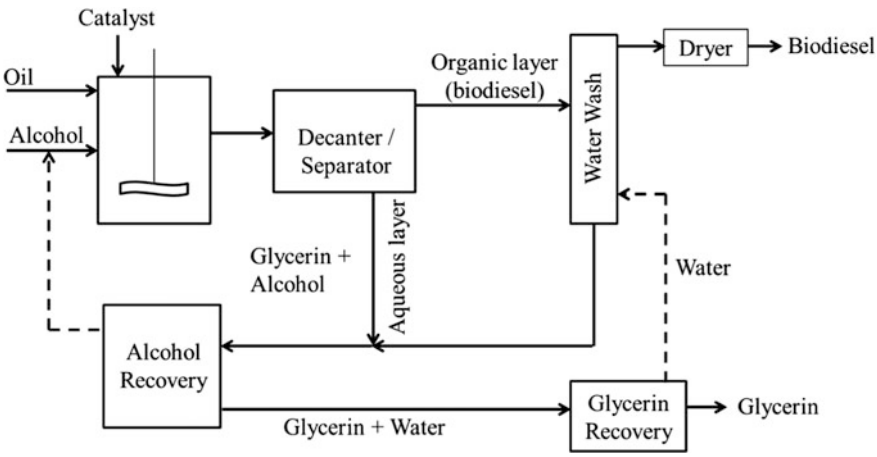

Fig. 4 Schematic diagram for homogeneous catalytic transesterification process

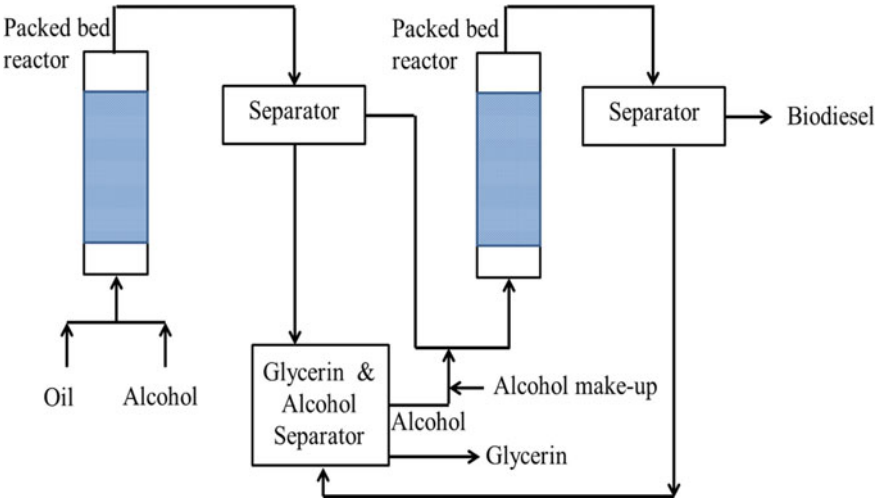

Fig. 5 Flow diagram for heterogeneous catalytic transesterification process

2.2 Heterogeneous Catalysis Process

The heterogeneous acid catalysis has some specific benefits such as easy product separation, recycling, more stability, usage of continuous packed bed reactor and in reducing waste generation. The flow diagram of heterogeneous catalysis process for biodiesel production is shown in Fig. 5. In this process, an alkaline catalyst such as MgO (Wang and Yang 2007), CaO (Granados et al. 2007), SrO (Liu et al. 2007), Mg/La_2O_3 (magnesium–lanthanum mixed oxide) (Seshu et al. 2008), KOH/Naxzeolite (Wenlei et al. 2007) or KF/A_2O_3 (Amish et al. 2009) are used for transesterification process. However, the solid heterogeneous alkaline catalyst reduces the yield of biodiesel, if the feedstock contains higher FFA and water content as similar to homogenous alkaline catalyst. Thus, the heterogeneous acid catalyst could be used instead of heterogeneous alkaline catalyst to overcome these issues. The heterogeneous acid catalyst is capable of accomplishing both transesterification and esterification reactions together (Halder et al. 2013). Even, if the oils contain a higher amount of FFA, it could be converted into a methyl ester.

Catalysts such as zinc oxide, zirconium oxide (Matthew et al. 2008), Zine oxide supported on tungstated zirconia (Furuta et al. 2004), vanadium phosphate (Di Serio et al. 2007), zeolites, silica and alumina are widely used in heterogeneous acid catalysis process. These catalysts should possess highly interconnected system such as large pore sizes and moderate to strong acid sites. It is also inferred that heterogeneous acid catalysts give better conversion without accelerating any side reactions. The comparison of homogeneous and heterogeneous catalyst for production of biodiesel is given in Table 1.

Table 1 Comparison of homogenous and heterogeneous catalysts

Property	Homogeneous	Heterogeneous
Reaction rate	High	Low
Temperature (°C)	Moderate (<80)	High (>150)
Thermal stability	Poor	Good
Catalyst recovery	Difficult and expensive	Easy and inexpensive
Selectivity	Excellent	Good

Influence of co-solvent

In spite of all the advantages, one of the demerits with both heterogeneous acid and the alkaline catalyst is the formation of different phases in presence of alcohol (aqueous phase) and oil (organic phase) which leads to diffusion limitations thus lowering the rate of the reaction. The organic phase of oil and aqueous phases of methanol in a transesterification system of homogeneous and heterogeneous process are immiscible. The mass transfer rate between reactants and catalyst is limited. The methanol is a significant factor for enhancing the rate of reaction. Although the miscibility could be enhanced by increasing the temperature, it is highly an energy consuming process (Guan et al. 2009). This limitation can be overcome by adding co-solvent which has the following advantages (Sakthivel et al. 2013).

(i) The disappearance of inter-phase mass transfer resistance enhances the rate of separation of two phases (biodiesel and glycerol) more easily
(ii) Suppresses the rate of formation of soap significantly
(iii) Increases the biodiesel yield.

Recently (Calgaroto et al. 2013; Encinar et al. 2016), many researchers have studied extensively on transesterification of oils with the presence of co-solvent using homogeneous, heterogeneous and supercritical methanol processes. It is suggested that co-solvent could significantly influence the reduction of operating temperature, pressure and molar flow ratio for the supercritical methanol and enhance the reaction rate for homogeneous process. Various co-solvents like propane, hexane, heptanes, tetrahydrofuran (THF), carbon dioxide (CO_2), mixture of THF and hexane, alkyl ether (dimethyl ether, diethyl ether, etc.) are used to increase the biodiesel yield due to enhancement of the miscibility of the two phases. However, in spite of the increase in yield of biodiesel, the operating cost increases, particularly resulting from separation of co-solvent from methanol or the final product. To overcome the above-mentioned issues is to use "biodiesel" as co-solvent. As we know, the biodiesel is an effective green solvent to oil due to its methyl ester component, which considerably lowers the viscosity and biodegradable nature. Sakthivel et al. (2013) conducted the experiments in both batch/and continuous modes to investigate the effect of co-solvent on the

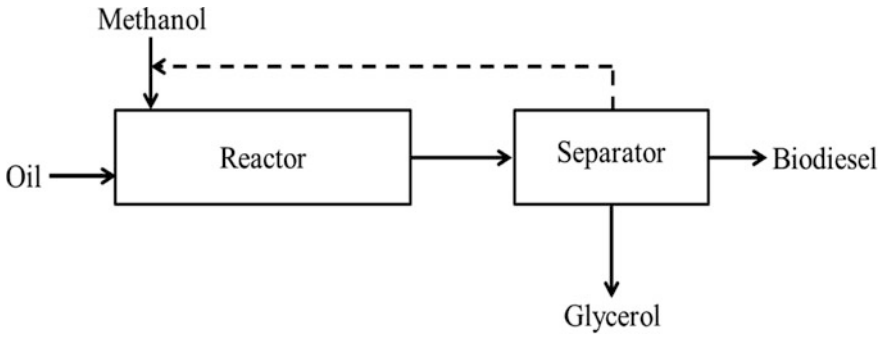

Fig. 6 Schematic diagram of SCM process

production of biodiesel from Jatropha oil. The result shows that the co-solvent (added in the range of 5–15% wt of biodiesel) enhances the mass transfer between triglyceride and methanol and, thereby the rate of reaction. It was also observed that there was about 20% increase in the yield of biodiesel with the presence of co-solvent.

2.3 Supercritical Methanol (SCM) Method

Supercritical fluids exhibit the properties of both liquid and gases when their temperature and pressure are above the critical point. The beneficial effect of supercritical fluid (alcohol) also is exploited in the production of biodiesel. One of the main advantages is the transesterification reaction between supercritical alcohol and triglycerides could proceed in the absence of any catalyst (Demirbas 2002). The significant decrease in hydrogen bond energy between alcohol molecules in the supercritical region allows them to be an acid catalyst (D'Iipolito et al. 2007). The schematic diagram of SCM process is shown in Fig. 6.

The SCM has several merits compared to that of homogeneous/heterogeneous catalytic process. This includes high production efficiency and eco-friendly process (Sawangkeawa et al. 2010). Besides supercritical alcohol, carbon dioxide and acetic acid at supercritical conditions have also been added to improve the process conditions. The supercritical carbon dioxide reduces the reaction temperature, while acetic acid reduces the glycerol by-product and increases the hydrolysis of fatty acids. The transesterification yield of 97+ % and the product composition with FAME content of >96% were obtained at a reaction temperature of 280 °C and a pressure of 20 MPa (Wei et al. 2013). Also, feedstock with high FFA and water

content could be handled at supercritical conditions as it won't affect the process yield. Overall, the SCM process gives a higher yield than the other conventional processes.

2.4 Enzymatic (Biochemical) Process

The enzymatic process is able to convert high-FFA feedstock by a two-step process. The glycerides are hydrolyzed to FFA, and then the FFA reacts with methanol to form biodiesel through esterification reaction. In the esterification step, water is produced and it is absorbed into the heavy phase, which also consists of glycerol, excess methanol and enzymes. Lipases (intracellular or extracellular lipase) act as a catalyst for biodiesel production at ambient temperatures and at atmospheric pressures. The enzymatic route is providing higher yield with purity, easy separation of product and by-product than the catalytic process of alkali/acid. This enzymatic process can be handled for feedstocks that are high in both FFA content and water. However, the enzymatic process has a few demerits for production of biodiesel such as slow rate of reaction, long residence time without the presence of a solvent, more expensive lipases and the frequent possibility of de-activation at the end of the reaction. The enzyme immobilization as a biocatalyst is one of the suitable catalysts and it would overcome the above-mentioned problems.

Recent studies report different types of enzymes like MucorMehei, *Geotrichum candidum*, *Candida antarctica*, *Pseudomonas fluorescens, Pseudomonas cepacia* and *Candida rugosa* available for biodiesel production. Generally, esterification and transesterification of triglycerides are to form methyl ester by methanol by using lipase (Fjerbaek et al. 2009). The polar short-chain alcohols affect the performance of the enzymatic process and inactivate the lipase (Shimada et al. 2002). To overcome the above problems three different options such as (i) methanol stepwise addition, (ii) acyl acceptor alterations and (iii) usage of solvent were suggested.

3 Technology Comparison

As many parameters, such as feedstock availability, process conditions, product quality, purification cost (removal of glycerol, catalyst and alcohol), operating cost and capital cost, are involved in the different technologies employed for biodiesel production, the comparison of the technologies with respect to these parameters are shown in Table 2.

Table 2 Comparison of technologies for production of biodiesel (Marchetti and Errazu 2010)

Variable	Alkaline Catalyst	Acid catalyst	Supercritical	Lipase catalyst
Temperature (°C)	50–70	60–80	240–350	35–50
Pressure (MPa)	0.1	0.1	High (35 MPa)	0.1
FFA in raw material	Saponified products	Ester formation	Ester formation	Ester formation
Yield	Normal	Normal	High	High
Product separation	Difficult	Difficult	Easy	Easy
Recovery of glycerol	Difficult	Difficult	Easy	Easy
Removal for purification	Methanol and catalyst	Methanol and catalyst	Methanol	Methanol and lipase
H_2O in raw material	Low yield	Low yield	No influence	No influence
Purification of methyl esters	Repeated H_2O wash	Repeated H_2O wash	Easy	Easy
Cost of catalyst	Inexpensive	Inexpensive	None	Expensive
Process	Complicated	Complicated	Simple	Complicated
Catalyst loss	Low	Low	None	High

4 Current Status in India

Currently, India has five to six large bio-refineries plants, with the capacity of producing 10000 to 250000 metric tons (mt) per year. At present, the total production of biodiesel is only 130–140 million litres from multiple feedstocks like non-edible vegetable oils, unusable edible oil waste (used-once) and animal fats. India has long preferred to produce biodiesel from the non-edible oils such as *J. curcas, P. pinnata, M. indica* and *A. indica* in India. Based on the literature survey, *J. curcas* and *P. Pinnata* have been identified as the potential feedstocks for biodiesel production in India. It is estimated that the potential availability of such oils in India is about 2 million tons per year (Agarwa 2007; Dwivedi et al. 2014).

The biodiesel production (in terms of yield) process mainly depends on free fatty acid (FFA) content, feed quality, type of alcohol and molar ratio (oil:alcohol), catalyst type and its concentration, reaction temperature and time (Puneet and Sharma 2016). But the cost of biodiesel depends on the raw material, processing cost, catalyst cost, nature of products separation, purification and storage. Typically, the feedstock consumes around 80–90% of the total operating cost of biodiesel production (Demirbas 2007). Baskar and Aiswarya (2016) have mentioned that the choice of catalyst and feedstock are the most important criteria for the effective production of biodiesel. In addition, the biodiesel producers must consider the following points to optimize the product price:

(i) Price of feedstock oil, alcohol (mostly methanol) and catalyst

(ii) Capital and operating costs of the plant, including services, product storage and buildings
(iii) By-product of glycerol, which provides secondary revenue to biodiesel producers.

In contemporary India, the commercial production cost of biodiesel is higher than the petroleum-derived diesel due to the cost of feedstocks and their operating costs including product wash. The academic and industrial researchers have to focus on cultivation and the feasibility of producing biodiesel at commercial scale from tree-borne oil seeds such as *Jatropha*, as pongamia (*P. pinnata*), neem (*A. indica*), kusum (*S. oleosa*), mahua (*Madhuca longifolia*), as well as waste edible oils.

5 Closing Remarks

The non-edible plant oils of *J. curcas, P. pinnata* are significant and promising feedstocks as per Indian climate conditions and they can be grown in dry climatic conditions with less maintenance, less water and less soil fertility. However, these seed oils have more than 10% of FFA that could hinder the yield as well as efficiency of the process. This is the biggest challenge for a biodiesel producer/ manufacture to get maximum yield in a cost-effective manner.

In a Technology viewpoint, the homogeneous (acid/alkaline) catalyst is mostly used for commercial production of biodiesel from batch and continuous processes. In addition, the homogenous methods have high rate of reaction, moderate temperature (<80 °C) and good selectivity. It could also be operated by a semi-skilled operator. The main disadvantage of a homogeneous method is the practical difficulty of removing the catalyst. The removal of catalyst from the product creates a large amount of wastewater, which increases the overall cost of the process. Thus, the total cost of the biodiesel production based on homogeneous catalysis, is not yet sufficiently competitive as compared to the cost of the diesel produced from petroleum.

The heterogeneous (acid/alkaline) catalysis is most favourable for the continuous processes with environmental friendly. The heterogeneous system requires more than 150 °C of reaction temperature and their respective methanol vapour pressure also to be considered, which could increase the operating and maintenance costs. Particularly, acid-catalyzed transesterification reaction requires higher alcohol to oil molar ratios than an alkaline catalyzed process. The low-quality feedstocks could be effectively converted into biodiesel by using heterogeneous catalyst methods. Other methods of Supercritical methanol process and enzymatic (biochemical) process are not yet economically viable for commercial production in India

Presently (2017), Indian commercial producers are using a two-stage homogenous process. The market price of biodiesel is Rs. 55 (±2), excluding tax. This price is a little high in the market due to the cost of feedstocks which makes up to

80–90% of the total production costs, operating costs and the capital cost in the view of business experts. The market price is almost near to the petroleum diesel price (approximately Rs. 60). However, in using biodiesel, there are enormous benefits towards the environment-friendly factors such as fewer emissions like CO, SO_2, NO_x and less particulate matter.

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Kinetic Modeling of Ethanol Production for Substrate–Microbe System

S. Suresh, V. C. Srivastava, S. Sakthivel and S. Arisutha

Abstract Ethanol, as an alternative energy resource, has become a subject of great interest due to the current surge in price of crude oil. Environmental concerns have promoted new applications and markets for ethanol. Kinetic modeling of ethanol production is very important from design and scale-up aspects of fermentors. In the present work, a kinetic model has been developed for the batch fermentation of crude whey for ethanol production by *Kluyveromyces marxianus*. Parameters of the kinetic model have been determined based on experimental data given by Zafar and Owais (Biochem Eng J 27, 295–298, 2006). Results have been compared by carrying out computer simulation. The kinetic model proposed in this study provides good predictions for growth of biomass, substrate consumption and ethanol production for all types of substrate-microbe systems.

Keywords Ethanol · Fermentation kinetics · Monod's equation
Substrate limitation · Product inhibition · Modeling

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Nomenclature

K_{ipx}	Inhibition constant of growth by product (g/l)
K_s	Monod growth constant for the substrate (g/l)
K_{sI}	Inhibition coefficient for cell growth on glucose
K_p	Saturation coefficient for cell growth on ethanol
K_{pI}	Inhibition coefficient for cell growth on ethanol
$K_{p/s}$	Monod product constant for the substrate (g/l)
K_r	Monod growth constant for the specific biotin concentration (g/l)
m_s	Maintenance coefficient (g substrate/(g cells h))
P	Product (L-glutamic acid) concentration (g/l)
$q_{p,max}$	Maximal specific production rate (1/h)
S	Substrate (glucose) concentration (g/l)
t	Time (h)
X	Biomass concentration (g/l)
X_m	Maximum cell concentration (g/l)
$Y_{x/s}$	Yield coefficient biomass from substrate (g/g)
$Y_{p/s}$	Yield coefficient product from substrate (g/g)

Greek letters

μ	Speicific growth rate (1/h)
μ_{max}	Maximal specific growth rate (1/h)
α	Growth-associated product formation coefficient (g/g)
β	Non-growth associated product formation coefficient(g/g h)

1 Introduction

Ethanol has tremendous applications in chemical, pharmaceutical and food industries in the form of raw material, solvent and fuel. The annual production of industrial ethanol is about four million tons, 80% of which is produced by fermentation. Biological fuel production might serve as a sustainable, carbon-neutral energy source compatible with current engine technology. In an effort to offset increases in consumption and to limit the fossil fuel-related negative impacts on the environment, the US Department of Energy has established the goal of supplanting 30% of gasoline consumption with cellulosic ethanol by 2030 (Bonkers 2006). With the increasing shortage of petroleum, urban air pollution and accumulation of carbon dioxide in the atmosphere, ethanol is expected to play a more significant role in the future. Government of India through a notification dated September 2002 made 5% ethanol-blending mandatory in petrol, in nine states and three Union Territories (MPNGR 2002). In the next phase, supply of ethanol-blended petrol

would be extended to the whole country and efforts would be made to increase the percentage of ethanol mixture in petrol to 10% (Suresh and Chandrasekhar 2009; MPNGR 2002). The increased realization of the finite nature of the world's oil supplies and vagaries in oil prices have rekindled interest in production of potable and industrial alcohol by fermentation of carbohydrate containing raw materials. Brazilian effort to reduce petroleum imports by adding ethanol to motor fuels is an interesting attempt in this direction.

1.1 Production of Ethanol by Using Fermentation

Ethanol Fermentation is a biological process in which sugars like glucose, fructose and sucrose are converted into cellular energy along with production of ethanol and carbon di oxide. Because yeast perform anaerobic conversion in the absence of oxygen. In ethanol fermentation, one glucose molecule breaks into two pyruvates. The energy released from this exothermic reaction is utilized in binding inorganic phosphate Adenosine di phosphate (ADP) and convert Nicotinamide Adenine Dinucleotide (NAD^+) to Nicotinamide Adenine Dinucleotide Hydrogen (NADH). The two pyruvates are then broken down into two acetaldehydes and give off two CO_2 as a waste product. The two acetaldehydes are then converted to two ethanol by using the H-ions from NADH; converting NADH back into NAD^+.

Many of the researchers were developed and optimized the non-linear mathematical models of fermentation of ethanol using different microbios (Starzak et al., 1994; Veeramallu and Agrawal, 1990; Chouakri et al. 1994; Cazzador and Lubenova 1995; Farza et al. 1997). Further, Baltes et al. (1994) demonstrated sensitivity analysis of different parameters over production. Tao et al. (2005) evaluated ethanol production by an acid-tolerant *Zymomonas mobilis* under non-sterilized condition. They found theoretical yield of ethanol from glucose was 0.488 g/g. In general, most of non-linear kinetic model established from batch experimental observations to evaluate concentration profiles for fermentation process may not perfectly fit. However, Wang and Sheu (2000) applied multi-objective optimization to estimate the kinetic model parameters of batch and fed-batch fermentation processes for ethanol production using *Saccharomyces diastaticus* in the 5 L fermentor. They found that estimated model was fitted through hybrid differential evolution of parameters.

1.2 Technologies for Ethanol Production

Chemical Route: Ethanol for use as an industrial feedstock or solvent (sometimes referred to as synthetic ethanol) is produced from petrochemical feed stocks, mainly by the acid-catalyzed hydration of ethylene:

$$C_2H_4 + H_2O \rightarrow CH_3CH_2OH$$

The catalyst used in this process is mostly phosphoric acid, adsorbed on a porous support like silica gel or diatomaceous earth. Shell Oil Company was the first to use this catalyst for the production of ethanol on large scale in 1947. The reaction takes place in the presence of high pressure steam at 300 °C (572 °F) where ethylene to steam ratio is kept 1.0:0.6. In the U.S., Union Carbide Corporation and other such industries used this process, but now it is used only commercially by Lyondell Basell.

In an older process, ethylene was hydrated indirectly to produce sulfovinic acid (ethyl sulfate) by reacting ethylene with sulfuric acid (conc. H_2SO_4), which was further reacted with water to hydrolyze it to produce ethanol along with regeneration of sulfuric acid. Union Carbide in 1930 first practiced this process on industrial scale but now this process is completely obsolete.

$$C_2H_4 + H_2SO_4 \rightarrow CH_3CH_2SO_4H$$
$$CH_3CH_2SO_4H + H_2O \rightarrow CH_3CH_2OH + H_2SO_4$$

Biochemical Route: Ethanol used in alcoholic beverages and as a fuel is produced through fermentation. Some particular species of yeast (e.g., *Saccharomyces cerevisiae*) metabolizes sugar to produce ethanol and carbon dioxide. The chemical equation given below clearly depicts this conversion:

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2$$
$$C_{12}H_{22}O_{11} + H_2O \rightarrow 4CH_3CH_2OH + 4CO_2$$

Fermentation is the process of culturing yeast under favorable thermal conditions to produce alcohol. This process is carried out at about 35–40 °C (95–104 °F). Toxicity of ethanol to yeast restricts the concentration of ethanol obtainable by fermenting. Therefore for higher concentrations distillation or fortification is used. A maximum of 18% ethanol concentration can be tolerated by the most ethanol-tolerant yeast strains. For the production of ethanol from the starchy material such as cereal grains, first starch must be converted to sugars. In production of beer, this has traditionally been done by letting the grain to germinate, or malt which then produces the enzyme amylase. When the germinated grain is mashed, the amylase converts the remaining starches into sugars.

1.3 Crude Whey for Ethanol Production by Kluyveromyces marxianus

The dairy industry represents an important part of the food processing industry and contributes significant liquid process residues that can be used for the production of ethanol (Ghaly and El-Taweel, 1997). Cheese whey (CW), a by-product of the

cheese manufacturing process whose major components are lactose (45–50 kg/m^3), proteins (6–8 kg/m^3), lipids (4–5 kg/m^3), and mineral salts (8–10% of dried extract), constitutes an inexpensive and nutritionally rich raw material for the production of different compounds (Gonzalez Siso 1996; Panesar et al. 2007). Ethanol production by bioconversion of whey is an alternative of great interest for reuse of this industrial by-product (Dragone et al., 2009).

Biological Oxygen demand (BOD) reductions of higher than 75%, with the concomitant production of biogas, ethanol, single cell protein or another marketable product, have been achieved and about half the whey produced nowadays is not a pollutant but a resource. However, annual world cheese-whey production is increasing and new bio-productions are being sought through biotechnology in order to get full use of the whey produced. Siso (1996) reviewed that application of cheese whey being exploited for production of ethanol.

Sansonetti et al. (2009) investigated the feasibility of bio-ethanol production by batch fermentation of ricotta cheese whey (Scotta), a dairy industry waste characterized by lactose concentration ranging from 4.5 to 5.0% (w/w) and, with respect to traditional (raw) whey, by much lower protein content. The microorganism used to carry out the fermentation processes was the yeast *Kluyveromyces marxianus*. The experimental data have demonstrated the process feasibility: scotta is an excellent substrate for fermentation and exhibits better performance with respect to both raw cheese whey and deproteinized whey. Complete lactose consumption, indeed, was observed in the shortest time (13 h) and with the highest ethanol yield (97% of the theoretical value).

Kluyveromyces marxianus KD-15, called flex yeast, is a strain that is in sensitive to catabolite repression and has the capacity to produce ethanol efficiently from a mixture of beet molasses and whey powder. Oda et al. (2010) conducted in 50 ml of a medium containing 200 mg/ml of sugar as sugar beet thick juice diluted with an arbitrary amount of crude whey, strain KD-15 produced over 99 mg/ml ethanol in all the media tested, and ethanol formation decreased in proportion to the volume of whey by *K. marxianus NBRC* 1963, the parental strain of KD-15, and *Saccharomyces cerevisiae NBRC 0224*, the reference strain for conventional ethanol production. Fermentation of thick juice diluted with whey alone by strain KD-15 at 30 °C initially proceeded slower than that at 33–37 °C but finally bore the highest level of ethanol. The maximum ethanol concentration obtained in 1.5L of a medium using a 2-L fermentor was elevated by aeration of 15–50 ml/min and reduced by that in excess of 100 ml/min. Under optimized conditions in 72 h, strain KD-15 converted all of the sugars derived from thick juice and whey to ethanol at 102 mg/ml, corresponding to 92.9% of the theoretical yield.

Cheese whey powder (CWP) is an attractive raw material for ethanol production since it is a dried and concentrated form of CW and contains lactose in addition to nitrogen, phosphate and other essential nutrients. Dragone et al. (2011) investigated that proteinized CWP was utilized as fermentation medium for ethanol production by *Kluyveromyces fragilis*. The individual and combined effects of initial lactose concentration (50–150 kg/m^3), temperature (25–35 °C) and inoculum concentration (1–3 kg/m^3) were investigated through a 23 full factorial central composite

design, and the optimal conditions for maximizing the ethanol production were determined. According to the statistical analysis, in the studied range of values, only the initial lactose concentration had a significant effect on ethanol production, resulting in higher product formation as the initial substrate concentration was increased. Assays with initial lactose concentration varying from 150 to 250 kg/m^3 were thus performed and revealed that the use of 200 kg/m^3 initial lactose concentration, inoculums concentration of 1 kg/m^3 and temperature of 35 °C were the best conditions for maximizing the ethanol production from CWP solution. Under these conditions, 80.95 kg/m^3 of ethanol was obtained after 44 h of fermentation.

Kuznetsova et al. (2015) investigated liquid extract (lupin whey) and its perspectives for biofuel production. The optimized multienzyme complex was composed of 1.1 ± 0.2 units/g of cellulase, 5.2 ± 0.4 units/g of xylanase and 2.5 ± 0.2 units/g of α-amylase. The enzymatic treatment resulted in 19% increase of the total sugar content of lupin whey versus to the control whey obtained without enzyme addition. The lupin whey was condensed by evaporation to 48–50% dry matter content. Condensed whey was used as nutrient medium for cultivation of yeasts *Saccharomyces cerevisiae*. After fermentation the yield of bioethanol reached 1.6 g/l. The proposed technology of complex processing of vegetable raw materials allows to obtain lupin protein concentrates with a crude protein content up to 63.2 ± 1.3% on dry matter basis and lupin whey with a total sugar content of up to 29% on dry matter basis. The lupin whey could be used as an organic substrate for biofuel production.

It is known that the fermentation process performance is affected by operational conditions such as temperature, stirring rate, initial inoculum and substrate concentrations, dissolved oxygen, among others. A suitable control of these variables is of great importance for a good process performance and obtainment of high-quality products. So optimize the conditions for ethanol production from CWP through different optimized numerical methods. Some of the factors are selected as process variables such initial lactose concentration, temperature and inoculum concentration; ethanol concentration, substrate consumption and fermentative parameters (ethanol yield factor, $Y_{P/S}$; ethanol volumetric productivity, Q_P; ethanol yield per cell, $Y_{P/x}$; and bioconversion efficiency, h).

In the present work, a kinetic model has been developed for the batch fermentation of crude whey for ethanol production by *K. marxianus* respectively. Parameters of the kinetic model have been determined based on experimental data given by Zafar and Owais (2006). Results have been compared by carrying out computer simulation.

2 Mathematical Model

In the present study, it was observed that the kinetic model used for gluconic acid fermentation by *Aspergillus* niger (Liu et al. 2003) may be used after suitable modifications. The logistic equation given by Eq. (1) can be used to model the cell

concentration, X. The logistic equation is a substrate independent model and there is inhibition of biomass on the growth model (Gong and Lun 1996).

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

The product formation rate given by Eq. (2) depends on both the instantaneous biomass concentration, X and growth rate, dX/dt in a linear manner and that the amount of carbon substrate used for product formation is negligible. The product formation kinetics is based on the Luedeking–Piret equation (Luedeking and Piret 1959).

$$\frac{dP}{dt} = \alpha \frac{dX}{dt} + \beta X \tag{2}$$

When, $\alpha \neq 0$, $\beta = 0$, the product formation is associate-growth.

The substrate is used for the production of cells and metabolic products as well as for the maintenance of cells (Znad et al. 2004). Therefore, the amount of carbon substrate used for product formation has also been included in the substrate model for the present study.

$$-\frac{dS}{dt} = \frac{1}{Y_{x/s}} \frac{dX}{dt} + \frac{1}{Y_{p/s}} \frac{dP}{dt} + m_s X \tag{3}$$

In addition, the kinetic equations (4)–(6) previously given by Bona and Moser (1997) for L-glutamic acid production were also used for kinetic modeling for ethanol production. These equations were developed in analogy to the equations given in the literature (Bajpai and Reub 1981; Moser and Schneider 1989).

$$\frac{dX}{dt} = \mu_{max} \frac{S}{S + K_s(1 + P/K_{ipx})} X \tag{4}$$

$$\frac{dS}{dt} = -\frac{1}{Y_{x/s}} \frac{dX}{dt} - \frac{1}{Y_{p/s}} \frac{dP}{dt} \tag{5}$$

$$\frac{dP}{dt} = q_{p,max} \frac{S}{K_{p/s} + S(1 + S/K_r)} X \tag{6}$$

2.1 Estimation of Kinetic Parameters

In order to estimate kinetic parameters given by eqs. Given in previous section, it is required to search those values of parameters which predict values of X, S and P close to the experimental values, X_{exp}, S_{exp} and P_{exp} within acceptable accuracy at

all times during fermentation process. The following objective function given by Nandasana and Kumar (2008) has been used for the simulation of the kinetic data.

$$Q = W_X^2 \sum_{\text{Alltimes}} (X - X_{\text{exp}})^2 + W_S^2 \sum_{\text{Alltimes}} (S - S_{\text{exp}})^2 + W_P^2 \sum_{\text{Alltimes}} (P - P_{\text{exp}})^2 \quad (7)$$

W_X, W_S and W_P are weighting factors, which were assumed as the reciprocal of the maximum concentration for respective components, viz. X, S and P. Model differential equations were solved by minimizing the objective function using regression procedure or numerical technique with Microsoft EXCEL 2007 to obtain values of X, S and P.

3 Results and Discussion

The variation of experimental and simulation values of X, S and P with respect to time is shown by data points in Figs. 1, 2 and 3, respectively. The initial value of X and S from Zafar and Owais (2006) for the experimental value were 2.82 and 35, respectively. Ethanol fermentation by *K. marxianus* showed a classical growth trend. After a lag phase, the cells entered the exponential growth phase. The strain started to form ethanol when the cells entered the exponential phase, and therefore, cell growth and ethanol took place simultaneously. The values of parameters of Zafar and Owais (2006) as determined by them are given in Table 1. Simulation lines of X, S and P with respect to time as per Bona and Moser (1997) and present model are shown by dotted and solid lines in Figs. 1, 2 and 3, respectively.

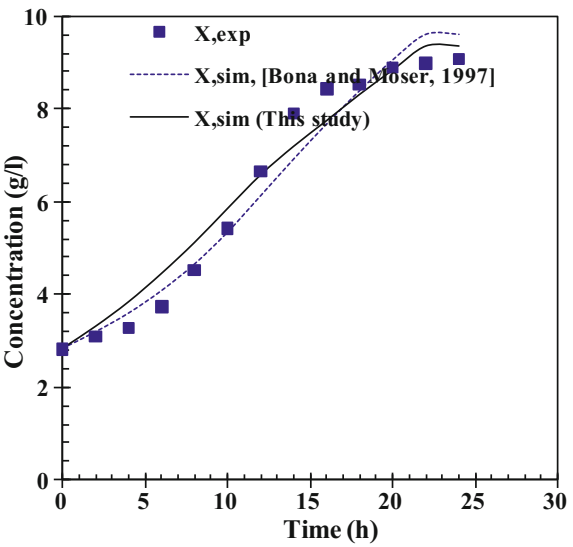

Fig. 1 Simulation of the experimental data collected from Zafar and Owais (2006) in an aerobic batch culture of *Kluyveromyces marxianus* for cell mass concentration

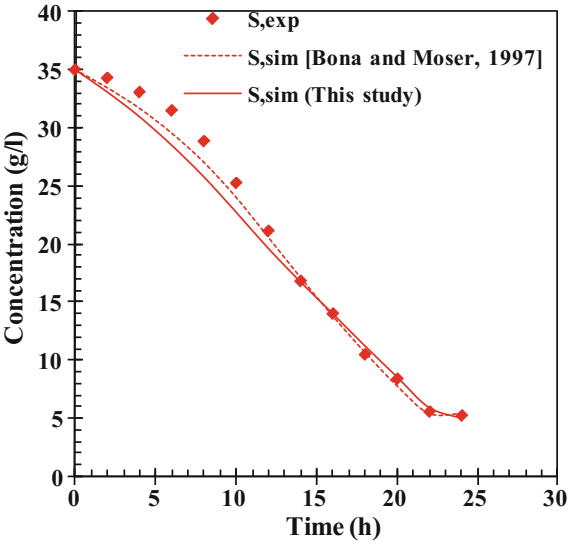

Fig. 2 Simulation of the experimental data collected from Zafar and Owais (2006) in an aerobic batch culture of *Kluyveromyces marxianus* for substrate concentration

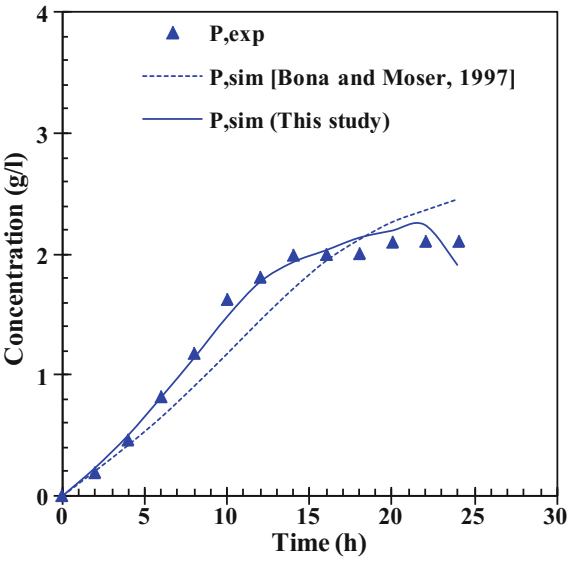

Fig. 3 Simulation of the experimental data collected from Zafar and Owais (2006) in an aerobic batch culture of *Kluyveromyces marxianus* for Ethanol concentration

The experimental values were fitted to the present model taking X_m = 11.864 g/l. The simulated values of the parameters are given in Table 1. A comparison of calculated value of X by Bona and Moser (1997) and present model along with the experimental data is given in Fig. 1. Both models seem to well-represent the experimental data. Similarly, Bona and Moser (1997) and present model well represent the S versus t experimental data given by Fig. 2. The present

Table 1 The kinetic values of model parameters as obtained from the present model and Bona and Moser (1997) model for the ethanol production experimental data given by Zafar and Owais (2006)

Kinetic parameter	Bona and Moser (2006) model	Kinetic parameter	Present model
Biomass production model			
μ_{max}	0.065	μ_{max}	0.095
K_{ipx}	4987.4		
K_s	6.21		
Substrate utilization model			
$Y_{x/s}$	0.229	$Y_{x/s}$	151.2
$Y_{p/s}$	27,203.4	$Y_{p/s}$	0.217
Ethanol production model			
$K_{p/s}$	54,0369.6	X_m	12.8
K_r	47,621.2	m_s	0.135
$q_{p,max}$	530.6	α	0.733
		β	0
Error or (Q value)	0.217	Error or (Q value)	0.100

model seems to better represent the P versus t experimental data given by Zafar and Owais (2006) as compared to Bona and Moser (1997) model (Fig. 3). Since, $\alpha \neq 0, \beta = 0$, the product formation is associate-growth Values of objective function (Q) by the Bona and Moser (1997) and present model were found to be 0.217and 0.10, respectively. Therefore, it may be concluded that overall present model better represents all experimental data.

4 Conclusion

Fermentation is a very complex process, and it is often very difficult to obtain a complete picture of what is actually going on in a particular fermentation. The model presented in this work is able to fit the experimental data with minimum value of the objective function (Q) of 0.10. The kinetic model proposed in this study provides good predictions for growth of biomass, substrate consumption and ethanol production by batch fermentation of crude whey by *Kluyveromyces marxianus.*

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