# Chapter 8 Mixed Lignocellulosic Feedstocks: An Effective Approach for Enhanced Biofuel Production



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Abstract Additional energy needs in today's world have led to the exploitation of fossil fuels like gasoline. Fossil resources are not only on the verge of extinction but also create environmental and economic problems. For these reasons, the ethanol production has been promoted as sustainable alternative to address the crisis related with increasing global warming, rising prices of crude oil and declining fuel stores. The lignocellulosic biomass leads to the production of second--generation (2G) ethanol through its hydrolysis, followed by bacterial fermentation and regeneration of product. Agricultural biomass produced as waste during or subsequent to the processing of the agricultural crops is one of such renewable sources and lignocelluloses of rich biomass sources found in abundance for ethanol production. Lignocellulosic biomasses are potentially sustainable feedstocks for the production of biofuels due to its availability, low price and high sugar content. Nonetheless, the costly processing necessities of lignocellulosic materials and the high cost of feedstock supply hamper the growth of biorefinery. A mixed feedstock system usually involves simultaneously handling and conversion of two or more than two different lignocellulosic feedstocks in equal or varying ratios for production of an interested product instead of using a single feedstock. This chapter aims to reveal the significance of the usage of mixed lignocellulosic feedstocks along with its potential advantages and limitations for enhanced biofuel production.

Keywords Biofuel · Lignocellulosic biomass · Mixed feedstocks · Bioethanol · Paddy straw

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# 8.1 Introduction

Today, the global transportation system relies heavily on the provision of mineral oil in spite of the quick development of other technologies, for example, (CNG) compressed natural gas vehicles, electrical vehicles, or biofuels (mainly ethanol and biodiesel) (International Energy Agency [2018](#page-26-0)). The utilization of fossil fueloils has led to the number of issues like environmental concerns, reduction of crude oil stores, and local disputes of source management (Oke et al. [2016](#page-28-0); Ge and Li [2018\)](#page-26-0). Carbohydrate polymers of lignocellulosic feedstock are renewable energy sources in petroleum-based fuels. The transition to renewable carbon from fuel-oils is largely determined by the shocking global demand for energy, which is being cut off by 80% by burning fuel, while 58% order comes from the transport sector only (Zabed et al. [2016\)](#page-30-0). In such approach, lignocellulosic materials, i.e., grasses, energy crops, industrial wastes, agricultural crop, and forest residues are expected to be considered as the potential raw supplies for the marketable production of 2G bioethanol. The supply of these materials is inexpensive as compared to 1G biomass (starchy and sugar-rich materials) and are basically not consumed by human beings because of extremely uneatable lignocellulosic substances. As a result, lignocellulosic waste materials do not interfere with human food stores and therefore avoid food versus energy clash. Also, some types of lignocellulosic waste biomass are highly poisonous and therefore cannot be utilized as fodders. Such indigestible varieties prevail over food, fodder versus energy conflict, and could therefore supply as an important feedstock in producing bioethanol in domestic and international markets.

A key to mass biofuel production based on lignocellulosic waste biomass is stable and constant, year-round trade of lignocellulosic feedstocks from a diversity of resources (Shi et al. [2015\)](#page-29-0). Nevertheless, in actual, the delivery of feedstocks in the United States reflects variations from area to area, year to year, or season to season. In a particular area, the quantity, quality, and cost of lignocellulosic feedstocks differ depending on climatic conditions, management, storage, and variety of crops (Milbrandt [2005\)](#page-27-0). As of an economic viewpoint, biorefinery have to be capable of effectively converting whichever waste residues offered at reasonable prices and indispensable levels to maintain profitability and productivity (Oke et al. [2016\)](#page-28-0). The amount of feedstock energy plays major function in estimating the price and energy for the production of biofuel. Low-energy density waste biomass is less efficient to convert it into biofuels than high-energy ones as a result of higher energy necessities for the transport, storage, and allocation of the waste lignocellulosic biomass from the grassland to the gateway of biorefinery.

As accounted by "Turkish Republic Ministry of Energy and Natural Resources" (TMENR [2011](#page-30-0)) (Imamoglu and Sukan [2014](#page-26-0)), overall utilization of fuel-oil was 22 million tons, out of which was three million tons of benzene and 160 thousand tons of bioethanol. According to Turkish Statistical Institute (TUIK) 900,000 tons of rice was collected from 99,000 hectares of plantations as well as 180,000 tons of its hulls were removed. Besides this, 2.60 million tons (MT) of cotton was produced from 481,000 hectares of plantations and 15.50 MT of its stalks were achieved in Turkey. Thus, as an outcome, the ratio of waste into product (W/P) was 6/1 for stalks of cotton and  $1/5$  for rice hulls in Turkey. Lim and Lee  $(2013)$  $(2013)$  reported chemical composition of rice husks and cotton straw with cellulose content of 28.60% and 47.10%, hemicellulose content of 28.6% and 24.1%, lignin content of 24.4% and 22%, and extractive matter of 18.4% and 6.3%, respectively. Thus, both of these feedstocks can be effectively used for enhanced biofuel production.

Till date, bulk of the existing studies on conversion of lignocellulosic biomass has paid attention on the use of single feedstock, and very less consideration for the effectiveness of converting combinations of different substrates to fermentable sugars as well as biofuels. This would necessitate re-examine as well as enhancement of current technology that is primarily based on utilization of single biomass feedstock. This chapter thus aims to focus on the probable advantages plus possible drawbacks about the use of mixed lignocellulosic feedstock approach for enhanced biofuel production. Different measures are also proposed to overcome the challenges and improve the processing of mixed lignocellulosic feedstocks (MLF) for the production of biofuels.

## 8.2 Biofuels

The vast raise in air contamination, shortage of fuel resources as well as rising global oil prices have sparked international interest in exploring renewable energy resources. The transport zone is the one that can apply renewable energy resources through replacement of fuel-oils with biofuels (Fig. 8.1). Biofuels can thus be



Fig. 8.1 Concept of biomass to biofuel



Fig. 8.2 Generation of biofuels

categorized into biodiesel, bioethanol, biohydrogen, biomethanol, and biogas. Bioethanol as well as biodiesel are the popular biofuels which are produced on industrial scale (Rodionova et al. [2017](#page-28-0)). Biodiesel and bioethanol are the main commodities in the world biofuel trade with an expected 25% biodiesel and 75% bioethanol of the total sales of biofuel. Five percent of the globe's bioethanol is provided by Europe, 39% by Brazil, and 50% by the United States (Heinimo and Junginger [2009](#page-26-0)). Bioethanol is ethanol formed through biochemical conversion of waste biomass through saccharification followed by fermentation. The different feedstocks which can be utilized are starchy grains, lignocelluloses, and microalgae that can be ultimately used to convert them into biofuels. There are four generation of biofuels which are shown in Fig. 8.2. The most expensive pretreatment method finds the yield of sugar that waste biomass can generate in the process of enzymatic saccharification, and this yield is generally less than 20% without prior treatment, while it can raise by more than 90% with pretreatment (Galbe and Zacchi [2007\)](#page-26-0). After pretreatment, the next step is saccharification followed by fermentation; both of which can be performed separately or concurrently. The technology for the production of bioethanol from lignocellulosic waste biomass has been developing quickly. Nonetheless, there are number of uncooperative buttonholes like complexity in pretreatment, production of highly resistant yeast inhibitors, and attempts to lessen the cost of enzyme and principal costs in producing complex manufacturing process that hinders trade (Banerjee et al. [2010;](#page-25-0) Talebnia et al. [2010](#page-29-0)). The method to decrease the expenditure of bioethanol production on industrialized level is by

decreasing the cost of untreated material, growing effectiveness, and promoting improved production systems (Jonker et al. [2015](#page-26-0)). Pretreatment is a major challenge to find the most excellent sugar raw substance for the process of saccharification and fermentation of lignocellulosic feedstock. The resultant outcome is not just on the point of lignocellulosic constituent, but also at the level of tissue as well as lignocellulosic biomass cells (Hendriks and Zeeman [2009\)](#page-26-0). Efforts are being made to improve the pretreatment method so as to get the lignocellulosic feedstock with higher porosity and larger surface area which ultimately makes cellulose largely available to enzymes as a result of removing disturbing substances (Agbor et al. [2011\)](#page-24-0). Different pretreatment methods have their benefits and drawbacks for their use at the industrial level. According to the relevant studies available, the effectiveness of pretreatment is to check the equilibrium between the inhibitor's production and the dissolution of feedstock (Parawira and Tekere [2011](#page-28-0)). In addition, pretreatment is the major cost-efficient process in terms of obtaining reducing sugars (Chandel et al. [2007](#page-25-0)). So to commercialize the production of biofuels at the industrial level, pretreatment is necessary.

# 8.2.1 Bioethanol

Bioethanol represents a closed carbon-dioxide  $(CO<sub>2</sub>)$  sequence as after the ethanol is burned, the released  $CO<sub>2</sub>$  is recycled reversely into the plant matter as they utilize  $CO<sub>2</sub>$  to produce cellulose during the photosynthesis cycle and thus, no net  $CO<sub>2</sub>$  is added in the atmosphere (Singh and Tiwari [2013](#page-29-0)). Also the toxicity of released emissions from bioethanol is lesser than that of the fossil fuel sources (Wyman and Hinman [1990](#page-30-0)). Bioethanol does not contain any mono-aromatic or poly-aromatic hydrocarbons, making bioethanol a clean and environment friendly fuel. Other advantages of bioethanol include its use as an octane enhancer in unleaded gasoline and as an oxygenated mixture of fuel for cleaner gasoline combustion, thereby dropping tailpipe pollution and improving ambient air quality (Kang et al. [2014](#page-26-0)).

### 8.2.2 Biobutanol

For blending of gasoline, a 4-carbon alcohol, namely butanol is much more attractive than bioethanol because of its elevated energy density, lesser Reid vapor pressure and hygroscopicity, enhanced mixing capacity, and utilization in conventional fire engines devoid of any alteration (Karimi et al. [2015\)](#page-27-0). Apart from the fuel extender, this biofuel can be utilized as an ultimate feedstock for the production of various marketable products (Mascal [2012](#page-27-0)). Fermentative production pathway, for example, by means of microorganisms under Clostridium genus attracts more attention environmentally and renewably than the petro-chemical path. These microorganisms usually produce combination of various liquid chemicals, primarily consisting of acetone, butanol, and ethanol; therefore, the process is thus known as "acetonebutanol-ethanol" (ABE) fermentation (Karimi et al. [2015\)](#page-27-0) Conversely, the key ultimatum in microbial butanol production is its low titer due to product inhibition. A number of approaches have been recorded to tackle these issues like metabolic as well as genetic alteration of microorganisms and significant integrated continuing culture technologies with proficient product revival techniques, for example, utilizing frameworks of metal-organic (Cousin et al. [2011\)](#page-25-0), gas stripping (Qureshi and Blaschek [2001\)](#page-28-0), liquid–liquid extraction (Sreekumar et al. [2015\)](#page-29-0), and pervaporation technique (Liu et al. [2014](#page-27-0)). Butanol can be produced through different engineered and metabolic ways using several different substrates. Starch or sugars be able to convert into biobutanol through clostridial pathway which involves "pyruvate: ferredoxin oxidoreductase", "glycolysis", "crotonase", "3-hydroxybutyryl-CoA dehydrogenase", "thiolase", "butyraldehyde/butanol dehydrogenase" as well as "butyryl-CoA dehydrogenase" The conversion of waste biomass to butanol also follows similar pathway after their conversion to pentoses and hexoses in the foregoing pretreatment and the enzymatic hydrolysis steps. The productions of lignocellulosic butanol have gained lots of attention, and more recently it has become the focal point of many studies (Morone and Pandey [2014\)](#page-27-0). However, the low titer and yield of butanol and necessity of additional pretreatment and hydrolysis steps are various key challenges in the lignocellulosic biobutanol formation. Likewise,  $CO<sub>2</sub>/H<sub>2</sub>$  or syngas also could be fermented to butanol through clostridial route (Bertsch and Müller [2015\)](#page-25-0).

# 8.2.3 Biodiesel

Biodiesel is a combination of "fatty acid methyl esters" (FAMEs) which can be synthesized via trans-esterification of animal fats or edible oil. It has recently received much interest as a renewable energy resource (Talebi et al. [2013\)](#page-29-0). However, this production source does not meet up the high demands for the transport fuel and requires renewable and sustainable energy source. On the other side, the oleaginous microorganisms can accumulate intracellular lipids, commonly called single cell oil (SCO), mainly triacylglycerols (TAGs). Microbial oils, such as unprocessed feedstocks for production of biodiesel, are advantageous as compared to vegetable oils due to shorter life cycle, are less labor intensive, less sensitive to location, climate, and season, and are easier to expand. Several oleaginous microorganisms, like yeasts, bacteria, microalgae as well as fungi, have been shown to produce considerable quantities of SCO with 20–50% dry cell mass) (Garay et al. [2016](#page-26-0)). Nevertheless, it is likely to enhance the accumulation of lipids in such oleaginous microorganisms through the technology of metabolic engineering, which involves different approaches, viz. enrichment of fatty acid and TAG synthesis, control of associated TAG biosynthesis circumvent, blockage of competitive routes, and multigene pathway (Satari et al. [2019\)](#page-29-0). An array of carbon resources from lignocellulosic-based carbohydrates along with other low-priced wastages from industries like foodstuff processing wastes, glycerol, and contaminated water has been incorporated by oleaginous organisms to make lipids. Supplementary nutrients like nitrogen (N) and phosphorous (P) are found in the waste streams. Conversely, accumulation of lipids in such organisms is often caused through deficiency of nutrients, for example, N or P, which is related to the carbon source (Jin et al. [2015\)](#page-26-0). The production of lipids from lignocellulosic waste has drawn considerable interest in present years and much research has been centered on its trade though major method enhancements plus reductions in production costs is necessary (Satari et al. [2019](#page-29-0)).

# 8.2.4 Biogas

In addition to aqueous biofuels, feedstock with higher organic contents is able to convert into biogas by anaerobic metabolism. In this method, the organic stuff decomposes naturally by the variety of microorganisms in the  $O<sub>2</sub>$ -deprived state and produces biogas (approximately  $25-50\%$  CO<sub>2</sub> and  $50-75\%$  CH<sub>4</sub>) (Kashi et al. [2017\)](#page-27-0). The process of anaerobic digestion can be categorized into four stages: (1) breakdown of proteins, lipids, and carbohydrates into amino acids, long chain fatty acids and sugars, respectively; (2) conversion of these formed products and monomers to "volatile fatty acids" (VFAs) and other small products, for example, alcohol by acidogenic bacteria; (3) conversion of VFAs to acetate,  $CO<sub>2</sub>$ , as well as H by acetogenic microbes; and (4) synthesis of methane through methanogenesis from the other phase products (Zheng et al. [2014\)](#page-30-0).

### 8.2.5 Biohydrogen

Hydrogen has found significant coherence as a substitute to fossil fuels due to several benefits of higher energy density of about 143 kilo joules per gram, with no carbon emissions plus numerous storage forms (Aruwajoye et al. [2020a\)](#page-25-0). Besides fuel compliance, hydrogen product has significant industrialized uses in synthesis of ammonia  $(NH<sub>3</sub>)$  and methanol. Biohydrogen has currently become of great significance as a carrier of renewable source, as the use of hydrogen intended for combustion, electricity production, or in fuel cell does not produce carbon emissions. Biohydrogen can be synthesized by photo-fermentation, bio-photolysis, and dark fermentation methods (Priya et al. [2020;](#page-28-0) Cárdenas et al. [2019](#page-25-0)). During dark fermentation process, biohydrogen is formed by fermentation of whole organic matter like lignocellulosic waste as well as industrial wastewater with thermophilic and mesophilic organisms. This process uses renewable raw stuffs and is not as much of energy intensive when comparing it to the thermo-chemical pathway and has therefore received a lot of consideration as the most favorable way for biohydrogen formation. However, fermented dark biohydrogen formation is still a major challenge due to low yield, low production rate, and low substrate conversion efficiencies (Pandey et al. [2019;](#page-28-0) Sekoai and Daramola [2015\)](#page-29-0).

### 8.3 Lignocellulosic Biomass (LB)

Lignocellulosic biomass (LB) refers to the plant biomass consists of cellulose, hemicellulose, lignin, and many other constituents like silica, ash, and pectins. The biomass is more gradually recognized as an important material, as it is a substitute to petroleum for the biofuel production. Bioethanol from renewable energy sources has been of great interest in current decades as an alternative to the existing fuel-oils (Belal [2013](#page-25-0)). The lignocellulosic materials are potentially sustainable feedstocks for biofuel production due to its accessibility, higher sugar content, and lower price (Saini et al. [2015](#page-28-0)). On an average, lignocellulosic waste biomass consists of 40–50% cellulose, 20-–30% hemicellulose, and 10–25% lignin (Shahzadi et al. [2014\)](#page-29-0). Cellulose is a main plant storage polysaccharide containing a linear sequence of D-glucopyranose units joined by  $\beta \rightarrow (1,4)$ -glycosidic bonds to each other and accountable for mechanical power, whereas hemicellulose macromolecule polymers are repeated polymers of pentoses (C5), hexoses (C6), and a variety of sugar acids. Lignin is an aromatic polymer of three alcohols, namely sinapyl alcohol, coniferyl alcohol, and p-coumaryl alcohol) produced by biosynthetic processes and forms a protective covering around cellulose and hemicellulose (Iqbal et al. [2013\)](#page-26-0).

The lignocellulosic waste biomass can be utilized for the production of biofuels only after a pretreatment process which helps in weakening the natural recalcitrant structure to open up the accessible fermentable sugars (Bosma et al. [2013\)](#page-25-0) in the shape of monomeric sugars (C5 and C6). Pretreatment methods include chemical, physical, and biological processes or a combination of all these (Fig. [8.3\)](#page-8-0). Among these pretreatments, the chemical treatment of dilute sulfuric acid is very efficient in opening the recalcitrant arrangement of agricultural biomass (Brodeur et al. [2011\)](#page-25-0). In this pretreatment, numerous inhibitory compounds are produced, as well as hydrolyzed sugars, which usually inhibit cell growth and fermentation. These inhibitory compounds are different salts, furfurals, acetic, ferulic, phenolic compounds, glucuronic acid, coumaric acid, and hydroxymethyl furfurals (HMF). Thus, their formation and removal becomes a vital parameter that needs to be performed for any aspect of lignocellulosic waste biomass in production of biofuels. In present years, extensive studies have been done on biobutanol production (Zhen et al. [2020\)](#page-30-0). Biobutanol is a better-quality fuel than bioethanol due to its higher energy content, improved air-to-fuel proportion, more explosive, less volatility, higher ash point, and lower vapor pressure. Commercial butanol is usually chemically synthesized from fuel-oil. However, with the reduction in fossil fuel and environmental barriers emerging, production of biobutanol through fermentation has become more beneficial.

<span id="page-8-0"></span>

Fig. 8.3 List of pretreatment technologies for lignocellulosic feedstock conversion

# 8.3.1 Lignocellulosic Biomass (LCB) Sources

Lignocellulosic biomass is divided into different categories according to its source as follows: shrubs; wood, for example, hardwoods as well as softwoods; non-food agricultural crops like rapeseed, kenaf; agricultural residues, viz. maize cobs, paddy straw, its hulls and husk, wheat straw, maize cobs, sugarcane tops and bagasse, as well as municipal solid wastages (MSW) related to gardening, tapering, road repairs, etc. The waste biomass involved in the generation of bioethanol chiefly involves agricultural wastes (Niju et al. [2020](#page-28-0)). The majority of organic products is formed while processing the by-products during the harvest of agricultural crops and in addition is classified as primary along with secondary residues. The primary ones are the remaining elements obtained during crop harvesting in the field (e.g., wheat straw and sugarcane tops) whereas secondary remains are massed while the procedures are described (e.g., sugarcane bagasse). Although the primary ones are utilized for animal feed as well as fertilizers, their use in energy generation is very little and restricted. In the meantime, the secondary ones found in bulk on its affluent place; in addition, it can be stored as energy sources. A lot of agricultural remains are not used and burned openly, which has a huge effect on the atmosphere with significant energy loss. Agricultural wastes, viz. wheat straw, paddy straw and

husk, maize cobs, sugarcane bagasse, dry sugarcane tops and leaves, etc. is widely available in the grassland and is believed as a key source of bioethanol production.

#### 8.3.1.1 Paddy Straw: The Most Abundant Lignocellulosic Feedstock

Paddy, a monocotyledon belongs to the *Oryza* genus consists of two cultivable species, namely Oryza glaberrima as well as O. sativa commenced from Africa and Asia, respectively (Khush [1997](#page-27-0)), the former is limited to only West African area while the later that has enhanced yield which is cultivated nearly in 112 countries. In most of the Asian countries, paddy is the main staple food, and it generates a large amount of paddy straw as agricultural crop residue in the fields (Singh et al. [2016\)](#page-29-0). In terms of total production in the world, paddy is ranked third important agricultural crop after wheat and corn (Binod et al. [2010\)](#page-25-0). India produces 100 million metric tons (MMT) paddy per year against the global manufacturing of 727 MMT (Kocher and Kalra  $2013$ ). About 1–1.5 kg of paddy straw is formed in every kilogram of grain harvested (Maiorella [1985\)](#page-27-0).

Among different states of India, rice is a major crop grown in Punjab on an area of 29.75 lakh hectares with total production of 177.34 lakh tons of paddy (118.23 lakh tons of rice). The average yield in terms of paddy in Punjab is 59.61 quintal per hectare (23.84 quintal per acre) (Anonymous [2017](#page-25-0)). Punjab alone produces 17 million tons of paddy straw yearly, from which around 15 million tons is cleaned off from the grounds by burning (Anonymous [2014](#page-24-0)). Farmers remedy to the activity of open field burning of paddy straw because of the following reasons: (1) short period between harvesting of paddy and wheat plantation; (2) to kill soil-borne deleterious pests and pathogens; (3) lack of harvesting machinery; (4) high transportation costs; (5) high processing cost; (6) high labor charges for the handling of straw (Singh et al. [2016\)](#page-29-0). The uncontrolled burning of paddy straw in the open field causes greenhouse gas emissions like methane  $(CH_4)$ ,  $CO_2$ , carbon monoxide (CO), nitrous oxide  $(N_2O)$ ; emissions of other gaseous contaminants like sulfur dioxide  $(SO_2)$ , nitrogen oxides  $(NO_x)$ , HCl and, to some level, furans and dioxins  $(Oanh et al. 2011)$  $(Oanh et al. 2011)$ . These pollutants have notable toxic properties and can be potential carcinogens (Gadde et al.  $2009$ ). Furthermore, emissions of  $CO<sub>2</sub>$  from open burning entail severe risk to the atmosphere as it adds to the issue of global warming to the greater level (Mandal et al. [2004](#page-27-0)). Burning of paddy straw causes loss of nutrients, reduction of soil organic material, and decrease in valuable soil biota. The brutality of harm caused by such pollutants can be experimented from the reality that paddy straw burning causes almost complete loss of nitrogen, phosphorus losses of about 25%, potassium losses of 20%, and sulfur losses of 5–60%. The amount of nutrients lost depends on the method used to burn the straw. The huge quantity of heat released through burning of straw have a direct effect on the properties of soil by reducing the humidity level as well as microflora. Therefore, the adverse impact of burning the straw calls for its management and utilization.

There are several options which are being practiced and/or being tried for management of straw such as fuel for power generation in brick kilns, biofuel production, surface retention and mulching, incorporation in soil for improvement of soil health, use as feedstock, mushroom cultivation, bailing, and removing the straw (Mandal et al. [2004\)](#page-27-0). In 2016–2017, to attain the above-mentioned aims, the state government had set the target of utilization of 5.73 million tons of rice straw (Anonymous [2014\)](#page-24-0).

Rice straw can be converted into various forms of biofuels. Thus, there is a requirement to "deoxygenate" the lignocellulosic waste and the most important methods for conversion of paddy straw to synthetic fuels are: (1) hydrogenation to produce pure hydrogen, (2) pyrolysis to produce bio-crude, (3) anaerobic digestion for biogas production, (4) gasification for syngas, and CO production, (5) biochemical conversion to bioethanol (Demirbas et al. [2011\)](#page-25-0). Among these, the ethanol production from paddy straw is an attractive option because the straw has higher contents of cellulose and hemicelluloses which can be easily hydrolyzed to fermentable sugars (Binod et al. [2010\)](#page-25-0). Thus, the paddy straw can be mixed in different proportions with other lignocellulosic feedstocks for enhanced and efficient bioethanol production.

#### 8.3.1.2 Rice Husk

In 2005, the rice consumption in Indonesia arrived 54 million tons. Of such amount, rice husks of about 10.8 million tons can be separated (Rahardjo et al. [2021\)](#page-28-0). According to other theory, the process of grinding rice typically yields about 50–63% of the milled rice; 20–30% of the husk; and 8–12% of the bran. The husk generally consists of cellulose, hemicellulose, lignin, and ash with value of 42.20%, 18.47%, 19.40%, and 15%, respectively.

#### 8.3.1.3 Coconut Husks

Coconut is tropical plant mainly found in almost all countries, especially in Indonesia where its production reaches around 28 lakh tons per annum. This highest production is associated with the higher waste biomass production in the type of coconut husks. The coir is the outmost part and when the fruit of coconut is removed from its coir, this will obtain 35% of the coconut weight (Khatiwada et al. [2016](#page-27-0)). It is thus expected that the coir production is around 10 lakh tons in 2016. Coir of coconut is a waste lignocellulosic feedstock consisting of crude fiber. The cellulose as well as lignin content in husks is extremely high, with value of 26.72% cellulose and 41.19% lignin, while the hemicellulose content is only about 17.73% (Sangian et al. [2015](#page-28-0)).

#### 8.3.1.4 Sugarcane Bagasse (SCB)

Saccharum officinarum (sugarcane) is the agricultural crop commonly originated in Indonesia, principally on the Java Island. It belongs to the tall evergreen true grasses of family Andropogoneae. Out of the top ten manufactures of sugarcane worldwide, its production in Indonesia was 29 million tons in 2012 (Rahardjo et al. [2021\)](#page-28-0). It is raw product for sugar derived through the extraction of sugarcane plants. It consists of three key components, i.e., extract of sugar, bagasse, and molasses. Molasses is an integrated sugar produce that can be utilized in the production of ethanol. Five percent sugar and 90% of bagasse content is found in sugarcane. Sugarcane bagasse consists of cellulose, hemicellulose, and lignin with content of 52%, 20%, and 24%, respectively (Wahono et al. [2014](#page-30-0)).

#### 8.3.1.5 Sugarcane Tops (SCT)

Sugarcane plant is a renewable energy source and is widely grown in countries like Australia, Brazil, Thailand, India, and China. Sucrose is the most important produce of sugarcane that accumulates in the stalk's internodes and thus able to be extracted as well as refined in particular sugar industries, followed by its utilization in the food industry or fermentation for ethanol production. An entirely full-grown plant of sugarcane has about 75% stalks plus 25% of the tops and leaves (Sherpa et al. [2019\)](#page-29-0). Sugarcane tops (SCT) include leaves that consist of sugar in cellulose form and thus are cellulosic-rich substances. Thus, ethanol could be extracted from the tops through its higher cellulosic potential and massive scale feedstock accessibility. The production of bioethanol from the leaves and tops of sugarcane will not have any effect on food supply and also not adversely affect the juice extorted from the stalks. The tops of sugarcane are found in abundance during collection but are burned in the ground itself (Sukumaran et al. [2010](#page-29-0)) and are generally utilized as an animal fodder prior to the leaves begin to rot. "Polyaromatic hydrocarbons" are released in the process of burning, while other matter released into the atmosphere can be mutagenic or carcinogenic. In 2010, the production of sugarcane is around 1700 million tons per year globally as documented by the "Food and Agricultural Organization" (FAO). This produces a large number of post-harvest residues, especially SCT, which is a cheap and easily accessible resource for lignocellulosic feedstock. Typically, 0.30 million tons of the sugarcane tops was produced during harvesting of one million tons of sugarcane (Sindhu et al. [2011](#page-29-0)). Attempts can thus be made to use the tops as an easily available substrate for enhanced ethanol production. The cellulose, hemicellulose, and lignin content in the sugarcane tops is 29.85%, 18.85%, and 25.80%, respectively (Sindhu et al. [2013\)](#page-29-0) while in the sugarcane leaves the content is 36%, 21%, and 16%, respectively (Moodley and Kana [2018\)](#page-27-0). In addition, the capacity of dry SCL is equivalent to ten tons of coal per hectare. The tops are rich in cellulose which contains higher glucan and xylan

contents and is found as a prominent raw material that can be utilized for bioethanol production.

#### 8.3.1.6 Maize Stover

Maize (Zea Mays) is an important food crop worldwide. In Indonesia, the area under harvest was 3.5 million ha with the average manufacturing of 3.47 tons per hectare, whereas the national corn production was about 11.7 million tons in 2006. The stems as well as dry leaves are the wastes from these agricultural crops have the production of 3.46 tons per hectare. The total agricultural wastage varies from 12.10 million tons. The chemical composition of maize stover is as follows: 37.10% cellulose, 22% hemicellulose, 2.5% arabinan, 1.6% galactan, 20.70% lignin, 7.8% protein, and others (Rahardjo et al. [2021](#page-28-0)).

#### 8.3.1.7 Palm Oil Empty Fruit Bunch

Elaeis guineensis is the central raw substance for the production of palm oil. The world's second leading producer of palm oil is Indonesia where number of its fields reaches around 67 lakh ha that reach across 22 zones. The world can generate around 310 lakh tons of palm oil per annum. Around 26% weight of the whole palm oil production is from its empty bunches which is a waste product and are abundantly present. Only 10% of bunches are utilized as petroleum for compost and boilers; accordingly, there is still much of the lignocellulosic waste left which is capable of being converted into useful products. The chemical composition of the empty bunches is 33.25% cellulose, 23.24% hemicellulose, 25.83% lignin, 56.49% holocellulose, 4.19% extractive matter, and 8.56% water (Rahardjo et al. [2021\)](#page-28-0).

# 8.4 Mixed Lignocellulosic Feedstock (MLF) Theory

The theory of mixed lignocellulosic feedstock (MLF) usually entails the simultaneous handling as well as conversion of mixture of two or more than two different substrates in equal or varying ratios for the formation of an interested product instead of utilizing single lignocellulosic feedstock (Fig. [8.4](#page-13-0)). The substrates can have the identical or different origins (resource as well as supply chain), might comprise similar or contradictory features, and may have need of same or different handing methods in their conversion.

The concept of combining two or more different substrates in the mixed lignocellulosic feedstocks approach can ultimately enhance the production of biofuels (Oke et al. [2016](#page-28-0)). The preference of substrates should be primarily based on the necessity to avoid additional nutrient uptake, the proximity of various feedstock to the collection hub or processing capability, and in general economic performance

<span id="page-13-0"></span>

Fig. 8.4 Single biomass v/s mixed biomass approach to lignocellulosic bioprocessing

depending on the quantity associated with the lower feedstock costs (Fan et al. [2019\)](#page-26-0). Table [8.1](#page-14-0) represented the different studies using mixed lignocellulosic biomass for bioethanol production. A combination of different lignocellulosic compounds was investigated by mixing substrates listed either as under similar class (e.g., mixed hardwood) (Lim and Lee [2013](#page-27-0)), grasses mixtures (Martín et al. [2008\)](#page-27-0), or different class (sugarcane bagasse and straw (Moutta et al. [2014](#page-27-0)) paddy straw and wheat bran (Qi et al. [2007](#page-28-0))). Meanwhile, the incorporation of starch or sugar-based constituents in the bioethanol production has also been investigated, in which part of the mix includes first-generation lignocellulosic feedstock (e.g., corn kernels, wheat meal, and starch or sugary wastes (e.g., extracted residue of Dioscorea composita (DER), sugarcane molasses). Thus, this method helps us to lessen production costs and enhance cellulosic ethanol production.

Fan et al. ([2018\)](#page-26-0) studied integrated molasses in the production of sugarcanebased ethanol which ultimately improved the final production of ethanol and showed that the optimal ratio for the fermentation of molasses and sugarcane bagasse was 1: 1. The productivity ratio inconsistency makes molasses unable to meet the conditions of bagasse-based ethanol production. To prevail over this issue of feedstock inequality, DER and cassava were thought to replace half of the molasses because they both are rich in starch and are grown worldwide in tropical and subtropical climates. Nevertheless, cassava as one of the mainstays for the industrial production of starch is used as a staple crop in a number of areas such as Latin America and South Asia, thus eliminating its high demand for biofuel production as feedstock.

Singla et al. ([2018\)](#page-29-0) evaluated cellulase production using different agricultural biomass for hydrolysis of rice straw. In their experiment, they used different ratios of soybean pod husk and paddy straw. The main focus of their study was to make use of paddy straw due to its abundance and the health problems linked with paddy straw burning. The combination of soybean pod husk and paddy straw as substrates in equal ratio showed higher enzyme activities as compared to 3:1 ratio and paddy straw alone as substrate under solid-state fermentation at different incubation time. The addition of soybean pod husk to the rice straw, thus, stimulated Aspergillus fumigatus for high cellulolytic enzyme production. Soybean pod husk was observed

Mixture constituents	Configuration/ Fermenting microorganisms	Mixture pretreatment	Objective	Results	Source
Ricotta whey and sugarcane bagasse	Simultaneous saccharification and fermenta- tion: Kluyveromyces marxianus CCT 7735	Separate and acid pretreatment given to sug- arcane bagasse	Optimization of bioethanol production	Highest ethanol yield of $49.65$ g/ L was observed at optimum conditions	Ferreira et al. (2015)
Wheat straw and waste paper	Simultaneous saccharification and fermenta- tion (SSF); Sac- charomyces cerevisiae "NCYC 2826"	Joint pretreatment of steam explosion	Effect of waste paper as co-substrate <sub>on</sub> pretreatment as well as production of ethanol	Decrease in the levels of inhibi- tor and high yield of ethanol after 24 h	Elliston et al. (2015)
Wheat straw: wood wastes, and waste papers	Separate hydro- lysis and fer- mentation (SHF); Saccha- romyces cerevisiae	Combined pretreatment of acid- assisted steam explosion	Effect of pretreatment on ethanol production	Theoretical eth- anol yield of 80%	Nguyen et al. (1999)
Cotton stalks and rice hull	SHF: Escherichia coli "KO11"	Separate acid pretreatment	Effect of sin- gle versus dif- ferent sub- strate mix- tures on bioethanol production	7:3 ratio of rice hulls and cotton stalks gave highest ethanol yield with value of $0.44$ g etha- nol/g sugar)	Imamoglu and Sukan (2014)
Wheat straw and municipal household waste	SSF; Saccharo- myces cerevisiae	Combined pretreatment of wet oxidation	Effect of pretreatment as well as enzyme loads on bioethanol production	60-65% yield of bioethanol obtained at optimized con- ditions and with reasonable enzyme loadings	Lissens et al. (2004)
Sugarcane residues (hops, straw, and bagasse)	SHF; Saccharo- myces cerevisiae "CAT-1"	Joint acid pretreatment	Bioethanol production on single versus combined biomass	25% high etha- nol yield on mixed residues as compared to sugarcane bagasse only	Pereira et al. (2015)

<span id="page-14-0"></span>Table 8.1 Different studies using mixed lignocellulosic biomass feedstocks for bioethanol production

to possess high crude protein content and therefore, the supplementation of soybean pod husk with paddy straw resulted in high enzyme titer with balanced proportion of different enzyme activities. The carbon-to-nitrogen (C:N) ratio of paddy straw is very high, i.e., about 80:1 (Goyal and Sindh [2011\)](#page-26-0) as compared to other agricultural waste biomass. Therefore, addition of soybean pod husk to paddy straw declined the C:N value of the resulting mixture, thus improving the conditions for fungal growth and cellulase production (Delabona et al. [2013\)](#page-25-0). As a result, C:N ratio is an essential factor to find out the efficiency of solid-state fermentation process for enzyme production (Krishna [2005\)](#page-27-0). Soybean pod husk residue was found to provide nearly all necessary nutrients for the growth and production of enzymes by A. fumigatus, in this manner reducing the necessity for adding up of costly supplements to the paddy straw for enzyme production. Hence, studies showed that by using better fungal strain and plant-based agricultural biomass, industrially relevant enzyme titers and productivities could be achieved for enhanced saccharification of paddy straw for ethanol production. This can, thus, help to reduce the overall expenditure of ethanol production from paddy straw.

Sherief et al. ([2010\)](#page-29-0) studied different fungal species for cellulolytic enzyme production and among these Emericella niveus, Aspergillus fumigatus, and A. terreus showed high yield of carboxymethyl cellulase, exogluconase, and xylanase activities. The enzyme production by A. fumigatus was tested on mixed lignocellulosic biomass, i.e., paddy straw and wheat bran added in different ratios (9: 1, 7:3, 1:1, 3:7, and 1:9 ratios). The maximum xylanase, CMCase, and endogluconase activities of 49.30, 14.70, and 0.68 U  $g^{-1}$ , respectively, were found in mixed culture of paddy straw and wheat bran in 1:1 ratio, while higher β-glucosidase (8.5 U g<sup>-1</sup>) and exogluconase (0.93 U g<sup>-1</sup>) activities were detected in mixed culture of rice straw and wheat bran in the 7:3 ratio. Thus, this study showed that paddy straw mixed with wheat bran can serve as superior substrate for cellulase and xylanase production.

Similarly, Reddy et al. ([2015\)](#page-28-0) studied different combinations of natural lignocelluloses for enzyme production by A. niger. The combination of wheat bran and rice bran (1:1) served best combination for maximum production of cellulases with filter paper, CMCase, and β-glucosidase activities of 29.81, 25.20, and 32.18 U g<sup>-1</sup> under solid-state fermentation, respectively.

# 8.4.1 Biofuel Production from Mixed Biomass

Although literature on mixed biomass use is often inadequate, a significant rise in the publications related to the production of biofuel from MLF was recorded over the past 5 years. Lignocellulosic feedstocks from a diversity of sources have been utilized in mixture with other feedstocks or first-generation residues for ethanol production. Numerous reports have shown that the production of ethanol in mixture of components is superior compared to that found in single lignocellulosic biomass. Where different lignocellulosic feedstock can be utilized in mixture for production of bioethanol, individual substrates possibly will be pretreated first and then hydrolyzed independently or in combination. In separate pretreatment or breakdown, the products of hydrolysis from the single feedstocks combined before fermentation (Imamoglu and Sukan [2014\)](#page-26-0); but for combined pretreatment or hydrolysis, the emerging hydrolysates of the raw combination is utilized directly for fermentation (Lissens et al. [2004;](#page-27-0) Elliston et al. [2015](#page-25-0)). In the case of starch-based substrates, hydrolysates of starch can be diluted prior to use or can be directly used with the cellulosic hydrolysate (Brandberg et al. [2007](#page-25-0); Erdei et al. [2013](#page-25-0)). Simultaneous saccharification and fermentation (SSF) and separate hydrolysis and fermentation (SHF) are often reported for the production of ethanol from mixed feedstocks. The implementation of saccharification and simultaneous co-fermentation (SSCF) and consolidated bioprocessing (CBP) is rarely reported. High bioethanol production in the 90–99% range is widely reported with SSF for the combinations of the first- and second-generation feedstocks (Erdei et al. [2013](#page-25-0); Ji et al. [2015\)](#page-26-0). Complete lignocellulosic mixtures usually have low yields for SSF in 70–80% range (Lissens et al. [2004;](#page-27-0) Martín et al. [2008\)](#page-27-0) and SHF (Pereira et al. [2015;](#page-28-0) Imamoglu and Sukan [2014\)](#page-26-0).

# 8.4.2 "Mixed Starch-Based Agricultural Waste" (MSBAW) for Integrated Production of Biofuel

"Starch-based agricultural waste" is a prospective component of bio-fuel production as it is cheaper, inexpensive, and less competitive. In addition, they contain a highstrength starch polymer together with usual holocellulose backbone that is common throughout the lignocellulosic biomass. Additionally, the use of such high-energy wastes of the world frees the environment from possible health risks related to their disposal as well as decay (Aruwajoye et al. [2020a](#page-25-0)). Utilizing biofuel production from these components in commercial quantities is also possible if the common concern of "waste-based biofuel production" is adequately addressed due to the MSBAW route. The drawbacks of commercial waste-based biofuels estimates are mainly based on the high cost of feedstock delivery and the technological complexity associated with low-throughput and advanced processes (Banerjee et al. [2010\)](#page-25-0). Feeding the supply chain arises from the cost related to the collection plus transport of feedstocks to the biorefinery machine. Moreover, the seasonal accessibility of single feedstock from which the wastage is produced disturbs its immediate delivery to the biorefinery. On the other hand, the utilization of MSBAW in equal or varying ratios is a possible way out to the above-mentioned challenges. Even though there is inadequate literature available on bioenergy production from MLF, but they offer numerous benefits as compared to the use of single feedstock (Aruwajoye et al. [2020a](#page-25-0)). For example, the use of MSBAW in particular has the potential to produce a higher output during pre-processing. In addition, the seasonal feedstock that normally requires wide storage of the single feedstock is avoided owing to the presence of different starchy wastages from different seasons which ultimately lessens the



Fig. 8.5 A planned design of integrated biohydrogen and bioethanol production

time frame for the collection of biomass and the apparatus use, workers, and sources required under the strict use of single feedstock (Rentizelas et al. [2009\)](#page-28-0). Furthermore, the utilization of MSBAW for the integrated production of bioethanol and biohydrogen meets the basic criteria for mixed feedstock utilization for biofuel production. These include similar features, given the high yield of sugar that can be fermented (existence of starch), hence bioethanol or biohydrogen (Aruwajoye et al. [2020a](#page-25-0)), and cheap and abundant. However, after designing an efficient production system from mixed starchy wastage, it is essential to select handling methods that will suit all components of the mixture throughout the main stages of the process. This is important as a result of the existence of different features of mixed starchy wastes such as ash, moisture, and chemical composition. The proposed process for the integrated production of bioethanol plus biohydrogen from mixed starch-based wastages is presented in Fig. 8.5. Modifications related with ethanol from mixed starchy waste biomass followed by biohydrogen should be accompanied by major phases of lignocellulosic biofuel production. The three crucial stages of ethanol generation from waste biomass are pretreatment, saccharification, and fermentation (Aruwajoye et al. [2020b\)](#page-25-0).

In an experiment reported by Fan et al.  $(2019)$  $(2019)$ , the pretreated sugarcane bagasse (SCB), molasses, and DER with various proportions were studied with a low solid load of 12%, with an optimal combination proportion of 1:0.5:0.5 for the pretreated bagasse/molasses/DER was tested for ethanol yield and concentration. However, it has been observed that yield of ethanol declined from 79.19 to 62.31% as the solid loading raised from 12 to 44% in batch modes, except for the fact that threecomponent fermentation was done under the appropriate conditions described above. Therefore, various fermentation methods like fed-batch and fed-batch with Tween 80 have been developed to promote the improvement of ethanol concentration along with its production at high solid loads between 36 and 44%. A high ethanol concentration rate of 91.82 gram per liter (69.33% of theoretical yield) was achieved with fed-batch with Tween 80 mode during SSF at high solid loading of 44%. Additionally, after the ethanol recovery, the remains were milled for the production of bio-methane and finally produced 320.72 mL per gram of volatile solids.

# 8.4.3 Advantages of Mixed Approach and Its Different **Studies**

Many enhancements in feedstock supply chain and conversion technology could be achieved while using mixed feedstocks in a biorefinery machine. In addition, this advancement can have a positive impact on the environment. These improvements could bring about reduction in the cost in the usage of lignocellulosic feedstock as major barriers to its bioprocessing research are linked with logistics and bioconversion methods (Banerjee et al. [2010\)](#page-25-0). In general, there is a need for literature concerning the benefits of cost of the MLF approach. The few that are published are focused solely on the high efficiency of MLF use and in applications other than bioethanol production. Even if the use of MLF for bioethanol production has established on a laboratory scale, still there are presently no strong studies focusing on potential price benefits. Furthermore, since upstream methods like transportation are unusual to most lignocellulosic purposes, its ethanol biorefinery might also find other benefits available in these categories.

First of all, in marking the challenge of waste biomass logistics for the production of biofuels, the selection of feedstocks will be taken into consideration as an important aspect. Non-food resources, for example, agricultural wastes can significantly drive the production of cost-effective biofuel. Several agricultural residues including forest by-products, industrial activities along with municipalities are considered to be rich and renewable biofuel sources owing to the occurrence of energy rich compounds such as polysaccharides with various carbohydrate products. Large amounts of renewable energy thus can be generated from agricultural waste because of its lignocellulosic properties (Li et al. [2014](#page-27-0)). Agricultural waste residues containing starch, for example, peels of cassava and potato, often produced in large quantities every year worldwide. They are a repository arsenal of essential polysaccharide components that can be bound to bioenergy and biofuels. Starch-based waste biomass can be assessed for ethanol production using appropriate strategies aimed at improving productivity and process efficiency. Waste-based and lignocellulosic feedstock-integrated production of biofuel has been accounted for the production of biodiesel, biohydrogen, bioethanol, and biogas. Such fuel-oils are reportedly produced by a mixture of unadulterated lignocellulosic substrates and starch-free waste biomass (Shamala et al. [2012](#page-29-0); Kenney et al. [2013](#page-27-0); Sangkharak and Prasertsan [2013\)](#page-28-0) or a mixture of food crops and agricultural waste (Shamala and Sreekantiah [1986\)](#page-29-0). Therefore, the feedstock logistics challenge to biofuel methods can be solved through the utilization of random blends of mixed waste biomass. Further strategies to deal with the present challenges for the production of biofuel are investigating the potential for multiple streams of biofuels from MSBAW in the integrated procedure. Combined production of biofuels permits the production of two or more biofuels from energy-rich waste biomass, thus enhancing the effectiveness of the production method. Agricultural residue-based biofuel processes produce pollution, which is a trouble to ecosystems and microorganisms. Such waste streams include inorganic and organic materials which can be used for further biofuel products, thus rising the productivity of the biorefinery.

#### 8.4.3.1 Associated Reductions in Cost

When several feedstocks are integrated into the existing biorefineries based on single biomass feedstock, there will be an increase in throughput due to additional processing of feedstock. This could translate into a reduction in the cost of a biorefinery machine. This can also increase the level of the biorefinery and fetch the economy with a profitable scale. Presently, no biorefinery functions at a higher capacity than 7000 tons of dry matter per day caused by feedstock problems (Oke et al. [2016\)](#page-28-0). The use of mixed feedstock will make certain nonstop availability thus allowing for the development of the biorefinery capacity. Production costs can be reduced due to the economic optimal size of the biorefinery on a large scale (Sultana and Kumar [2011](#page-29-0)). In a single biomass method, seasonal allocation requires widespread storage of a large amount of feedstock for large time to attain the year-round operation of the handling facility. As the time framework for collecting this large amount of feedstock is insufficient, so the requirement for sources like apparatus, storage, and personnel also becomes regular, and this leads to increase its cost and lessen its use. But, with multiple feedstocks systems, seasonal accessibility is avoided; hence, an increase in resource costs will also be avoided. Moreover, in the storage case, significant costs can be incurred because the required space will be reduced due to the smooth flow of biomass throughout the year (Rentizelas et al. [2009\)](#page-28-0). The supply of feedstock to biorefinery machine can also be very consistent and reliable as various feedstocks can balance each other as inputs in the biorefinery machine at some point in the short supply time of either of them (Nilsson and Hansson [2001](#page-28-0)). The biomass feedstock delivery stability will lead to better efficiency which will ultimately reduce labor plus cost of apparatus. In addition, condensed storage necessities and enhanced supply of feedstock will allow for even and continuous operation of the biorefinery throughout the year, thereby increasing the utilization of biomass and processing area scale. The continued operation of machinery may also mean that less expensive alternatives could be used for mixed approach. This is due to the fact that the feedstock would not require being stored for longer periods of time which would require the usage of more exclusive storage services. The utilization of MLF can also bring savings in costs attributable to the combination of integrated lignocellulosic feedstocks.

A powerful simulation report (Nilsson and Hansson [2001\)](#page-28-0), designed at reducing the costs in the feedstock utilization for regional heating purposes, it was estimated that up to 15–20% of the reduced costs could be obtained using wheat straw and reed canary grass (RCG) as a source of fuel rather than using wheat straw only. The authors also point out that the lowest cost can be obtained by combining a mixture of straw of wheat and woodchips with RCG. They say this about the proficient use of equipment, storage space, and the optimum fuel ratios. The corresponding consequence of RCG has also been a major factor in reducing costs by dropping the quantity of costly fuel which is commonly used when straw is the only fuel resource. One more advantage of the MLF method is that it can make sure the efficiency of the feedstock supply. The majority of the biomass as agricultural products are at high risk, for example, weather uncertainty, diseases, insects, floods, hurricanes, etc. The exploitation of different combinations and various biomass varieties will be able to reduce the risk allied with single feedstock by providing a buffering outcome on the delivery of feedstock during disturbances (Vera et al. [2015\)](#page-30-0). The reductions in cost related to the feedstock transport could also be attained when mixed biomass feedstock are utilized in the biorefinery machine. It has been revealed that the supply cost for combinations of agricultural and woody biomass was less than the price of transporting single feedstock varieties (Rentizelas et al. [2009](#page-28-0)). Such low delivery charges can be obtained if the right types (loose biomass, pellets, barley, chips) for each type of biomass at definite concentrations are used. The investigators found that the supply of a combination of feedstock consisting 30% agricultural waste biomass bales plus 70% woodchips in a biorefinery machine was more reasonable than whole of the either type was supplied (as shown below in Table [8.2](#page-21-0)).

#### 8.4.3.2 Environmental Advantages

The use of mixture of lignocellulosic biomass feedstocks in biofuel production has some advantages in terms of environmental sustainability. Solid municipal waste collection (MSW) is a major problem in many urban areas around the world. A number of actions taken to dispose of this waste, includes burning, often increase environmental contamination. The waste disposal option is subject to certain restrictions, with the European Union limiting the use of landfill and the amount of decomposing MSW used for this purpose (Li et al. [2007](#page-27-0)). Besides, the cost of land replenishment has been increasing in recent times (Elliston et al. [2015\)](#page-25-0). Therefore, the conversion of biological components of this waste into biofuel is another effective method for the management of MSW. In Malaysia, free burning of waste is not legally permitted which requires additional control measures (Siddiqui et al. [2009](#page-29-0)). Earlier studies have shown the possibility of converting mixture of lignocellulosic feedstocks from municipal wastage resources into bioethanol (Oke

Lignocellulosic			
biomass			
feedstocks	Applications	Advantages	Source
Sugarcane resi-	Production of	55% high enzymatic conversion plus	Pereira et al.
dues (straw,	bioethanol	25% high bioethanol yield achieved	(2015)
bagasse, and hops)		with combination than with only	
		bagasse	
Wheat straw and	Production of	Decline in levels of inhibitor in the	Elliston et al.
waste paper	bioethanol	combination as compared to only one	(2015)
		feedstock; declined lag phase in fer-	
		mentation for combination	
Rye grass and	Production of	Higher cellulose conversion to	Martín et al.
clover	bioethanol	bioethanol accomplished in the combi-	(2008)
		nation without any requirement for the	
		supplementation of urea	
Clover grass and	Production of	Higher bioethanol yield obtained in the	Thomsen and
wheat straw	bioethanol	mixture without extra nitrogen	Haugaard-
mixture		supplementation	Nielsen $(2008)$
Rice waste resi-	Production of	Endoglucanase production was 1-7	Pal et al.
dues and palm oil	endoglucanase	folds higher on mixture of substrates	(2013)
		than on single substrate	
Hybrid poplar and	Production of	Mixed biomass feedstock gave higher	Vera et al.
wheat straw	fermentable	sugar recovery after pretreatment,	(2015)
	sugars	decreased sugar degradation, and higher	
		yield of sugar after enzymatic hydroly-	
		sis than any of the single biomass	
		feedstock	
Sesame oil cake	Production of	SOC-WB combination gave high yield	Roopesh et al.
(SOC) and wheat	phytase	of phytase than any of the single	(2006)

<span id="page-21-0"></span>Table 8.2 Technological advantages and improvements related with utilization of mixed feedstock

et al. [2016\)](#page-28-0). Additionally, there is a potential for better energy stability when mixed feedstock sources are used for bioethanol than when singles are processed individually. The efficient operation of the process and the efficient use of equipment and resources are likely to produce more energy from the method compared to when processed one by one. This prospect, however, requires testing from intensive experiments and life cycle testing because there is shortage of literature in this view. Since it is one of the objectives of a 2G biofuel to lessen the usage of fossilderived products, the utilization of lignocellulosic biomass feedstocks can make it feasible. It has shown that mixture of certain types of feedstock reduces the necessity for the adding up of fossil derivative detoxifying substances or supplements of mineral nutrients during fermentation (Erdei et al. [2013](#page-25-0); Tang et al. [2011](#page-30-0)). This is due to the dilution of inhibitors that occur when products of hydrolysis from different feedstocks are combined, resulting in the effect of self-detoxification. In addition, such mixed hydrolysates may have adequate levels of the natural nutrients present in each of the feedstock.

# 8.4.4 Disadvantages of Mixed Lignocellulosic Feedstock Approach

In addition to biogas production through anaerobic metabolism pathway (Li et al. [2014;](#page-27-0) Appels et al. [2011](#page-25-0)), the quantity of literature existing regarding the usage of combined lignocellulosic feedstock resources for bioenergy purposes is very limited when compared to single biomass feedstocks. This may be due to the restrictions and challenges allied with this advancement (Rentizelas et al. [2009](#page-28-0)). These restrictions are basically associated with logistics and technical concerns which occur from a variety of biomass resources. Since the components of mixtures can have very different features such as moisture content, cellulose, hemicelluloses, lignin, ash, bulk density, particle size, and distribution (Williams et al. [2015](#page-30-0)), selecting the appropriate method for processing which will be optimum for all the combined components may be complex to formulate as different feedstock has different optimum conditions for pretreatment, saccharification, and fermentation. In addition, mixed lignocellulosic waste residues have a higher level of pollution as compared to single biomass (Faaij et al. [1997\)](#page-26-0). Identifying the best pretreatment and saccharification conditions that will release optimal amounts of fermentable sugar and produce low inhibitors from a mixture of feedstock may need many initial studies that are very time consuming; and in fact, compromise should be made. It is possible to decrease the efficiency and profitability of the method. A list of profitable enzymes that must be well combined with the various and distinct polysaccharide compounds of the combinations must be given to ensure good yield of sugar for them. This may add to higher costs of production. Alternatively, mixed sources of carbon from combined substrates can lead to catabolite repression problems for fermenting organisms particularly when carbon resources are available at higher concentrations. Some of the organisms be likely to use carbon resources merely when the waste biomass are present in limited quantities, but when they are found in high quantities, they use substrates in sequence (Harder et al. [1982\)](#page-26-0), in the literal sense, which can lead to reduced production. This is a daunting challenge to the benefit of the whole process as higher concentration of sugar is required in order to obtain a good yield of ethanol. Similarly, as combined feedstocks may have a different lignin structure, the activity of a fermenting microorganism can be decreased with the lignin of one of the feedstocks despite of whether it works well in another component (parts) of the mixture. The same is true of enzymatic saccharification. Lignin is known to act as barrier for cellulases and microorganisms (Gao et al. [2014;](#page-26-0) Rahikainen et al. [2013\)](#page-28-0). This crisis can make the procedure less economical as yield and production will be affected. Asset management can be difficult when many feedstock resources are used in the ethanol production process. This is particularly true when feedstocks are acquired in improper forms and in lower bulk density (Sultana and Kumar [2011\)](#page-29-0). Different types of biomass have need of different types of tools for collection, management, loading, and shipping. Where multiple biomass feedstocks are affected, asset management can be a major challenge and the expenditure of this can undermine the possible savings related to the system (Rentizelas et al. [2009\)](#page-28-0). For

seasonal waste biomass species, collecting biomass at a different time than its actual harvest day, with the aim of creating different constituents of the mixed biomass feedstock obtainable at the biorefinery at the same time for simultaneously handling can affect the biomass quality for production of ethanol. This is for the reason that harvest time persuades the chemical properties and ethanol possible production of certain biomass varieties (Godin et al. [2013](#page-26-0)). Adapting to obtainable cellulosic ethanol plants for processing of mixed feedstock can also be hard to attain. This is just because the ethanol plants are often cited near the source of an existing feedstock where its location may be farther away than any other feedstock to be mixed with. This is expected to lead to an increase in shipping costs. Alternatively, existing equipment might be designed to fit only a particular type of feedstock processing and utilizing for any other type of feedstock or combinations of feedstocks can yield unsatisfactory results. Other systemic features such as planning, availability variations, storage, and backup of biomass from time to time are problems that need to be investigated in more detail (Faaij et al. [1997](#page-26-0)).

# 8.4.5 Overcoming the Barricades of Mixed Biomass Approach to Biofuel Production

Since literature addressing the approach of mixed biomass is generally limited, studies of effective testing strategies for overcoming the impact of cost of MLF logistics are yet very uncommon. However, laboratory research on the production of ethanol from MLF shows that the expected challenges of MLF usage can be prevail over if strategic measures are taken. While any current approach may seem exploratory, the fact that a number of the actions discussed here have successfully been implemented in the other MLF systems which makes the application of mixed feedstocks for promising ethanol production.

The central behavior for the handling of lignocellulosic biorefineries to manage and process large amounts of biomass in one area is a main challenge in the production of ethanol. In the scenario of mixed feedstock, this difficulty is exacerbated by the variety of feedstocks, supply disruptions, and the cost of the materials involved in each feedstock type. The organization of "regional biomass processing depots" (RBPDs), which procure, pre-process or pretreat, consolidate plus transport feedstock to the biorefinery industry and return products like animal feed to the end users (Eranki and Dale [2011\)](#page-25-0) have been proposed as a way to address the system's barriers central processing. RBPDs can process several lignocellulosic feedstock streams from each site to have similar features before they are finally transferred to the main biorefinery for pretreatment, saccharification followed by fermentation. The relative life cycle analyzes have revealed that this decentralized scheme can produce the similar energy as the centralized scheme with lower emissions of greenhouse gases.

<span id="page-24-0"></span>The expenditure and effectiveness of MLF processing can be enhanced by using suitable technologies which can withstand the different features of feedstock combinations (Morales-Vera et al. [2016\)](#page-27-0). Such technologies are already available for mixed biomass gasification (Faaij et al. [1997\)](#page-26-0). It is likely that these types of technologies were introduced for the production of bioethanol. At present, different pretreatment technologies are appropriate for only certain feedstock types (Alvira et al. 2010). It would be remarkable to have pretreatment methods that can produce optimum sugar yield with a variety of feedstocks for consequent fermentation.

# 8.5 Conclusion and Future Prospects

The expectation and success of replacement of fossil fuels with lignocellulosic waste biomass for biofuel production is challenged by the high cost of feedstock provide logistics and the complexity of the conversion technology. The utilization of various feedstocks in the biorefinery has potential to significantly reduce costs and thus gain more attention recently. It is thus possible that the use of mixed lignocellulosic feedstock approach may result in significant reductions in operating costs, but further studies are needed to set up this. This approach can also have positive ecological impacts to be demonstrated through life cycle assessments and energy balance explorations. Challenges related to processing technology and the unique features of the mixed lignocellulosic feedstocks (MLF) supply logistics may limit the implementation of this method. However, the adoption of strategies aimed at the supply of feedstock such as delivery of feedstock in suitable forms and ratios, strategic planning, and redistribution of the biorefinery and mixed plantations may alleviate the expected limitations. Similarly, strategies related to technology can facilitate the utilization of MLF in bioethanol production. Such strategies include the special design of feedstock, the use of optimum mixture proportions, the development of flexible technologies that can handle various aspects of the feedstock, etc. The ethanol outlook from MLF is promising. The quick increase in the number of studies in this area is likely to be demonstrated in the upcoming years. Researchers require looking for more innovative measures to ensure the sustainability of the lignocellulosic biofuel sector.

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