Clean Energy Production Technologies
Series Editors: Neha Srivastava · P. K. Mishra

Neha Srivastava
Maqsood Ahmad Malik *Editors*

Food Waste to Green Fuel: Trend & Development

Springer

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Neha Srivastava, Department of Chemical Engineering and Technology, IIT (BHU) Varanasi, Varanasi, Uttar Pradesh, India

P. K. Mishra, Department of Chemical Engineering and Technology, IIT (BHU) Varanasi, Varanasi, Uttar Pradesh, India

The consumption of fossil fuels has been continuously increasing around the globe and simultaneously becoming the primary cause of global warming as well as environmental pollution. Due to limited life span of fossil fuels and limited alternate energy options, energy crises is important concern faced by the world. Amidst these complex environmental and economic scenarios, renewable energy alternates such as biodiesel, hydrogen, wind, solar and bioenergy sources, which can produce energy with zero carbon residue are emerging as excellent clean energy source. For maximizing the efficiency and productivity of clean fuels via green & renewable methods, it's crucial to understand the configuration, sustainability and techno-economic feasibility of these promising energy alternates. The book series presents a comprehensive coverage combining the domains of exploring clean sources of energy and ensuring its production in an economical as well as ecologically feasible fashion. Series involves renowned experts and academicians as volume-editors and authors, from all the regions of the world. Series brings forth latest research, approaches and perspectives on clean energy production from both developed and developing parts of world under one umbrella. It is curated and developed by authoritative institutions and experts to serves global readership on this theme.

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Editors
Neha Srivastava
Department of Chemical Engineering and Technology
Indian Institute of Technology (BHU)
Varanasi, Uttar Pradesh, India

Maqsood Ahmad Malik
Department of Chemistry
King Abdulaziz University
Jeddah, Saudi Arabia

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Foreword

Bioenergy production from renewable resources is the most sustainable way out for environmental preservation in today's scenario. However, there are some rigid roadblocks such as cost and lack of technology that hamper the smooth arrival of these renewable bioenergies to replace limited and environmentally toxic fossil fuels. From the past many years until now, continuous efforts are being made to make this sustainable mode energy practically and commercially feasible, and there is a need to explore more to make it viable. Therefore, there is an urgent need to search, evaluate and summarize the most feasible and sustainable solution to resolve the existing roadblock in a green manner to make it an advanced and cost-effective process.

Publication of the book entitled *Food Waste to Green Fuel: Trend & Development* is one of the potential steps towards the sustainable green energy production based on low technology. I am writing this message with complete satisfaction as a researcher working in this area. This book holds ten potential, innovative and very informative chapters which explore in-depth and thoroughly discuss the availability and feasibility of maximum utilization of food waste for green and sustainable energy production. In my view, this book will definitely be used as one of the matchless assets in the bioprocess and allied research area and industries.

I appreciate the efforts of Dr. Neha Srivastava and Dr. Maqsood Ahmad Malik for bringing out the book entitled *Food Waste to Green Fuel: Trend & Development*. This book is completely sufficient to fill the existing research and technology-based gap. I congratulate the editors for their hard work and bringing a final shape to this book.

Research and Scientific Studies Unit,
College of Nursing and Allied Health Sciences,
Jazan University,
Jazan, Saudi Arabia

Shafiul Haque

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The editors are thankful to all the academicians and scientists whose contributions have enriched this volume. We also express our deep sense of gratitude to our parents whose blessings have always prompted us to pursue academic activities deeply. It is quite possible that in a work of this nature, some mistakes might have crept in text inadvertently and for these we owe undiluted responsibility. We are grateful to all the authors for their contribution to this book. We are also thankful to Springer Nature for giving this opportunity to editors and the Department of Chemical Engineering and Technology, IIT (BHU) Varanasi, U.P., India, for all technical support. We thank them from the core of our heart.

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About the Editors

Neha Srivastava has received her PhD in Biotechnology from the Department of Molecular and Cellular Engineering, SHIATS, India, in 2016 in the area of bioenergy. She worked in the Department of Chemical Engineering and Technology, IIT (BHU) Varanasi, India. She has published more than 28 research articles in peer-reviewed journals of SCI impact factor and have filed 03 patents, one technology transfer and seven published books of internationally renowned publisher. She has many popular social scientific articles in reputed newspapers and has 11 potential deposited microorganisms in her credit. Presently, she is working on bioprocess technology and biofuels production (microbial screening and enzymes; production and enhancement, biohydrogen production from waste biomass, bioethanol production).

Maqsood Ahmad Malik is working as Associate Professor in the Department of Chemistry, Faculty of Science, King Abdulaziz University, Saudi Arabia. He authored more than 40 publications and has membership of prestigious scientific societies. His research expertise is related to nanomaterial synthesis and its various applications.

Chapter 1
Utilization of Food Waste for Biofuel Production

Kamini Pandey, Ashok Kumar Yadav, and Charu Goel

Abstract Food waste can be significantly used as a raw material for the production of biofuel using various suitable techniques. Carbohydrate, lipid, and other nutrient-containing materials present in food waste can be converted to bioethanol, biodiesel, hydrogen, and methane. In this chapter, processes for manufacturing of biofuels from food wastes have been discussed. Due to the limited availability of petroleum, there is an increase in the demand of biofuels worldwide. In this respect, biodiesel and bioethanol are the most popular biofuels that can be commercially used. Industrial production of biodiesel, bioethanol, hydrogen, and methane from food waste can be helpful to resolve many issues like waste disposal, energy scarcity, pollution, and availability.

Keywords Food waste · Bioethanol · Biodiesel · Methane · Biofuels · Petroleum

1.1 Introduction

Food waste can be defined as any by-product or waste product generated at various steps of the food supply chain such as handling, processing, and supply of food including fruits, vegetables, cereals, uncooked raw materials and edible materials from wet markets, and wasted foodstuff from houses and restaurants (Ong et al. 2018). Food waste disposal is increasingly becoming challenging. Most of these food wastes are dumped directly in landfills every day. Biochemical decomposition of food waste results in unpleasant smell and formation of unhealthy degraded products.

K. Pandey · A. K. Yadav (✉)
Food Processing and Management, DDU Kaushal Kendra, Rajiv Gandhi South Campus
Banaras Hindu University, Mirzapur, Uttar Pradesh, India
e-mail: ashok.yadav4@bhu.ac.in

C. Goel
Defence Food Research Laboratory, Mysore, India

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Table 1.1 General composition of food waste

Fractions	% w/w
Soluble materials	33.81
Starch	10.68
Glucose	4.39
Cellulose	10.31
Hemicellulose	11.32
Fructose	3.47
Sucrose	4.38
Pectin	3.27
Total reducing sugar	12.54
Protein	0.54
Fat	11.91
Lignin	6.75
Ash	5.16

Food waste has become one of the major economic, social, and environmental issues. Bio-wastes and the organic fractions of municipal solid waste like garden, kitchen, and food waste account one-third of the total waste and are considered to be a valuable resource that could be converted into high value-added products. The food waste composition is not always uniform and shows significant variations depending on the season of the area, harvesting time, and the dietary habits of the population of that region. The general composition of food waste has been represented in Table 1.1.

Although dumping of food waste in landfills is regarded as one of the easier and economic ways of disposal, it is unsustainable and environmentally unfriendly. In this context, better management of food products at food industries and eateries level will certainly help to bring down the amounts of food waste produced. Currently, there are various technologies available for the utilization of food wastes such as (1) composting of food waste, (2) preparation of animal feed, and (3) biogas production, which are adopted for food waste valorization (Karmee 2016).

Biomass, which includes fuel, wood, charcoal, and animal waste, continues to provide a vital source of energy in many parts of the world. Bioenergy is the most important source of energy basically for cooking for most of the world's population who live in extreme poverty. More progressed and effective change advances presently permit extraction of biofuels in liquid or gaseous form from sources such as wood, crops, and waste material (The State of Food and Agriculture 2008).

Food waste is simply disposed of in landfills/incinerators without being used much around the world. Food waste with varied compositions is a rich source of carbohydrates, sugars, proteins, fats, and lipids and some major and minor minerals, which make it suitable for the production of biofuels through microbial or enzymatic transformation. Out of these wastes, carbohydrate content is maximum in Indian food waste (Shakharkar 2018). Food waste management helps to partially reduce the dependency on crude oil as an energy source. The decomposition of food waste for

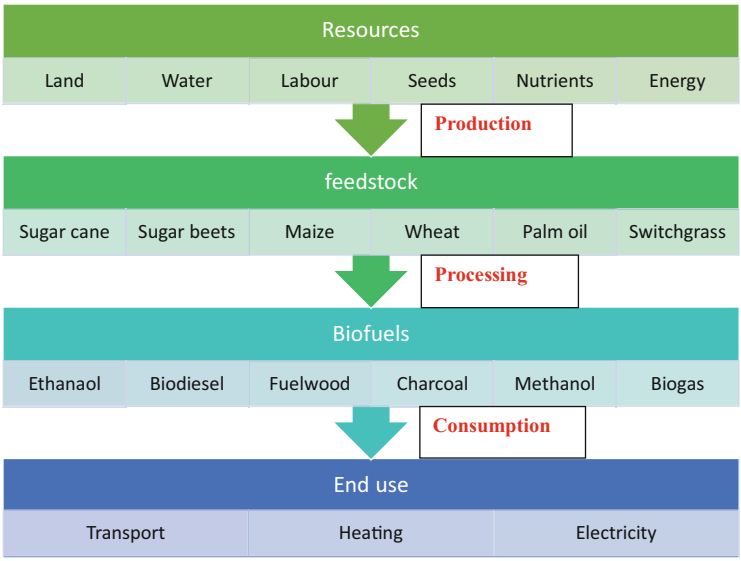

Fig. 1.1 Biofuels—from feedstock to end use. (Source: The State of Food and Agriculture 2008)

biofuel production is increasing with the 2030 Sustainable Development Plan set by the UN in 2015 (Prasoulas et al. 2020).

The term "biofuels" refers to enriched energy chemicals generated by biological processes and extracted from the biomass of living organisms. Biofuels are one of the prominent alternatives that can fulfill the increasing demand of energy source around the globe. For several years, fossil fuels have been a primary source of energy; however, their use is unsustainable, and the burning of fossil fuels creates environmental concerns (Allakhverdiev et al. 2009; Razzak et al. 2013; Voloshin et al. 2015).

For microbial biofuel production, the newer methodologies are well analyzed and perceived, and potential outcomes of the microalgal cultivation techniques for direct energy production to generate biofuels have been suggested. For example, biofilm treatment of microalgae or cyanobacteria may be the newer technique for decomposition of biomass for the production of biofuels (Demirbas 2009; Heimann 2016). The most popular source of biofuels is the plant biomass in the last few years. Sugar is the essential molecular substrate for the production of biomethanol and bioethanol (Demirbas 2009; Heimann 2016).

The resources like land, water, labor, seeds, nutrients, energy, etc., produced the feedstock including sugarcane, sugar beets, maize, wheat, palm oil, switchgrass, etc. (Fig. 1.1). After the processing of feedstock, biofuels such as ethanol, biodiesel, fuelwood, charcoal, and biogas are produced as the end uses of food wastes can be utilized in transportation, cooking, and other energy sources (The State of Food and Agriculture 2008).

Table 1.2 Classification of biofuels

	Secondary		
Primary	First generation	Second generation	Third generation
Firewood, wood chips, pallets, animal waste, forest and crop residue, landfill gas	Bioethanol or butanol by fermentation of starch (from wheat, barley, corn, potato) or sugars (from sugarcane and sugar beet). Biodiesel by transesterification of oil crops (rapeseed, soybeans, sunflower, palm, coconut, used cooking oil, and animal fats)	Bioethanol and biodiesel produced from conventional technologies but based on novel starch, oil, and sugar crops such as *Jatropha*, cassava, and *Miscanthus*. Bioethanol, biobutanol, and syndiesel produced from lignocellulosic materials such as straw, wood, and grass	Biodiesel from microalgae; bioethanol from microalgae and seaweeds; hydrogen from green microalgae and microbes

Source: Dragone et al. (2010)

Biofuels can be categorized into two types: primary and secondary biofuels. Table 1.2 depicts the classification of biofuels clearly. Primary biofuels are those that are obtained naturally from firewood, animals, plants, and forest waste and crop residues. Secondary biofuels are those that are derived from sources such as plants and microorganisms. The secondary biofuels can be classified into three distinct generations. Ethanol from starchy food like potato, corn, wheat, barley, and sugarcane and biodiesel from soybean, sunflower, and animal fat are examples of first-generation biofuels. Bioethanol and biodiesel from several species like *Miscanthus*, straw, jatropha, cassava, grass, and wood are considered as second-generation biofuels, and biodiesel produced by the action of microalgae and microbes is considered as third-generation biofuel (Seibert 2009; Poudyal et al. 2015; Slade and Bauen 2013; Chisti 2007; Carlsson et al. 2007; Lindberg et al. 2010; Carere et al. 2012; Cha et al. 2013; Razeghifard 2013; Singh et al. 2011; Atsumi et al. 2009; Tran et al. 2010; Gronenberg et al. 2013; Hasunuma et al. 2013; Verbeke et al. 2013; Ilmen et al. 2011; Tai and Stephanopoulos 2013; Buijs et al. 2013; Abdelaziz et al. 2013).

1.2 Background

Food waste is picking up expanded consideration among buyers and policy creators globally. Earlier production of energy using food waste has been considered as a least preferable option over disposal. But now when we look toward the principle of waste hierarchy (Fig. 1.2), we can say that this assumption is getting changed (Nordic Energy Research 2019). However, global warming, climate change, and the limitation of fossil fuels create a need to find any suitable alternative way to reduce the emission of greenhouse gases. In developing countries, transport segment

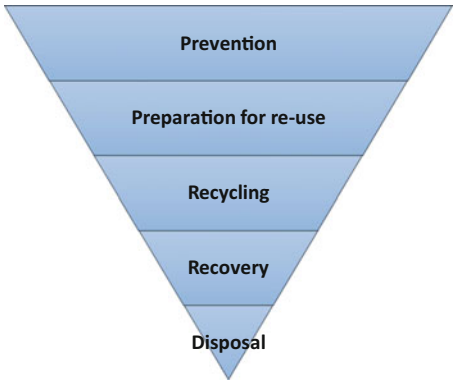

Fig. 1.2 Waste hierarchy and food waste

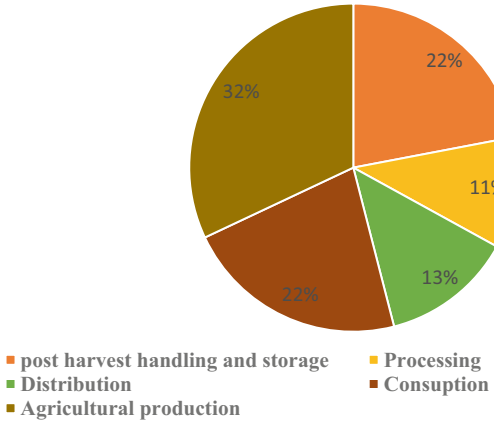

Fig. 1.3 Relative food wastage in the food supply chain

is the major division of greenhouse gas emission. As per the EU climate policy principles, biomass, non-edible parts of food waste are regarded as a solution for low emission of greenhouse gases production that can be utilized in transport sector. Therefore, the waste from food plays a vital role for low carbon emission transport fuel, assuming that the principles of waste hierarchy are as follows (The state of food and agriculture 2019).

1.3 Characteristics of Food Waste

- If we observe, we will find that food is wasted mostly at all stages of the food supply chain, from production to consumption.
- Food items are exceptionally troublesome to measure individually. Moreover, it is all wasted along the food supply chain (Fig. 1.3) (FAO, Food and Agriculture Organization of the United Nations 2014a).

Table 1.3 Total agriculture production and food wastage globally

Agricultural commodity	Examples	Production in million tons	Total wastage in million tons	Edible wastage in million tons
Fruits	Banana, apple, citrus fruits, grapes, etc.	550	340	300
Vegetables	All vegetables	900	360	280
Cereals	Wheat, paddy, Bajra, barley, maize, rye, millets, sorghum, etc.	2100	400	300
Milk and egg	Milk and egg products	730	100	100
Oil crops, pulses, and legumes	Oils and pulses	510	40	35
Poultry and meat	Poultry, chevon, beef, mutton, hog	350	50	50
Fish and seafoods	Fish, crabs, etc.	120	20	0
Roots	Starchy roots	700	300	240
Total		5960	1610	1305

Source: Shuang and Yang (2016)

- Food waste is divided into eight categories by food service operators: fruits, vegetables, cereals, milk and eggs, oil crops, pulses, and legumes, poultry and meat, fish and seafoods, and roots (Table 1.3) (FAO, Food and Agriculture Organization of the United Nations 2014a).
- These commodities are the major donors to the overall food wastage in each region so that there are a few contrasts among the region and it depends on the wage level.
- Approximately 85% of total food waste is from food crops such as fruit and vegetable items, and the remaining waste items come from animal waste.
- Meat wastage is higher in unorganized sectors than in organized sectors.
- The main components of food waste are carbohydrates, lipids, and proteins. Apart from supplying the majority of food waste volume, these three areas, such as wastewater loss, land contamination, and greenhouse gas emissions from waste decomposition, are the significant contributors (FAO, Food and Agriculture Organization of the United Nations 2014b).
- Wastewater can be considered nontoxic as it contains very few hazardous and nonbiodegradable compounds from food processing industries (Unido. Pollution from food Processing Industries 2001).
- Tofu wastewater is low in carbon but rich in nitrogen. It contains a small amount of polysaccharides, which makes pretreatment necessary before conversion via fermentation (Wang et al. 2006).
- Potato processing wastewater makes a good substrate for alcohol production as it is rich in starch (Chandrasekharan 2012).

1.4 Production of Biofuels

1.4.1 Biodiesel Production from Food Waste

Biodiesel is used as a fuel in Europe, the United States, and many other nations. Biodiesel is made up of fatty acid methyl esters (FAME) at a chemical level. Depending on the source of the feedstocks, such as plant oils, animal fats, and waste oils, biodiesel contains both saturated and unsaturated fatty acid methyl esters. Sunflower, palm, soybean, rapeseed, and other edible plant oils are generally used for biodiesel production. Biodiesel made from edible oils is expensive, so the current cost of biodiesel is higher than that of petroleum fuels. As a result, low-cost and non-edible oils are needed for biodiesel production. Non-edible oil plant species like *Jatropha*, *Pongamia*, *Mahua*, and others are already being researched for biodiesel production.

The extraction of lipids is required for the production of biodiesel from food waste. Food waste is first mixed with water to make a slurry (typically 100 g of food waste in 1 L of water) and then vigorously mixed with nonpolar organic solvents such as *n*-hexane and diethyl ether. This step is optional, but it can be performed. Afterward, the collected mixture is poured into a separating funnel. The organic layer is separated and evaporated under reduced pressure to acquire the organic solvent-free lipid (Karmee 2016).

Biodiesel is a mono alkyl ester of fatty acid or fatty acid methyl ester. It is a sustainable and clean burning fuel and is a renewable substitute for diesel and petrol (Yaakob et al. 2013; Karmee and Lin 2014). Biodiesel produced by utilizing food waste and used oil has low emissions and is nontoxic, biodegradable, and carbon neutral (Wan Omar and Saidina Amin 2011; Yaakob et al. 2013). Currently, it is one of the most widely accepted alternative fuels. Biofuel production from rendered fats has recently increased significantly in the EU and the UK (Lin et al. 2013). Direct transesterification by chemical catalysts, enzymes, or microalgae fermentation are some of the methods for producing biodiesel from food waste. A representation of possible ways for the preparation of different biofuels from food waste using different methods is shown in Fig. 1.4.

Transesterification of lipids into biodiesel is the turning point, despite the fact that oils and fats can be diluted and mixed with petro-diesel for direct usage in engines. Transesterification can be acid, base, or enzyme catalyzed. Food waste (like bone, ash, and shell) can be used to derive these catalysts. A study by Tan et al. (2015) showed that they utilized a calcinated egg shell as a heterogenous catalyst and utilized waste cooking oil as feedstock for producing biodiesel. Pretreatment methods ought to be optimized as different feedstocks have different compositions (de Almeida et al. 2015). The transesterification process can be supported by utilizing ultrasound and microwave methods to increase product yield. During the transesterification process, the device should be free from water to prevent saponification. Lately, the feasibility of valorization of food waste into biodiesel has been

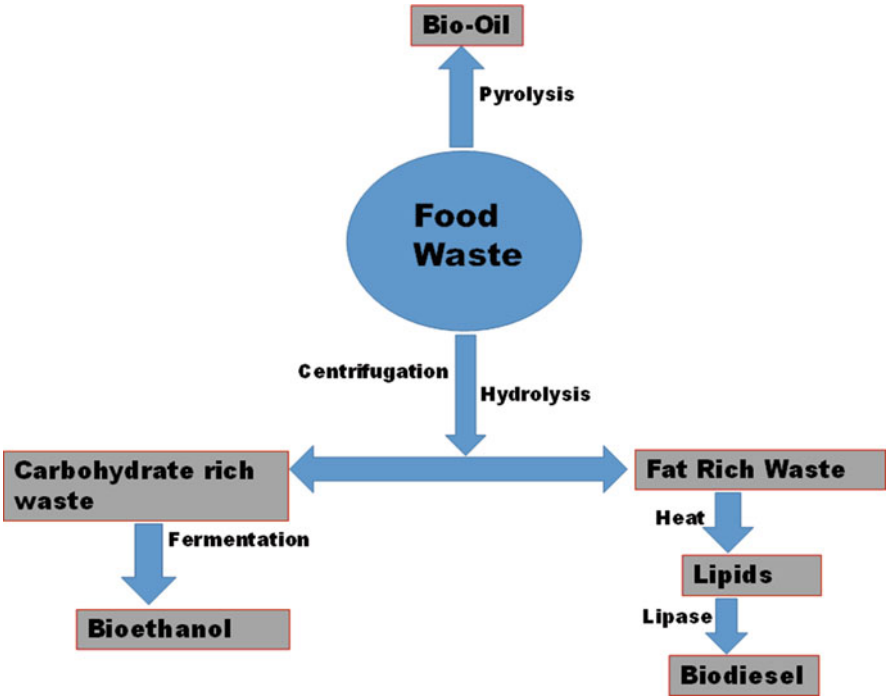

Fig. 1.4 A schematic representation of possible ways for the preparation of different biofuels from food waste

shown by some pilot plants like SENECA Green Catalyst S.L. in Spain and Brocklesby Ltd. in the UK (Lin et al. 2013).

SENECA Green Catalyst S.L. is a spin-off corporation situated in the south of Spain. The organization produces 3–5 tons of biodiesel each day by utilizing a novel dual technology. This method permits concurrent esterification/transesterification of waste oil in a single pot. Pre-esterification with NaOH and methanol was used to convert free fatty acid into fatty acid methyl ester under homogeneous conditions, followed by transesterification with an enzymatic process. The organization has developed an adsorbent for purifying biodiesel at the end of the process. The popularity of the method is influenced by factors such as the quality and variability of waste cooking oil, industry and market demands, location and cost-competitiveness of the operation, and so on (Woodgate and Van der Veen 2004).

The food wastes are exposed to the cell disruption process, followed by mincing and thermal treatment under 100 °C, which release the liquefied oil from the cells. Then the oil/fat portion is separated from the wet sludge, and water is treated under constant mechanical separation by means of tricanter centrifuge. Currently, the plant is converting 2000 tons per year of triglyceride-rich waste including animal by-products into biodiesel with 30% yield. A variety of substrates and products in wet and semisolid phase can be managed by this approach. Starchy and fiber-rich

residue is sent outside for composting. Carbohydrate-rich by-products could be utilized as a substrate for fermentation or as an alternate feedstock for microwave pyrolysis. Microwave pyrolysis has been shown to be a viable treatment for oil extraction and homogenization from food waste (Liu et al. 2014). Microwave pyrolysis is a popular technique for drying waste and delivering high-calorific-value biochar, in addition to obtaining bio-oil. The energy can be recovered, and solid food waste can be reduced.

Transesterification of microbial oils from various oleaginous microorganisms may be used to produce biodiesel from food waste (Chen et al. 2009; Mahmood and Hussain 2010; Papanikolaou et al. 2011; Pleissner et al. 2013; Yaakob et al. 2013). Food waste is used as a substrate for the growth of oil-rich microbes to absorb oil, which is then transesterified. Microbial oils can be delivered by numerous yeast strains or microalgae, and they can be utilized as plant oil replacement because of their similar fatty acid composition.

1.4.2 Bioethanol Production from Food Waste

Ethanol has been graded as an excellent fuel for modern combustion engines in motors. It has an octane number of 98, which is higher than gasoline, having an octane number 80, and it has less evaporative loss due to lower vapour pressure than gasoline. Ethanol is less flammable in air than gasoline, making it a safer alternative. Bioethanol is obtained as the product of fermentation of simple sugars in the biomass with the help of microorganisms performing enzymatic digestion (Awasthi et al. 2015).

Ethanol can be used as a transport fuel instead of gasoline. Thermochemical reactions are used to treat feedstock from the chemical industry and for fuel cells. Bioethanol is produced from starchy crops like corn, potato, rice, sugarcane, and many more. Utilizing ethanol as a substitute for gasoline is restricted since the feedstock used for ethanol production in the United States (corn) and Brazil (sugarcane) is edible. Due to an increment in fuel ethanol generation, the corn cost has hiked within the past decades. Currently, bioethanol produced from low-cost feedstocks has gained popularity.

Lignocellulosic biomass is a promising alternative energy source that can be utilized to produce ethanol. The conversion processes involve two steps: hydrolysis of cellulose in the lignocellulosic biomass for the production of reducing sugars and fermentation of sugars to ethanol (Awasthi et al. 2015). Pervaporation as a separation technology on food waste increases the yield and efficiency of the bioethanol produced (Shakharkar 2018).

1.4.2.1 Pretreatment of Food Waste

Perishable nature and difficulty in isolating food waste from whole waste are the challenges faced while utilizing food waste for biodiesel production. The food waste with rich composition is difficult to store and handle as it is easily contaminated by microbes. Additionally, high volume results from food wastes with high water content. Drying of food waste can increase its storage stability and minimizes storage space as well. The utilization of food waste for biofuel production without drying is preferred because decomposition with the help of microorganisms can be easily done with wet waste (Kim et al. 2005). Any food waste contains complex carbohydrates like cellulose and hemicellulose that are difficult to hydrolyze.

The amount of carbohydrate saccharification affects the efficiency of food waste conversion to ethanol (Tubb 1986). Glucose, fructose, xylose, maltose, amylose, sucrose, and arabinose are fermentable sugars that can be delivered within the saccharification process for consequent ethanol production. Protein is used explicitly in commercial applications or a defined blend of α and β amylase, and glucoamylase from various roots is used and these are more successful for nutrient waste saccharification. During cereal hydrolysis, starch is converted into glucose; cellulase and xylanase hydrolyze cellulose and hemicellulose, respectively, while pullulanase catalyzes the hydrolysis of α-1,6-glycosidic bonds, resulting in the discharge of straight oligosaccharides.

After saccharification, the nutrient-dense waste produces a sticky squash with a high glucose concentration, which activates yeast. Researchers employed granular starch hydrolyzing enzymes (GSHE) in synchronous saccharification and fermentation (SSF) at a lower temperature (48 °C) to overcome this issue. A large amount of ethanol (30%w/w) is generated from nutrient-rich waste through a vacuum recuperation framework.

Granular starch hydrolyzing protein diminishes the thickness of the maturation of broth and valorizes the glucose level, and it discharges the yeast. Glucose hindrance amid maturation increases ethanol production. Moreover, ethanol production increases by utilizing a vacuum recuperation framework. The procedure involves applying an aqueous pretreatment to the residual components in order to produce ethanol after ageing.

As the result of saccharification, various sugars like glucose, fructose, and xylose are produced. However, *Saccharomyces* spp. yeasts, which have been traditionally used in ethanol fermentation, cannot convert xylose to ethanol.

1.4.2.2 Process Strategies

Firstly, during storage, the food waste was introduced to lactic acid bacteria to produce lactic acid for 48 h. Subsequently, by adding glucoamylase, the food waste was saccharified, and the resulting liquid included glucose and lactic acid. Seventy percent of lactic acid may be converted to pyruvic acid, which is the final

solution utilized in ethanol production, by immobilizing lactate oxidase for 5 h. The addition of the enzyme improved ethanol output yield by 20% more than without the addition of enzyme. To achieve higher ethanol yield, a 23 factorial design was used to optimize fermentation conditions.

1.4.3 Hydrogen and Methane Production from Food Waste

1.4.3.1 Production of Hydrogen

Hydrogen is used as a compressed natural gas and is one of the best fuel sources of the future due to its high energy efficiency (142.35 kJ/g) and nonpolluting nature. Renewable biohydrogen production is explored using emerging techniques like photo-electrochemical splitting of water, solar thermal splitting of water, reforming renewable resources, and fermentation of sugary materials. Food waste rich in carbohydrate and of low cost could be an appropriate option for hydrogen generation. During the transformation of nutrient-rich waste into biofuels, pretreatment preparation is continuously essential. Pretreatment methods can increase the efficiency of fermentation processes. Carbon and nitrogen are also present in nutrient-rich waste sources, such as lignocellulose, starch, and protein. These ought to be removed or converted into simpler molecules.

1.4.3.2 Production of Methane

Primarily, methane is called biogas and is a renewable source of energy that has been used by humans since ancient times. Methane is also one of the major gases emitted from crop fields, which is obtained from the decomposition of waste in landfills. Since it is not a controlled reaction, the nutrients of the crops beneath the soil are converted into methane. With increase in the nutritional value of the field crop, the production of methane increases. In addition to this, the nutrient-rich waste can be utilized as fertilizer and soil conditioner. With a combined-stage framework, methane generation and hydrogen generation can be integrated. Almost any natural fiber can be utilized for anaerobic decomposition for methane production, including food waste, wastepaper and cardboard, grass clippings, various leftovers, mechanical effluents, sewage, and animal waste. Methane synthesis from food waste is becoming more feasible thanks to perishable nutritional waste including methanogens. The overall technological routes for the production of transport fuels from food waste is shown in Fig. 1.5.

Water vapour extraction, CO_2 removal, physical CO_2 absorption, chemical CO_2 absorption, cryogenic separation, and membrane processing are some of the purification procedures used for biogases (Malode et al. 2021).

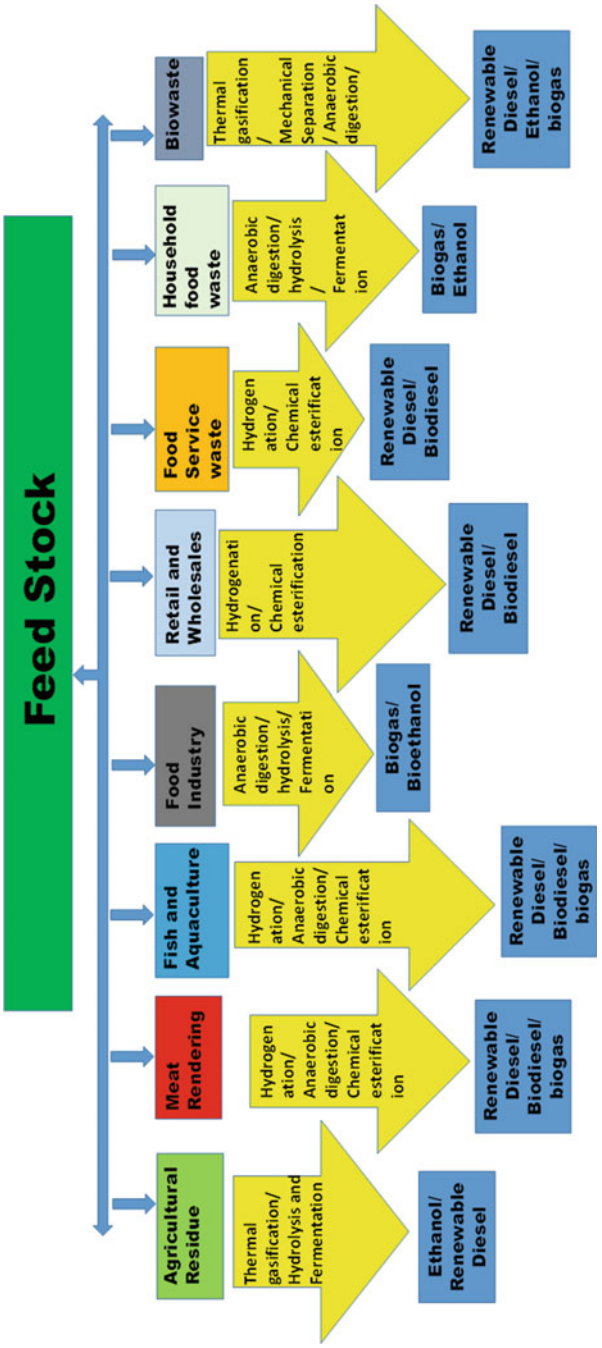

Fig. 1.5 Technological routes for transport fuel production from food waste

1.5 Biofuel Economics from Food Waste

Continents like Asia and Europe have defined their financial approaches keeping in mind the biofuel-based energy. In Table 1.4, food waste utilization in different forms across the world is shown (Nordic Energy Research 2019). There is an increasing demand of these biofuels among intellects in numerous states of the United States. In the coming decades, numerous nations in a similar way will try to develop these types of fuel, which could be a wealthy economy; biofuels will be a major driving force for financial development. There is more financial advantage for biofuel generation from food waste. Utilizing scholarly examination, financial device uncovers that biofuels can lower nursery gas outflow compared to customary fills (Hertel et al. 2010; Huang et al. 2013).

Besides, biofuel generation might diminish dependence on petroleum fuel, which may lower the production cost of biodiesel, bioethanol, methane, and hydrogen, and biofuel generation will too make the nations not dependent on others for energy. Generation of biofuels will have a positive response on economy.

Two fluid biofuels, biodiesel and bioethanol, are being used to replace diesel and gasoline. Transportation fuels like biodiesel and bioethanol from food waste will be useful. Availability and cost of the starting substances can be overcome by the cost of the biofuels generated from food waste. Generally, food waste is discarded without assistance, and it is labeled as no-cost resources. So the main cost is sorting, transportation, and pretreatment of the food waste from the resource point.

A combined study on technical and economic aspects will give data on the plan and price estimation of biofuel manufacturing plants. Advancement of the strategy, genuine advertised information, and money-related investigation of the generation office and cost of the biofuels need to be monitored (Karmee 2016). Due to the high economical value of substrates, biofuels are more expensive than customary petroleum energy sources; this can be the financial impediment for biofuel generation from food waste. The demand of biofuels can result in high food price, which can adversely affect the people's demand for food and also lead to a higher rate of lack of healthy food and food products within the developing nations (Skarlis et al. 2012).

Table 1.4 Estimated utilization of food waste across the globe

Form used	Percentage
Food and feed	21
Chemicals	02
Auto consumption	00
Compost	12
Biogas	22
Biofuel	01
Landfills	00
Incineration	30
Uncollected	11

1.6 Food Waste Applications from Different Industries

The utilization of food waste as biomass can serve as an energy source and reduce the associated disposal costs.

(a) From Banana Plantain and Pineapple Peels

Itelima et al. (2013) reported that banana peels, plant parts, and peels of pineapple can be used for bioethanol production by following saccharification and fermentation process carried out for 7 days in the presence of *Aspergillus niger* and *Saccharomyces cerevisiae* culture. Biomass yield, cell dry weight, reduced sugar concentration, and ethanol yield were estimated at an interval of 24 h. The results exhibited that after the fermentation process carried out for 7 days, biomass yield was highest for pineapple peels with 1.89 optical density (OD), followed by banana peels with 1.60 OD, while plantain produces the least yield with 0.98 OD. Ethanol yields were 8.34% v/v for pineapple peels, 7.45% v/v for banana peels, and 3.98% v/v for plantain peels. This indicates that ethanol yield was higher ($p < 0.5$) in pineapple and banana peels than in plantain peels. Fermentable sugars present in most of the fruit waste can be easily converted to useful fuel like bioethanol (Reddy et al. 2011; Reena 2016).

(b) From Mango

Mango fruit processing industries generate solid waste (peels, stones) and liquid waste (juice and water used for washing) whose utilization is both a necessity and a challenge. After fermentation, the mango peel contains a considerable amount 40% (w/v) reducing sugars, resulting in 5.13% (w/v) ethanol. Utilizing nutrients such as yeast, wheat bran, and peptone increased ethanol production up to 7.14% (w/v). The yeast *Saccharomyces cerevisiae* and the facultative bacterium *Zymomonas mobilis* can be used for industrial alcohol production. Out of these two, *Z. mobilis* is more preferable to *S. cerevisiae* with respect to productivity, efficiency, and tolerance (Ylitervo 2008; Reena 2016).

(c) From Orange Peel

Orange peels are thrown out in large quantities in both, industries and households. In any orange juice processing industries, half of the orange fruits as pulp are discarded as waste after juice extraction. The citrus peel waste includes a mixture of peels, rags, and seeds that are rich in pectin, cellulose, and soluble sugars. By fermentation process at 25–35 °C, ethanol could be produced, and to maximize ethanol production, yeast could be used.

Pourbafrani et al. (2010) investigated the production of ethanol, biogas, pectin, and limonene from orange peel waste by using an integrated approach. Citrus peels were hydrolyzed by dilute acid. The best sugar yield (0.41 g/g of the total dry waste) was obtained by dilute-acid hydrolysis at 150°C in 6 min residence time. At this condition, high solubilization of pectin took place, and 77.6% of the total pectin content from citrus waste could be recovered using the solvent recovery method. The sugars present in the hydrolysates were converted to ethanol using yeast, while an ethanol yield of 0.43 g/g of the fermentable sugars was obtained. Then, the stillage and the remaining solid materials of the

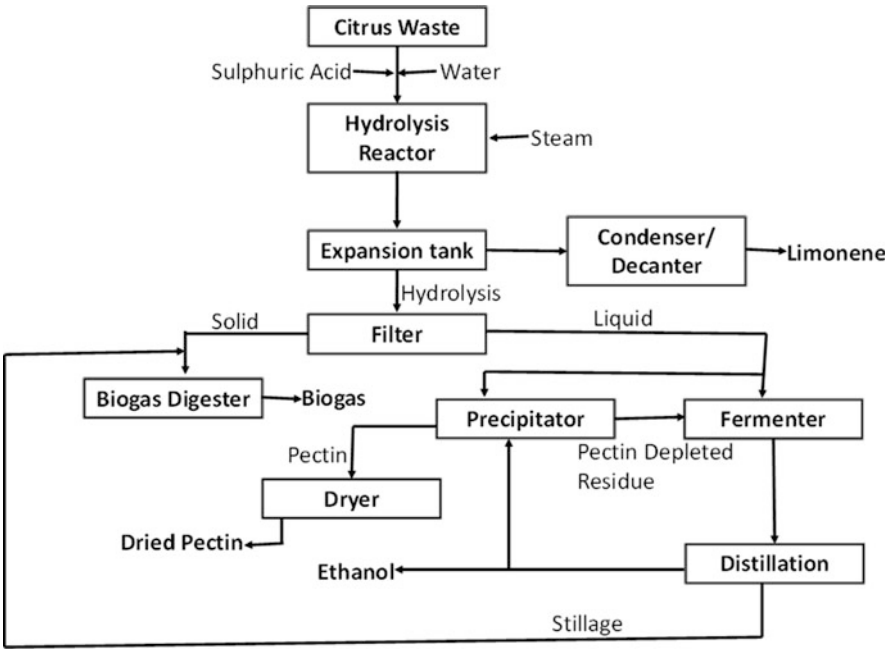

Fig. 1.6 Flow diagram for the production of ethanol, biogas, pectin, and limonene from citrus waste

citrus waste were anaerobically digested to obtain biogas. Concludingly, 1 ton of citrus waste with 20% dry weight resulted in 39.64 L of ethanol, 45 m^3 of methane, 8.9 L of limonene, and 38.8 kg of pectin.

(d) From Lemon Peels

The application of steam explosion and enzymatic hydrolysis pretreatments on lemon (*Citrus limon* L.) citrus peel wastes was studied by Boluda-Aguilar and Lopez-Gomez (2013) to obtain bioethanol, galacturonic acid, and other co-products, such as D-limonene and citrus pulp pellets (Fig. 1.6). Steam explosion pretreatment and recovery of lemon citrus essential oils were carried out at pilot plant scale. The effect of steam explosion on the lignocellulosic composition of lemon peel wastes was studied using the thermogravimetric method. The antimicrobial activity of lemon oil on *Saccharomyces cerevisiae* and its influence on ethanol production during fermentation were also studied. The steam-exploded lemon peel wastes were processed through sequential and simultaneous hydrolysis and fermentation. Concentrations of sugars, galacturonic acid, and ethanol were analyzed to measure the efficiency of these processes. The significant antimicrobial activity of lemon oils has been observed on *S. cerevisiae* at concentrations above 0.025%. The steam explosion pretreatment has shown an interesting effect on lemon peel waste processing for obtaining ethanol and galacturonic acid. This pretreatment reduces the residual content of essential oils below 0.025% and significantly decreases the hydrolytic

enzyme requirements. Ethanol production in excess of 60 L/1000 kg fresh lemon peel biomass can be obtained.

(e) From Apple Pomace

Molinuevo-Salces et al. (2020) assessed the use of apple pomace as a substrate for biofuel production. Two different types of pomace were tested for juice and cider manufacturing. First, bioethanol generation was performed, and its fermentation residues, together with available biobutanol fermentation residues, were studied for biogas production. Twelve different bacterial and yeast strains were compared for bioethanol production, obtaining bioethanol concentrations about 50 g L^{-1} by different strains of *Kluyveromyces marxianus*, *K. lactis*, *Lachancea thermotolerans*, and *Saccharomyces cerevisiae*, with yields of 0.371–0.444 g g^{-1}. Specific methane yields of the fermentation residues of bioethanol and biobutanol production were 463 and 290 mL CH_4 g^{-1} VS (volatile solids) added, respectively. Methane yield for the co-digestion of apple pomace and swine manure was 596 mL CH_4 g^{-1} VS added, with an apple pomace percentage of 14.6% and a substrate concentration of 9.38 g VS L^{-1}.

1.7 Advantages of Biofuels from Food Wastes

Waste fats and used cooking oil, which are otherwise sent to landfills or thrown down the drains (causing blockages), are converted into sustainable renewable biodiesel. Biofuel produces significantly lower carbon dioxide and greenhouse gas emissions than fossil fuels, reduces environmental pollution, saves landfill space, and reduces the maintenance costs of drainage/water treatment networks. Biofuels are renewable sources of energy as biomass like crop waste, legume plant waste, animal waste, sugarcane waste, grasses, etc., are continuously in use for the production of higher amounts of biofuel in almost all countries due to the presence of forests, the need of people, and the availability of animals. Biofuels emit less pollution when burned; therefore, they are considered as best alternatives to fossil fuels.

Biofuels that are manufactured from waste substrates are cheaper than other traditional fuels like gasoline, petrol, diesel, and kerosene. Bioethanol and biodiesel can be produced from lignocellulosic biomass and vegetable oil waste by the decomposition action of algae. Biofuels can be produced from renewable resources and thus can be used as an unlimited resource of greener and safer fuels for industries and the transportation sector. Bacteria decompose the organic waste (human, animal, and food waste) to produce biogas primarily consisting of greenhouse gases like methane and carbon dioxide. When biogas is used as fuel for cooking, heating, and transportation purposes, it keeps the fuel from dispersing into the air and reduces global warming and climate change.

1.8 Disadvantages of Biofuels from Food Wastes

Biofuels are considered as greener and safer fuels that can lower the level of greenhouse gas emissions that are released by vehicles, but there are some drawbacks in the technology, especially when they are produced from food crops. Currently, corn is widely used for bioethanol production. Higher ethanol demand may increase the price of corn. The utilization of cropland to grow biofuels' base ingredients can raise food costs and, in the long run, lead to food shortages.

Biofuels show significantly lower energy output than mineral fuels (gasoline, diesel) resulting in more fuel consumption in vehicles running on biofuels. The setup of plants for biofuel production from different biomass is also an expensive deal. However, operational plant produces biogas in a couple of days. Large amounts of biogas that can be used for cooking can be produced continuously if there is regular supply of food waste.

1.9 Challenges

Food waste is a low-cost resource that can be turned into liquid biofuel. However, there are many challenges that must be addressed. Since this is an emerging research field, a thorough understanding and discussion of various aspects of food wastes would aid in overcoming the limitations.

1.9.1 Unorganized Industry

Food waste collection remains a concern due to the unorganized nature of the industry. The general view of food waste is that it should be discarded; this mentality makes collection difficult. To emphasize the importance of food waste, a social campaign is needed. Food sectors, as well as urban planning and housing agencies, should formulate a proper plan for the smooth collection of food waste. Volunteers who are eager to deliver food waste to community recycling centers should be motivated to show up every day. Food waste disposal would be much quicker and simpler as a result of these measures (Karmee 2016).

1.9.2 Separation of Food Waste

Food waste is commonly combined with other solid urban wastes in many areas. For further processing and utilization, proper separation and sorting methods of food wastes from nonbiological wastes are needed. Since food waste is diverse and

complex, the separation strategy can vary depending on the types of food waste produced (Karmee 2016).

1.9.3 Nonrenewable Resource

Food waste is a nonrenewable resource. Food waste can be minimized as a result of better food processing and consumption management. Starting large-scale factories to recycle food waste would necessitate a steady supply of massive amounts of food waste. Maintaining a large industry dependent on food waste is not practical in this situation. As a result, large restaurants and food parks can be connected to small and medium biofuel production plants. This will also lower the cost of transporting food waste. (Karmee 2016).

1.9.4 Nonstandard Resource

Food waste composition is primarily determined by location, eating habits, and eating times. As a result, its chemical composition and water content must be calculated before it can be used as a resource for the production of biofuels. Food waste is more complex than traditional feedstocks like plant oils, corn, and lignocellulosic materials. As a result, a comprehensive chemical characterization system for various forms of food wastes is needed for reproducibility of results (Karmee 2016).

1.10 Future Prospects

Generally, edible feedstocks are traded for fulfilling biofuel demands. Alternatively, focus should be made to utilize non-edible food waste for biofuel production. Economic validation of "food waste to energy" methods needs to be analyzed very keenly to know the commercial, social, and economic viability. Food waste is complex and diverse in nature. Food waste composition is affected by a variety of factors such as food production area and methods, harvesting or collection timing, and food, diet habits of people in a certain area. New and cost-effective valorization strategies can be devised to convert food waste into high valued products (Yang et al. 2015). Teamwork among different research institutes related to chemo-catalysis, genetic engineering, biocatalysis, biotechnology, downstream processing, and environmental engineering can provide better and advanced state-of-the-art food waste valorization technologies. Public groups, municipal corporations, food industries, nongovernmental organizations, and local governments must raise public awareness regarding the importance of food waste, its recycling, and further utilization.

Furthermore, government agencies should frame rules and policies to encourage start-ups, entrepreneurs, and industrialists for the effective utilization of food wastes to build a strong and sustainable society.

The lesser yield and production rate of butanol and oils by the activity of microorganisms are the major hurdles in the commercialization of biobutanol. Recombinant DNA technology and genetic engineering techniques can be used to improve the strain's accessibility toward the solvents and to increase butanol production. Butanol recovery technologies such as liquid extraction and gas stripping could be coupled with fermentation technology to boost butanol production and yield.

In comparison to the production of solid biofuels or gaseous biofuels, fluid biofuels undergo continuous innovation. As compared to gas-based biofuel manufacturing methods, fluid biofuel manufacturing methods demonstrate the ability to perform more transformations, produce less waste, and use less space and water. The commercial viability of biofuel growth is determined by a number of factors, including feedstock prices, manufacturing infrastructure, product quality, and market demand. The use of organic trash as a feedstock for biofuel production is a promising option (Malode et al. 2021).

Lipids, carbohydrates, amino acids, phosphates, vitamins, and other nutrients are the major constituents of any food waste. Separation and purification of each component from food waste will be a very costly and time-consuming step. VOCs (volatile organic solvents) are used to separate components and are harmful to both humans and the environment. As an alternative and as the best substitute, the production of biodiesel, bioethanol, and bio-oil could be done by combining all the food waste constituents without any isolation, separation, and purification of each individual component. This will be a much more cost-effective and simpler approach to produce biofuels.

1.11 Conclusion

Food waste occurs at all stages of the food supply chain, which causes serious economic, environmental, and societal problems. Environmental damage caused by the emission of greenhouse gas and groundwater contamination due to food waste discarded in landfills should be avoided. Bioconversion of food waste can be done for energy recovery in the form of biodiesel, hydrogen, methane, and ethanol. Carbon source, nitrogen source, and fat subsequently were used as feedstock for microorganisms for the production of biofuels.

In spite of the fact that biofuel generation from nutrient-rich waste is actually and financially reasonable with nil or low cost of food waste, still concerns with respect to cost and transportation of food waste, time devouring process, and low efficiency got to be basically considered. It is clear that forces inquire about optimization process, performance efficiency, and interrelationship between generation forms and value-added items. For the moment, fat portion is utilized for biodiesel generation;

natural gas, hydrogen, and biogas are manufactured by carbohydrates and protein portions. This multidisciplinary approach may permit to realize low food waste economy and more feasible bio-based society. Valorization of food waste could be a promising feasible area where research facility or plant scale investigations ought to be performed to be backed by governments and business operators.

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Chapter 2
Bioenergy and Food Processing Waste

Aparna Agarwal, Memthoi Devi Heirangkhongjam, and Kanika Agarwal

Abstract Food wastage is a serious issue worldwide and has been anticipated to increase considerably in the subsequent 25 years because of the growth in economy and population across the globe. The biodegradable wastes discharged from several sources such as households, food industries, and hospitality sector are known as food wastes. Fresh fruits, vegetables, bakery products, meat, and dairy products are the chief food items lost throughout the food supply chain. In this chapter, we briefly discuss overall food wastage, focusing mainly on food processing wastes (FPW), the residuals which are left over after a primary product have been processed in the food processing industry. And it generates large proportions of solid and liquid wastes, resulting from the preparation, production, and finally consumption of food. These varied wastes consist of worthwhile different nutrients for valuable biomass generation and production. However, if these wastes are left without any treatment and management, their uncontrolled decomposition will in turn pollute the environment. Therefore, proper planning, management, and utilization of these food processing industry wastes in a more productive way are the need of the hour to mitigate some of the issues of hunger and undernourishment in India and around the world.

Keywords Bioenergy · Food processing waste · Fruit and vegetable waste · Meat and dairy product waste

2.1 Introduction

The human population currently faces multiple challenges on the global stage; energy and food security are two of the major challenges that need immediate attention before the situation becomes worse. As we all know, both energy and food are the basic necessities of our livelihood, so easy and affordable availability of both items is of great significance for the sustenance of human population at large.

A. Agarwal (✉) · M. D. Heirangkhongjam · K. Agarwal
Department of Food and Nutrition, Lady Irwin College, University of Delhi, New Delhi, India

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This chapter will shed some light on how food and food waste processing could mitigate the energy issues without compromising food security and balancing its interdependence. Owing to the fast increase in human population, the demand for foods and energy has increased manifold. Apart from the increased demand, the way we consume food and energy has also changed drastically, which led to new challenges as well.

In the last few decades, there has been a major shift in food choice behavior. More and more health-conscious people are moving toward organic foods and healthy processed food products than normally available foods. This food behavioral change ultimately resulted in unprecedented food wastage. According to the Food and Agriculture Organization (FAO), one-third of the food produced globally is wasted. Despite various efforts to reduce food wastage, still about 1.3 billion tons of foods are wasted including fruits, vegetables, meat, and dairy and bakery products. These wastes are generated at different levels, namely, agricultural farm produce, post-harvest, processing, storage, etc. Therefore, how these huge amounts of food waste could be productively utilized is gaining significance.

The most effective and productive way for utilizing these food wastes is in generating biomass as a source of bioenergy production. It can be used directly without any treatment for microbial growth or treat with enzymes for the production of bioenergy. The final products that are generated from these perishable wastes are either in gaseous or liquid forms. Therefore, it is very important to determine the quantity, quality, and characteristics of the feedstock beforehand as the type of process and its conversion to several types of biofuels vary. Preliminary screening and characterization of different biochemical wastes and its treatment and management processes can be further designed to retrieve utmost energy from waste nutrients (Singh et al. 2012).

Food and agricultural wastes are a kind of biodegradable waste mainly discharged from households, food processing units, and hospitality sectors. Dependence on biomass as the source for the production of energy or as a chemical feedstock is increasing considerably over the last few decades. Such biomass will be mainly used for non-food reasons; its use and production will, however, compete with other claims such as food and feed productions (Mahro and Timm 2007). Therefore, such food-feed-fuel conflict can be minimized by amalgamation of all kinds of food waste or biowaste for bioenergy production. In this context, food industries, in particular food processing sector, have the capacity to be a good reliable candidate as they inevitably generate huge amounts of biogenic residues every single day. The rapid growth of population and the ever-changing food habits among the younger generations will further strengthen the growth of the food processing industry/sector. As per a recent data, the average annual growth rate (AAGR) in the food processing sector is around 8.41%, and India's CAGR is 14.6%, which is much higher than the global growth rate (Invest India National Investment Promotion and Facilitation Industry 2021).

In view of the future growth prospects, food processing industry waste could become a key player in the growth of biomass and the production of bioenergy, thereby helping in mitigating a fraction of the energy security issues that the

common people face every single day. The very advantage of such biogenic food processing waste and residues are easy availability, and their collection is usually done in controlled conditions, as moist food residues are generally not suitable for incineration process and thermal recycling. In other aspect, it might help in reducing the overall costs for the disposal of waste.

In this chapter, we will study various possibilities to use biogenic residues from the food industry and its processing waste more efficiently and shall discuss their potential as a biomass resource for the production of bioenergy.

2.2 Present Scenario of Food Processing Waste in India and the World

Food wastage has now become a very serious issue considering the acute hunger and undernourished people prevailing across the globe. World food production is expected to feed 7.6 billion people across the globe, ironically, food wastage is one of the top reasons behind persisting hunger and undernourishment. According to the Food and Agriculture Organization, annually, an astounding 1.3 billion tonnes of food is being wasted. Further, the FAO states that about one-third of the total global food produce and manufacture is wasted, which costs about $750 billion of the world economy. The problem of wastage of food is more serious in more affluent countries; however, it is estimated that the prevalence of the same issue in developing countries is also rising. Fast-developing countries like India and China produce majority of household food waste every year, but the regular volume of food waste production per capita is less than 70 kg in these countries. In contrast, people in Australia nearly produce 102 kg of food waste each year on average. Figure 2.1 indicates approximate worldwide household food wastage of some selected countries.

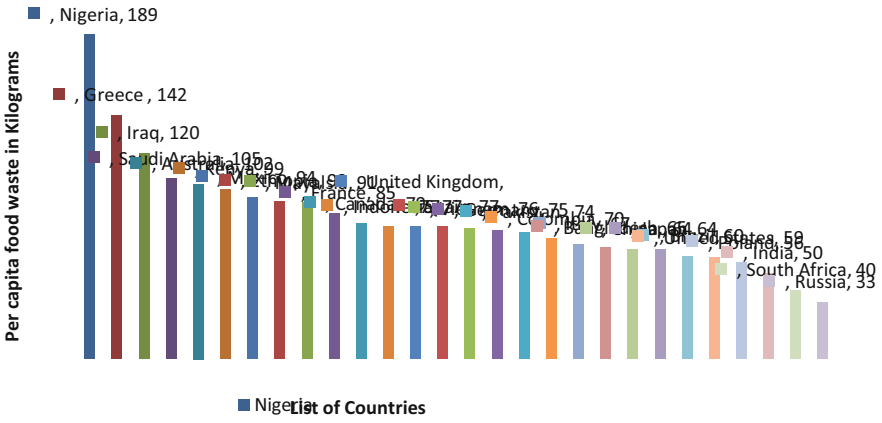

Fig. 2.1 Worldwide food wastage of selected countries in kilograms per annum, 2020

The biodegradable wastes discharged from several sources such as households, food industries, hospitality sector, etc., are known as food wastes. Fresh fruits, vegetables, bakery products, meat, and dairy products are the chief food items lost throughout the food supply chain (FAO 2012).

Food waste has been anticipated to increase considerably in the subsequent 25 years because of the growth in economy and population across the globe. Asian countries, particularly India and China, will be the epicenter. As per Melikoglu et al. (2013), the annual growth of food waste in urban areas of Asian countries could rise from 278 to 416 million tonnes from 2005 to 2025. A total of about 1.4 billion hectares of fertile and productive land (28% of the world's agricultural area) is annually used to produce foods that are wasted. Another major negative impact due to food wastage is in overall climatic condition. As a result of food waste, it is estimated to contribute to the emission of greenhouse gases (GHG) accumulating roughly 3.3 billion tonnes of carbon dioxide in the atmosphere each year. Moreover, normally, due to poor waste management, the major components of municipal solid waste are generally incinerated or discarded in open areas, causing serious health and environmental issues, especially in big and small cities (Pattnaik and Reddy 2010; Kumar and Goel 2009; Kumar et al. 2009; Talyan et al. 2008; Agarwal et al. 2005). Food waste containing high moisture releases dioxins if it is not properly maintained due to incineration (Katami et al. 2004), which may further deteriorate the area and the environment at large. Further, incineration results in the reduction of the economic value of the substrate as it largely hinders the recovery of valuable nutrients and chemical compounds from the substrate. Hence, suitable methods are needed to manage the food waste (Ma et al. 2009).

Among food groups, oil-bearing crops, roots, and tubers report the maximum level of loss, followed by fruits and vegetables. The highly perishable nature of fruits and vegetables incurs high levels of loss. Among all food groups, oil-bearing crops, roots and tubers loss, cassava and potato loss are the main contributors, given the significant amount of data reported for these commodities. In fact, cassava deteriorates in 2 or 3 days after harvesting, making it the most perishable. On the other hand, potatoes need careful management, handling, and proper storage to avoid large-scale losses, especially in many developing countries with warm and humid climates. Figure 2.2 shows a summary of the quantity and typical wasted food by commodities (FAO 2019).

As highlighted above, food wastage is a serious issue worldwide, and the situation in India is more concerning than the global trend. In fact, India being the second producer of cereals and fruits and the leading producer in livestock and marine production, it processes only 2% of the total produce. As per the United Nations Development Programme (UNDP), nearly 40% of the foods produced in India are wasted. Moreover, as per the Ministry of Agriculture, Government of India, around Rs. 50,000 crores worth of food produced every year is being wasted in the country. The total loss is around $9 billion to GDP due to food waste. Fruit and vegetable produce, oil seeds, and fisheries are the major contributors to the loss (MOFPI 2015).

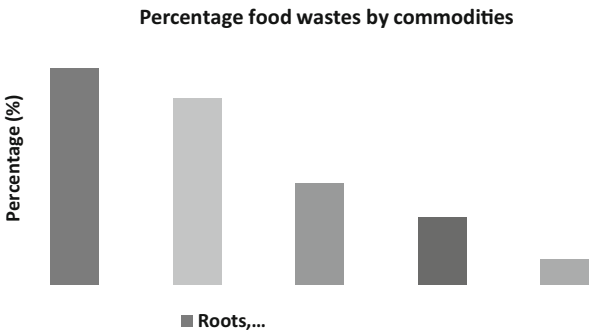

Fig. 2.2 Percentage food wastes by commodities

Furthermore, food losses are equally high in developed and industrialized countries and in developing countries, but there is a major difference in the loss between industrialized and developing countries. In developed and industrialized countries, over 40% of the food losses and wastages occur at retail sectors and consumer levels, whereas post-harvest and processing level loss in developing countries accounts for more than 40% of the food losses. Moreover, knowing the waste volume availability of the substrate is very important in waste conversion processes.

We have briefly discussed the overall food wastage; however, the chapter's main focus is on food processing wastes (FPWs). By FPWs, we mean the residuals that are left over after a primary product has been processed in the food processing industry. We are aware that food processing industries generate large proportions of solid and liquid wastes, resulting from the preparation, production, and finally consumption of food. These losses during processing are mainly due to contamination problems during storage, inappropriate packaging, and non-appropriate transport systems (Girotto et al. 2015). FPWs consist of almost all our daily-use items, such as fruit and vegetable peels, seeds, pits, cheese whey, bone, blood, process water, tofu whey, sludge, and wastewater treatment. These varied wastes consist of worthwhile different nutrients for valuable biomass generation and production. Food effluents are rich in biodegradable components with high biological oxygen demand (BOD) and chemical oxygen demand (COD) contents. If these wastes are left without any treatment and management, their uncontrolled decomposition will in turn pollute the environment as it releases toxic materials and methane (Waldron 2009). Therefore, proper planning, management, and utilization of these food processing industry waste in a more productive way are the need of the hour to mitigate some of the issues of hunger and undernourishment in India and around the world.

2.2.1 Biofuels from Food Processing Wastes

Biofuels are energy carriers that store energy derived from biomass and are a renewable source of energy. Liquid biofuels include biodiesel, bioethanol, bio-oil,

and biobutanol, while gaseous biofuels include methane, hydrogen, and hythane as presented in Fig. 2.3.

2.2.1.1 Liquid Biofuels

Bioethanol

Ethanol or bioethanol can be produced from any feedstock containing substantial amounts of sugar or food wastes containing starch or cellulose, which can be converted to sugar and used to produce ethanol. The feedstock for ethanol production includes sugarcane, sugar beet, sorghum, maize, wheat, cassava, and mixed food waste, that is, biomass containing easily fermentable sugars. In the case of food wastes containing cellulose or lignin, usually the steps involved include pretreatment, enzymatic hydrolysis/saccharification, fermentation, and then distillation to yield ethanol. The hydrolysis of starch can be done using amylases such as α-amylases and β-amylases, and then hydrolysate is subjected to fermentation.

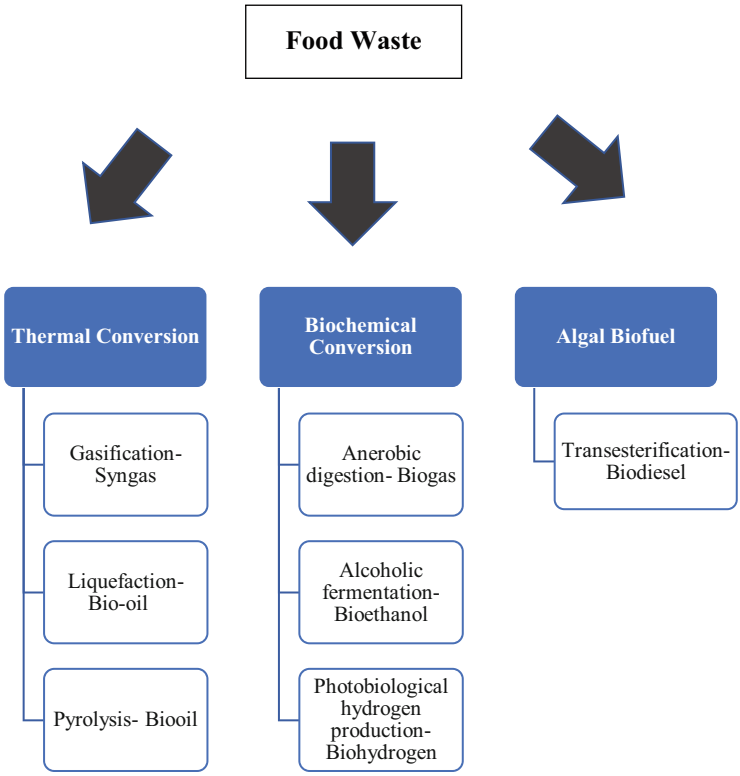

Fig. 2.3 Development of biofuel

Biodiesel

Biodiesel is composed of both saturated and unsaturated fatty acid methyl esters (FAME) depending on the source of feedstock. Edible plant oils including palm, soybean, sunflower, and non-edible oils from jatropha and mahua are usually used for the production of biodiesel. However, using edible plant oils as feedstock for biodiesel production may be a costly process, and alternatives such as food waste and waste cooking oil may be used for its production. Biodiesel can also be produced by the transesterification of microbial oils produced by oleaginous microorganisms including algae, fungi, and yeast. Food waste has been converted to biodiesel and fatty acids either by extraction of lipids by lipases or transesterification by alkaline or acid catalysts or transesterification of microbial oils produced by yeast strains. The use of biodiesel is advantageous since it is carbon neutral; that is, the fuel upon use does not produce carbon in the form of carbon dioxide (Dar et al. 2019).

Bio-oil

Bio-oil is a dark brown-colored liquid biofuel. Pyrolysis can be used to produce bio-oil from biomass agricultural residues, municipal biowastes, and forestry wastes. Food waste has also been used to produce bio-oil through pyrolysis and gasification.

Biobutanol

Biobutanol or butanol is a second-generation alcoholic fuel that can be used as a transportation fuel. It is produced by anaerobic degradation by Clostridium species to convert carbohydrates present in cereal crops, sugarcane, and sugar beet into acetone, butanol, and ethanol. Food processing wastes such as inedible dough, bread, and butter liquid are also used for the production of butanol (Dar et al. 2019).

2.2.1.2 Gaseous Biofuels

Biogas or Methane

Biogas or methane (CH_4) is produced by anerobic digestion of food processing wastes such as waste from fruit processing or meat industries, potato waste, and brewery waste (Dar et al. 2019). Methane is a renewable source of energy. Its production results in a nutrient-rich digestate that may be used as a fertilizer. The production of methane primarily involves four steps: hydrolysis (rate-limiting step due to lignocellulose, animal fats, and protein present in the biomass), acidogenesis, acetogenesis, and methanogenesis. The important factors associated with acidogenesis, acetogenesis, and methanogenesis include carbon-to-nitrogen ratio, temperature, pH, volatile fatty acids, ammonia, and long-chain fatty acids.

Hydrogen

After combustion of Hydrogen it results in water as the by-product (Zhang et al. 2016). Food processing wastes including tofu residue, cheese whey, rice slurry, wheat starch, jackfruit, apple pomace, and kitchen food waste have been used to produce biohydrogen. It has been noted that carbohydrate-rich wastes have a higher potential for hydrogen production than protein- and fat-based wastes. Hydrogen can be produced by light-dependent as well as light-independent processes. It has been noted that integrated photo-dark fermentation process is cost-effective for hydrogen production. The production of hydrogen is sensitive to food waste concentration, pH, temperature, volatile fatty acids, and partial hydrogen pressure.

Hythane

Hythane is a mixture of methane and 10–25% of hydrogen by volume, which is produced by anerobic digestion of food processing wastes.

2.3 Bioenergy Sources from Different Food Wastes

With the growing demand on fossil fuels for energy, biofuels produced biologically from renewable and waste organic substrates from microorganisms offer sustainable fuel source. They are renewable and biodegradable, may limit green gas emissions, and improve air quality. The utilization of food processing wastes to generate bioenergy will put the immense food wastage during processing to a sustainable use.

2.3.1 Cereal and Millet Wastes

Approximately 2–3% losses of cereal grains were reported at the time of harvest. Harvesting is the main cause for the critical loss point for all categories of food. In most African countries, for cereal grains and legumes, the major critical loss points consistently happens at the harvesting site and on-farm storage, irrespective of climatic conditions and location. Infestations and the effects of diseases, unfavorable climatic conditions, inappropriate harvest, and lack of labor or funds are the major causes of grain losses. With regard to on-farm storage losses, inadequate or improper storage amenities (e.g., inadequate ventilation) and lack of proper handling practices are the key causes (FAO 2019).

Crop residues are those plant parts that are left out after the economic parts of plants have been separated out. They may be categorized broadly in two groups: (a) field crop residues, those materials that are left out in agricultural fields after the harvesting of crops, such as stalks and stubble in the case of cereals; and

(b) processed residues, comprised of materials that are left out and further processed into a usable resource, for example, husk or hull in the case of cereals (Shahane and Shivay 2016).

The utilization of biomass as a feedstock to produce bioethanol is considered a more advanced technological way. In order to avoid competition, the utilization of straw or agricultural waste, for example, husk, is a better option between food and non-food use of cereals. The combined sweet sorghum (*Sorghum saccharatum*) and finger millet biomass conversion is another potentially promising research area.

Cereal by-products signify an unexploited resource of various compounds or fractions with high nutritional value. They could contribute as novel materials not only for the production of food and feed but also for the production of other nonfood products. Apart from the food industry, the use of cereal by-products could potentially be a reliable resource for the bioethanol industry. The milling industry is found to be the primary provider of cereal by-products. Grain screening performed before milling produces large quantities of cereal by-products as they do not fulfill grading specification. Other by-products obtained during milling are cereal bran and germ. The by-product germ is generally used for the production of cereal germ oil. However, the main by-product of the cereal milling process is the cereal bran. The bran consists of aleurone layer and seed coat from the milling and processing of the cereal grains after sieving out the endosperm. In the milling process, numerous steps are involved, such as cleaning, picking, and grading before processing the cereal grains and then the sifting method to obtain different fractions by using sieves, which is based on the size-exclusion separation method. Depending upon the type of mill used, the cereal grains are further broken into smaller pieces by cutting, grinding, or crushing to the desired size specification and depending on the final use. Hence, during the processing of cereals, these by-products are produced. According to Ma et al. (2009), it was found that a greater amount of damaged starch was observed when the particle size of flours becomes smaller. It also resulted in lower levels of sulfhydryl content of gluten protein, thereby altering the quality attributes of the flours.

In maize processing, two types of milling techniques are used, namely, wet and dry milling. The desired final product is the main criterion for determining the use of these techniques in maize processing. Normally, in order to obtain maize endosperm fraction, dry milling method is generally employed. The final product is used as flours, meals, or grits, thereby producing fractions of maize bran. Wet milling is employed to obtain the maize germ, which is a by-product mainly used for the production of oil. Apart from bran, the cake that is a residual by-product of cereal is commonly obtained from the extraction of oil during the process of de-oiling of maize germ (Papageorgiou and Da Rocha, n.d.).

The milling industry produces large amounts of maize bran by employing the dry milling process of maize in order to meet the growing demand of maize flour. Hence, it could represent a potential source of polymers (mainly composed of 50% heteroxylans and 20% cellulose) with high value added bioactive compounds such as phenolic acids, primarily diferulic and ferulic acids for the food industry. (Saulnier et al. 1995). Further, water-soluble maize bran gum is obtained by the

extraction of heteroxylans from maize bran, which is another by-product for the use in the food industry (Carvajal-Millan et al. 2007). Also, maize bran gum can be used as a carrier of bioactive compounds in the intestine in addition to numerous technological applications in food, nutraceutical, and pharmaceutical industries. Apart from the milling industry, the largest source for the production of bioethanol and other renewable biofuels such as biodiesel and biogas is from maize and its different by-products obtained from milling. So wastes of different grains and cereals in respective industries should not be treated as completely waste items as their abovementioned different by-products could potentially be used as an alternative resource for generating renewable biofuels by using the latest technology.

2.3.2 Fruit and Vegetable Processing Wastes

According to the FAO, fruit and vegetable waste occupies a major chunk in food waste. These are biodegradable substances generated in huge quantities; however, maximum wastes are dumped in open land to rot. This type of open dumping creates a lot of issues in and around the area due to emission of foul odor. Moreover, it also attracts birds, rats, pigs, etc., and creates big trouble by ultimately becoming vectors and carriers of various diseases. Apart from postharvest losses during transportation and lack of storage capacity, fruit and vegetable processing and packaging according to customers' specifications is also a major factor in waste generation. The rotten items, peels, shells, and scraped portions of vegetables or slurries are the major wastes, and they can be treated through fermentation under controlled conditions for the production of biofuel. Moreover, these wastes can also be used for composting in order to generate products with high humus content by natural decomposition due to microbes. Such carbohydrate-rich and naturally decomposed biomass can be a viable source for renewable energy generation as per many research works.

However, vegetable and fruit wastes are a special group of biomass. Therefore, proper characterization is needed in order to fully identify its composition and nature for its usage as a reliable source of raw material. Hence, a suitable and effective methodology could be proposed for its proper utilization. Therefore, proper understanding of waste composition is of great significance for the overall yield and kinetics of the biologic reaction during digestion. Like any other waste, fruit and vegetable waste can be characterized chemically, physically, or biologically. In solid wastes, physical characterization, estimation of volume, moisture, weight, total solid, volatile solid (VS), odor, ash, temperature, color, etc., are normally considered, whereas for liquid wastes, dissolved and suspended solids are estimated. In liquid waste characterization, turbidity is another important parameter that needs to be taken into account. In chemical characterization of food waste, measurement of hemicellulose, cellulose, starch, protein, reducing sugars, total organic compounds, COD, BOD, pH, halogens, toxic metals, nitrogen, etc., is checked. Moreover, apart from these biochemical parameters, calcium, carbon, phosphorus, sulfur, potassium, and magnesium can also be tested. Further, understanding the biochemical and

Table 2.1 Chemical characteristics of fruit and vegetable wastes

Food waste	Cellulose (%)	Hemicellulose (%)	Starch (%)	Protein (%)	References
Tomato	30–32	5–18	10–18	17–22	Schieber et al. (2001), Thassitou and Arvanitoyannis (2001)
Potato peel	17–25	10–15	30–40	3–5	Vallejo et al. (2004), Panda (2005)
Carrot	13–52	12–19	1–2	5–8	Llorach (2004), Ma et al. (2009)

Source: Utilization of vegetable wastes for bioenergy generation (pp 213–222) (Adapted from: Singh et al. 2012. Agricultural Research)

chemical parameters of different wastes provides an understanding and prospects on the applicability and usability of different wastes in specific energy production for employment generation.

Furthermore, the presence of pathogens and other organisms in biologic characterization of food waste indicates pollution. Some of the common features of different forms of food wastes are richness in protein, high COD, lipid biomolecules, and carbohydrates with clear pH variation. According to Joshi et al. (1999), wastes from vegetable processing industries including peas, tomatoes, and carrot, have a high BOD and are also a rich source of numerous vital nutrients like minerals, fibers, vitamins, etc. Therefore, a comprehensive study of waste characteristics is very important for determining its application and the economic feasibility of the process. Some chemical characteristics of fruit and vegetable wastes are given in Table 2.1.

With regard to fruit processing, 30–50% of the by-products are produced depending upon the type of fruit being processed (Chatanta et al. 2008). They can be distinguished broadly into two categories: (a) preprocessing by-products that include stalks, rotten fruits, and stems from sorting processes, and (b) by-products obtained from processing, namely, pulp, seeds, peels, and pomace. Moreover, in fruit processing plants, starches, pectin, sugars, vitamins, and other components of the cell wall are the main constituents of wastewater.

In the food processing industry, food manufacturing operations generate different types of waste. This reflects the diverse types of processes and ingredients being carried out due to such processing operations. For example, washing of root vegetables such as sugar beet gives rise to elevated levels of total soluble solids (TSS) in the effluent. Moreover, involvement of additional processing of vegetables, like dicing and/or peeling, can give rise to elevated dissolved solids (e.g., sugar in fruit processing).

Furthermore, around 80–90% of water is present in fruit and vegetable wastes, but the content of fat and proteins are very less (Mirabella et al. 2014). The by-products generated at the time of processing retain their natural chemical properties like that of the raw material. Different compositions of some common food wastes are listed in Table 2.2. As per a study, the potato peel contains 64.5% carbohydrates, 3.4% sugars, 13.5% proteins, 7.6% ash, and 11.2% moisture (Mabrouk and El Ahwany 2008). However, depending on geographical locations,

Table 2.2 Fruit and vegetable wastes, by-products, and their utilization

Vegetable	Part used	Resources utilized	References
Carrot	Pomace	Beta-carotene, coumarins, and hydroxycinnamates	Çinar (2005)
Potato	Peel	Chlorogenic, gallic, protocatechuic, and caffeic acids. Antioxidants	Salim et al. (2017)
Tomato	Crushed and dried seeds and skins of the fruit	Lycopene, beta-carotene, hydroxycinnamic acid derivatives, flavonols (quercetin derivatives), flavanones, and naringenin chalcone	Schieber et al. (2001), Baysal et al. (2000)
Onion	Scale tissues and external membranes	Flavonoids (quercetin) and organosulfur compounds	Erlund (2004), Tapiero et al. (2004)
Red beet	Pomace and peel	Betalains, betacyanins, betaxanthins, coumaric acid, cyclodopa glucoside derivatives, ferulic acid	Schieber et al. (2001)
Lettuce	Low-quality lettuce heads, stems, and external leaves	Caffeoylquinic acid; caffeoyl tartaric acid derivatives; flavones and flavonols; chlorogenic acid and chicoric acid	Llorach (2004)
Brassicaceae	Low-quality florets, stems, leaves	Hydroxycinnamates (sinapic acid), isothiocyanates, and glycosylated flavonoids	Vallejo et al. (2004)
Grape pomace	Skin, seeds, pulp, and stalks	PUFA, polyphenols, dietary fiber, flavonoids like catechin, proanthocyanidins, and epicatechins	Kammerer et al. (2005), Aliakbarian et al (2012)
Apple juice processing	Apple pomace, apple press cake	Flavonoids, chlorogenic acid, glycosides, pectins, natural sweeteners, antioxidants, essential oils and fibers	Bhushan et al. (2008)

growing conditions, potato varieties, and additional parameters, the composition may vary. Usually, the maximum portion of potatoes is used to feed livestock (Al-Weshahy and Rao 2012). Moreover, in cassava pomace, water- and insoluble dietary fiber contains the maximum amount, whereas in cassava peels, high crude fiber contains about 10–30% (dry matter basis) and protein content is less than 6% on dry matter basis. In fruit residues, elevated levels of carbon/nitrogen (C/N) ratio are present in comparison to vegetable wastes. Both vegetables and fruits show uniqueness in terms of sulfur and carbon/nitrogen contents. However, all the fruits and vegetable samples do not show optimal sulfur and nitrogen content as feedstock for anaerobic digestion. The optimal ratio of carbon/nitrogen (C/N) should be less than 25, and the optimal ratio of nitrogen/sulfur (N/S) must be 15–20 (Deublein and Steinhauser 2011). According to Dar et al. (2019), potatoes represent a separate category that reported 21.8% of total solids, 17.4% of volatile solids on a wet basis, and a C/N ratio of 23.

2.3.3 *Dairy Processing Wastes*

Several countries have noted a growth in the dairy sector due to a rise in consumer demand of dairy products. Processing of dairy products such as fermentation of milk or by-product processing, preparation of cheese, and preparation of whey concentrates from cheese whey result in processing wastes. The effluents of the dairy industry could include suspended solid and organic matter, sodium chloride residue, nitrogen, phosphorus, residue of cleaning products (detergent, sanitizer), by-products such as whey concentrates, oils, and greases. The waste, especially organic waste, generated from the dairy industry may pose a threat to the environment since it could lead to depletion of dissolved oxygen (DO). This depletion of DO could lead to breeding of mosquitoes and flies, thereby propagating diseases such as malaria, dengue, and chikungunya. Apart from this, nitrate, ammonia, and nitrogen present in raw milk are lost as the waste during processing may be converted to nitrite leading to contamination of groundwater. In addition, concentrated dairy waste has also been known to be toxic.

Ethanol or bioethanol production catalyzed by yeast has a lower energy intensity as compared to methane, hydrogen. Due to a higher production rate, it can be used as a transport fuel. Lactose-rich dairy waste has been used to produce ethanol by using yeast strains, which generate lactose hydrolysis enzymes. The studies using whey powder as a substrate for ethanol production are presented in Table 2.3.

Several studies have demonstrated that there is a lot of potential in using algal species in the treatment and utilization of dairy waste such as producing a high-quality biodiesel and at the same time reducing the pollution load (Ding et al. 2021; Dong et al. 2016; Kothari et al. 2012).

Apart from using yeast and microalgal strains to utilize dairy waste, some studies have reported using anerobic degradation of fatty wastes to produce biomethane (renewable source of energy). These fatty wastes usually consist of high concentrations of organic matter, mainly lipids and proteins, which may need pretreatment processes such as enzymatic hydrolysis, acid treatment, and saponification.

2.4 Factors Affecting the Production of Biofuels

There are several factors that affect the production of biofuels:

1. *Temperature*: Temperature plays an important role in biofuel production, especially in ethanol production, where fermentation using yeast should be done at an optimum temperature of 30 °C. Likewise, biohydrogen production carried out under mesophilic temperatures (30–37 °C) results in a higher yield at a lower energy cost (Dar et al. 2019). The activity of lactic acid bacteria is suppressed at higher temperature, while that of hydrogen-producing bacteria is enhanced. The optimum temperature for biodiesel production is between 50 and 60 °C with 55 °C being optimal since an increase in temperature beyond this point reduces

Table 2.3 Dairy waste and by-products and their utilization

Product	Waste	Processing	References
Bioethanol	Whey waste	Fermentation using *Kluyveromyces fragilis*	Ozmihci and Kargi (2007)
Bioethanol	Whey waste	Fermentation using *Candida inconspicua* W16	Minakshi and Shilpa (2012)
Bioethanol	Delactosed whey permeate	Medium optimization using *Corynebacterium glutamicum*	Shen et al. (2019)
Biobutanol	Whey	Cultivation using *Clostridium acetobutylicum* DSM 792	Foda et al. (2010)
Biomass and biofuel	Wastewater	Cultivation with *Chlorella pyrenoidosa*	Kothari et al. (2012); Lu et al. (2015)
	Wastewater	Cultivation using microalgae *Acutodesmus dimorphus*	
Biodiesel	Activated dairy sludge	Lipid extraction, refining, and optimization	Balasubramanian et al. (2018)

Adapted from Biofuels from Food Processing Wastes (p 260), Dar et al. 2019, Microbial Fuel Cells: Materials and Applications

the yield by causing vaporization of methanol. A temperature lower than 50 °C results in a lower yield, with an increase in temperature resulting in an increased conversion rate. Thermophilic anaerobic digestion (55–70 °C) for the production of methane is more advantageous than mesophilic digestion (37 °C) since it results in faster reaction rates and higher load-bearing capacity. However, it has several limitations including acidification, lower stability, poor methanogenesis, and sensitivity to environmental conditions.

2. *pH*: The optimal pH for ethanol production ranges from 4 to 5 (Dar et al. 2019), while that for methane production is 6.8–7.4 for anaerobic digestion. The optimal pH for methanogenesis is 7 with the growth rate of methane reducing when pH levels go below 6.6. The optimal pH for acidogenesis in methane production is 5.5–6.5.
3. *C/N ratio*: Anaerobic digestion for biogas production is sensitive to carbon/nitrogen ratio.
4. *Rate of hydrolysis:* The production of hydrogen is limited by the rate of hydrolysis with hydrolysis of carbohydrate-rich materials being faster than that of protein- and lipid-rich materials. Biodiesel production may involve fungal hydrolysis of food waste using *Aspergillus awamori* and *Aspergillus oryzae* to separate lipid and food hydrolysate rich in carbohydrates and amino acids. Also, lipases are used to catalyze hydrolysis in biodiesel production, and immobilized lipases can be reused for many cycles. Likewise in bioethanol production, hydrolysis is a key step where starch is hydrolyzed into carbohydrates.
5. *Pretreatment*: Pretreatment processes are important in the production of biofuels, especially hydrogen. The removal of hydrogen-consuming bacteria is an important step to favor the growth of hydrogen-producing bacteria. On the other hand, in ethanol production, harsh pretreatments are usually avoided before enzymatic hydrolysis because they can lead to partial degradation of sugars.

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Chapter 3
From Fruit and Vegetable Waste to Biofuel Production: Part I

Navodita Maurice

Abstract Vegetables and fruits play an important role in human survival, and their production all over the world has also increased in order to meet the demands of the expanding population. Increase in the production of fruits and vegetables has also increased their losses due to lack of proper handling methods. Vegetable and fruit losses not only indicate the fraction of food wasted without consumption but also represent wastage of water, land, fertilizer, energy, and effort. Disposal of fruit and vegetable wastes (FVW) has casted serious threat to the environment; for example, their dumping in the landfills results in the emission of greenhouse gases that are a threat to the environment, and also their decomposition emits foul smells and contaminates the air, water, and soil, thereby increasing the number of harmful pathogens. It has been investigated that FVW can be reused for the recovery of value-added and bioactive products, for example, antiviral, antibacterial, antimutagenic, etc. Researchers have discovered the potential of FVWs in the production of biofuels, namely, bioethanol, biohydrogen, biodiesel, and biogas, as they are rich in organic matter that can be easily hydrolyzed by microbes into biofuels by fermentation. The biofuels produced from FVWs can be used for cooking, transportation, as well as energy and electricity production.

Keywords Fruit and vegetable wastes (FVW) · Biofuels · Bioethanol · Biohydrogen · Biodiesel · Biogas

Abbreviations

CFU	Colony-forming units
CO	Carbon monoxide
CPW	Citrus peel waste
CSF	Consecutive saccharification and fermentation

N. Maurice (✉)
Prophyl Ltd., Mohács, Hungary

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CW	Citrus waste
DAP	Dilute acid pretreatment
DF	Direct fermentation
ETAAS	Electrothermal atomic absorption spectrometry
ET-ICP-MS	Electrothermal vaporization-inductively coupled plasma-mass spectrometry
FAO	Food and Agriculture Organization
FL	Food loss
FLW	Food loss and waste
FSC	Food supply chain
FSCW	Food supply chain wastes
FVW	Fruit and vegetable waste
FW	Food waste
GHG	Greenhouse gas
LCA	Life cycle assessment
LHW	Liquid hot water treatment
SF	Saccharification and fermentation
SSF	Simultaneous saccharification and fermentation
TSS	Total soluble solid

3.1 Introduction

The prevailing universal energy crunch has caused a serious concern all around the world. The world population is expanding radically day by day causing a great pressure on the demand of energy sources. The immediate sources of energy have been fossil fuels for decades, but their excessive utilization has raised questions about their availability in the future (Guo et al. 2015). The consistent usage of fossil fuels and their by-products alters the environment due to greenhouse gas (GHG) accumulation in the atmosphere resulting in climatic shifts, exalted sea levels, and upturns in temperature (Voloshin et al. 2015). Petroleum fuels are the ultimate source of energy for the transport of goods as well as humans, and their dependence is increasing every day. The global consumption of gasoline is more than 80% at present, and electricity as well as heat production releases a higher fraction of greenhouse gases (Gunay et al. 2019). Diesel and gasoline are highly utilized in the transportation of goods and people, which causes a threat for the depletion of fossil fuels for the future generation. Excessive utilization of fossil fuels as well as their by-products and their fast depletion have raised the demand for the alternative sources of energy. Biofuels have emerged as the renewable sources of energy that can replace fossil fuels. Tremendous research has been done and is still ongoing all over the globe in order to search resources that can serve as biofuels. Biofuels are eco-friendly and can limit the climatic alterations. Researchers have estimated that the utilization of biofuels is expected to rise to 30% by 2050 (Isah and Ozbay 2020).

It has been found that food waste (FW) can also serve as an alternative renewable source of biofuels, and a major fraction of solid waste that comes from municipalities is food waste. Gigantic fractions of FWs are generated all over the globe, and their safer disposition is one of the greatest challenges in the present era (Karmee 2016). Land fillings or incinerators are the major dumpyards where the FW is mainly disposed of. The Food and Agriculture Organization of the United Nations estimated that about 1.3 billion tons of FW is dumped without utilization. Tremendous increase has been noticed in the generation of FW with the increasing development and population all over the world with a global production of 1300 million tons (Melikoglu et al. 2013). The disposal of FW in the landfills has caused serious concerns in the society, for instance, contamination of air, leaching, and foul smells. Landfills not only generate carbon dioxide, methane, as well as other toxic gases but also occupy a larger space, which is a problem in metropolitan cities. Therefore, recycling and reusing of FW and exploration of the technologies that can transform FW into renewable sources of energy are a must (Katami et al. 2004). Researchers are trying to figure out accustomed FW valorization strategies to reuse FW by anaerobic digestion, burning, and animal feeds. Recently, it has been figured out that FWs can be biotransformed into biofuels and can be utilized as fuels either in pure forms or mixed with diesel engines (Pham et al. 2015). Biofuels are renewable sources of energy produced from waste organic matter by the activity of microbes. Biofuels are renewable and biodegradable and generate acceptable amounts of exhaust gases. They have emerged as sources that can limit emission of GHGs and improve the quality of air with significant energy production. Biofuels are needed for economic and environmental sustainability. Biotransformation of FW to biofuel appears to be a promising approach to decrease the energy cost (Fig. 3.1).

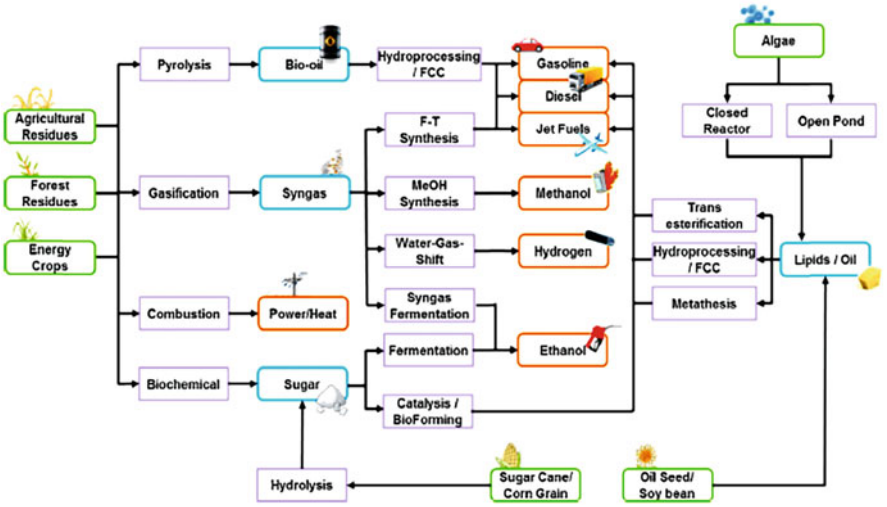

Fig. 3.1 Biomass conversion into biofuels. (Adapted from Yue et al. 2014)

3.2 Food Waste (FW) Definition, Generation, and Impact

Food waste (FW) is an inevitable portion of food eliminated from the food supply chain (FSC). FW is composed of biomass that can be either dumped into sewer lines, landfills, and sea or reutilized for composting, harvesting, bioenergy production, anaerobic digestion, and incineration (Lin and Tanaka 2006). FW includes those components of food items that are removed from the FSC for the production of bio-based chemicals and animal feeds. Therefore, it is wise to use the term "food supply chain wastes (FSCW)" that includes all types of wastes generated by the FSC. These FSCW can be used by the food processing industry for raw material production and for product distribution (Maina et al. 2017). Food loss (FL) refers to the deterioration of quality and quantum of food, while food loss and waste (FLW) is the total of deprived and removed consumable parts of food at all steps of FSC. The by-products of food can be transformed into value-added materials. Focusing on the fruit and vegetable wastes, the widely used term is fruit and vegetable waste (FVW), but different authors use different terms. According to the estimation of the Food and Agriculture Organization (FAO), 1.3 billion tons of food is dumped and famished every year globally, of which 88 million tons is produced in the European Union and the fractions are expected to increase 40% in the future (Plazzotta et al. 2017). FLW estimations are 170 million tons in North America. In the USA, the estimated FLW is 30–50% (Muth et al. 2019). The results of the FAO indicate that North America and Oceania rank first in the FLW values followed by Europe and the Russian Federation, Japan, China, the Republic of Korea, Latin America, North and West Africa, Central Asia, sub-Saharan Africa, and South and East Asia (Blakeney 2019). Considerable amounts of FVWs are generated during the harvesting and processing steps in the progressing countries; however, only 10% of FVW is produced after the consumption level. Reasons behind the major losses of the fruits and vegetables include bad storage conditions, poor processing facilities, and inappropriate infrastructure (Zhao et al. 2011). Fruit and vegetable damages during the harvesting and consumption levels are rather elevated in industrialized countries in comparison to the processing level (Girotto et al. 2015). FVW propagation is proportional to the nature of food standards set up by manufacturers and consumers; for example, in the USA, 45 million tons of fresh fruits, vegetables, grains, and milk products are wasted every year. Similarly, in India, this value is 5.6 million tons annually (Kosseva 2013). The commonly generated fruit and vegetable wastes include sugarcane bagasse, grape, sugar beet pulp, olive pomace, tomato, palm fiber, apple, palm kernel shells, potato pulp, cassava pulp, and pineapple and citrus peels (Pramanik and Rao 2005). The Committee on World Food Security and the High Level Panel of Experts (HLPE) on Food Security and Nutrition discussed the effect of FLW on the social, economic, and environmental magnitudes of suitability and on food nutrition and security (Supriyanto et al. 2019). Alternative studies have emphasized the financial, environmental, and social effect of FLW on nutrition and job losses (Blakeney 2019). Life cycle assessment (LCA) methods in general assess the effect of environmental factors on FLW by taking into account

water consumption, GHG emissions, chemical use, land use, biodiversity loss, and energy use. Studies can be conducted to assess the effect of financial factors on FLW from simple mathematical calculations to more complicated analyses. FLW estimates the overall losses of 490 billion US dollars and 23% of the agricultural area annually as per the report of the FAO. FLW is also liable for the 8% GHG emission as well as 23% fertilizer utilization globally (Blakeney 2019). The unsustainable recent practices of water, land, energy, and fertilizer utilization have posed a risk to food security, which is necessary to fulfill the demand of the increasing world population. This results in an imbalance between the demand and food production. Fluctuations in the production of food are under the influence of several factors, for example, climatic conditions, natural calamities, and pests. This generates the need for a stable food system. Nutrient losses are common throughout the FSC, and management of FVWs is challenging due to sanitary and environmental problems as any unchecked deterioration of the organic matter of the FW can alter the environment (Esparza et al. 2020).

3.2.1 FW Characteristics

FW is the organic matter that emanates from a vast array of sources, for instance, home and commercial kitchens, coffee shops, restaurants, and food processing sections (Kiran et al. 2014). As per the report of the FAO, the wastage of food is much higher than the human consumption. Throughout the whole FSC at all stages, food is either discarded or famished from the preliminary production to the last-minute usage by the final consumers. Bulk amounts of food produced have to undergo the steps of storage, handling, and consumption before being converted into waste. A considerable fraction of food can be categorized into escapable (evitable) and inescapable (inevitable) before being discarded. The key ingredients of FW include vegetables and their peels, fruits, eggshells, meat fragments, pulses, roots, grains, oil crop residues, seafood and fishes, milk and milk products, raw food stuffs, etc. The considerable fraction of FW is composed of fruits and roots of starchy plants. However, 85% of the FW is composed of vegetables, while 15% is derived from the animals and their allied products (Li and Yang 2016). FW is considered as a useless resource as it is thrown away without consumption. FWs are rich in carbohydrates, vitamins, lipids, phosphates, amino acids, and essential carbon elements. Various factors are responsible behind the generation of FW; for example, in developed countries, FW is produced by the consumers who purchase a considerably higher amount of food products and discard them without eating. Similarly, lack of proper harvesting methods and processing units and inappropriate marketing information in developing countries also generate a huge bulk of FWs. Generation of FW can be avoided if instead of throwing the quality food into the trash, it is given to the needy and through utilization of FW management strategies, but unfortunately, 95% of food ends up in the landfills (Lin et al. 2013). According to the report of the FAO, food production needs to speed up to 60% by 2050 in order to fulfill the hunger of the

world population. Increments in oil prices have elevated the cost of food items as well. Therefore, the requirement of approaches for the management of FW is a must in order to avoid environmental and human health problems (Ma et al. 2009).

3.2.2 Current FW Management Avenues

With the expanding human population and advancement of the global economy, the production of FW is also augmenting at a frightening rate. Disposal of FW is now a major challenging issue. The commonly used FW management practices include the avoidance of food from being disposed of, feeding the hungry, feeding domestic animals, incineration, and landfilling. Although feeding the hungry instead of throwing the food into the trash is one of the major goals of the FW management practices, a significant amount of food that is wasted is inevitable. A number of factors are responsible for this, for example, mentality of society, hygienic conditions, moral values, and the need of edible food. In many countries, FWs are fed to stray dogs and domestic animals like cattle and pigs instead of being disposed of; however, a considerable amount still reaches the landfills due to the health regulations of the majority of European countries. FWs can be transformed into soil-like organic matter compost, but this process is rather long and needs stable environmental conditions. Composting process can cause aftereffects like foul smells if not carried out properly (Li and Yang 2016). Around 95% of the FW undergoes either landfilling or incineration without being utilized for sustainable energy production or other approaches (Melikoglu et al. 2013). FW incineration with other municipal solid wastes is a conventional practice where microbes and chemical content of the FWs are destroyed and a small fraction can be compensated as energy but burning of FW and municipal solid wastes generates CO_2, NO, SO_2, and other toxins (dioxins). In order to avoid such issues, many nations select the option of landfilling, but this approach has many advantages and disadvantages. Both burning and landfilling approaches have side effects; for example, burning produces energy but generates a lot of gases that are not good for the environment; similarly, landfilling alters the quality and texture of the land. Several countries have adopted the policy of "use less" and "waste less" to make the earth a happier and healthier place to live (Karmee 2016). Production of biofuels from FWs has emerged as an eco-friendly and safe technology in the past decade where, instead of throwing the food into the trash or burning or landfilling, biofuel can be produced that can be used for a wide variety of purposes (Fig. 3.2).

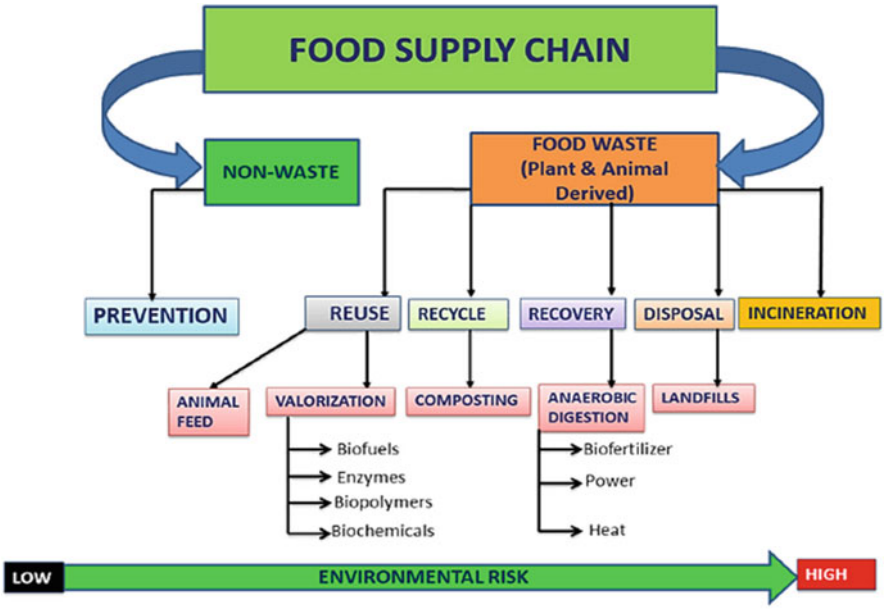

Fig. 3.2 FW management practices and their probable by-products. (Adapted from Dhiman and Mukherjee 2020)

3.3 Biofuels as Sustainable Energy Sources

Biofuels serve as energy sources and are either utilized in the form of heat or electricity or work generated from biomass along with its by-products. Biofuels are energy-loaded chemicals generated from live microbes and bioresources after undergoing a complex biological process with specific technology. The biomass generated by plants and microbes has been known as the distinguished biofuel source in the last decade in being environmentally friendly (Heimann 2016). Algae and plants have the potential to conduct photosynthesis by using solar power and CO_2 from the atmosphere to form biomass by sugar transformation (Voloshin et al. 2015). Biofuels have emerged as the sources of energy in developing countries. Biofuel production has undergone several ameliorations in the last decade, which can be classified into different generations (first–fourth). The first-generation biofuels were mainly produced from corn, wheat, oilseed, and barley where biodiesel was produced from sunflower and soybean (Pimentel and Patzek 2005). Sugarcane and raw corn fermentation along with fungal mycelia produces ethanol. Starch digesters, namely, *Saccharomyces cerevisiae* and *Rhizopus* sp., convert raw corn flour into ethanol (Wang et al. 2007). The industrial mass production of first-generation biofuels is based on starch or sucrose conversion by enzyme hydrolysis (Sheldon 2018). However, second-generation biofuels are derived from wood residues and organic and crop wastes utilized for bioethanol production, while

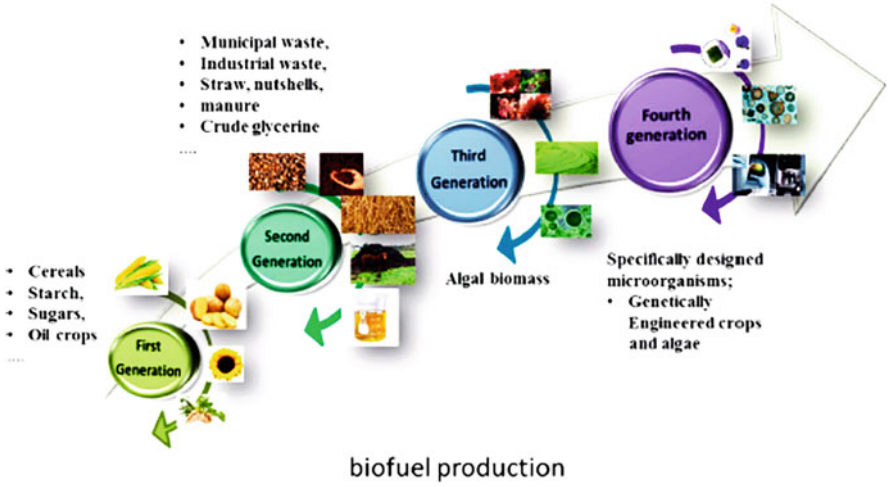

Fig. 3.3 Different generations of biofuels. (Adapted from Acheampong et al. 2017)

third-generation biofuels come from the metabolic activity of cellulolytic bacteria along with microalgae as well as other microbes for biodiesel production (Galbe and Zacchi 2002). Post-genome technique has helped in the metabolic alterations of the microalgae leading to the emergence of fourth-generation biofuels (Dutta et al. 2014) (Fig. 3.3). For the production of distinguished generations of biofuels (first to fourth), different technologies are being used. Biofuels can be categorized into liquid, solid, or gaseous fuels depending upon the physical status of their production (Kour et al. 2019). The common biofuels encompass biodiesel, bioethanol, biobutanol, biohydrogen, bio-oil, natural gas (syngas), and biochar. The common utilization of biofuels by humans includes cooking, heating, and electricity (Isah and Ozbay 2020). Biofuel production from the biomass of plants and microbes has emerged as a cheaper and eco-friendly sustainable energy source in the last decade that can cover up the expanding demand of energy globally. Since biofuels are produced by the activity of microbes, they curtail human dependence on nonsustainable sources of energy (Pleissner et al. 2014). The most important and crucial step for biofuel production is the selection of feedstocks as they not only contribute to the production (80–90%) but also determine the biofuel price. The everyday increasing global population has not only led to a crisis of food and fuel but also caused serious environmental threats; for instance, depletion of soil elements, alteration of land texture, and deforestation have led to atmospheric irregularities. Therefore, biofuels produced from the edible feed residues are rather expensive, and there is a big difference between demand and supply (Atabani et al. 2017). The higher need for feedstock supply for biofuel production causes a clash between food and fuels. In order to avoid such conflict, non-edible feedstocks are now used for biofuel production as they are cheaper, and several advancements have been made in the last few years by the biofuel industry in order to search for feedstocks. The

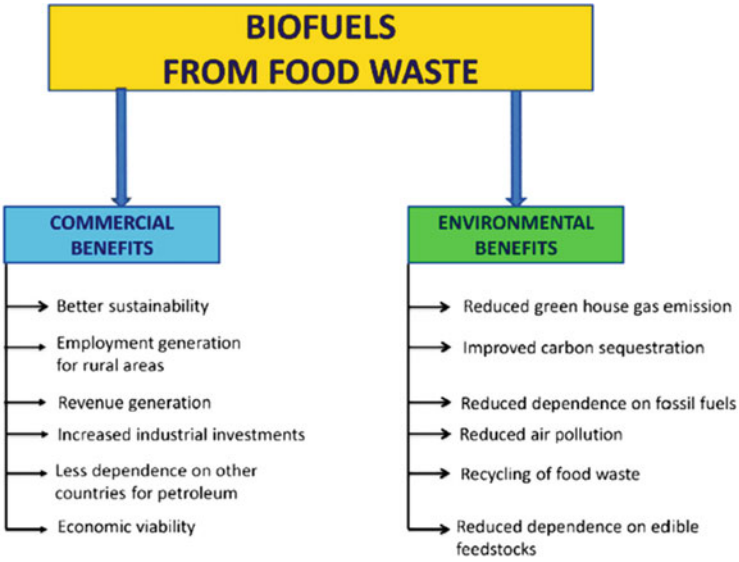

Fig. 3.4 Benefits of biofuel production from food waste. (Adapted from Dhiman and Mukherjee 2020)

commonly used feedstocks at present include fruit and vegetable wastes along with other FWs and waste cooking oils (Mahdavi et al. 2015) (Fig. 3.4). The utilization of FWs for biofuel production has been an issue of debate; however, FWs are organic matters composed of 35–69% of carbohydrates, 4–22% of oils, proteins, organic acids, and fats (Kiran et al. 2014). The protein and sugar content of FW cannot only be reutilized by fermentation but it also serve as a feedstock for microbes for the production of value-added products like biofuels, biochemicals, and enzymes (Karmee 2016). The conversion of biomass into biofuel not only solves the problem of fuel crises but also offers many benefits; for example, biofuel serves as a sustainable energy source that can be harnessed by humans and animals (production of animal feeds) (Lin et al. 2013).

In the present scenario, bioethanol, biogas, and biodiesel are only generated at an industrial scale contributing to the fraction of 90%. All types of biofuels must have specific physical and chemical properties in order to be used in engine operation and transportation. Biofuels, especially the liquid ones, can be easily hoarded, dispersed, and transported in trucks, cars, planes, and other means of transport without any trouble (Yanai et al. 2015). Transportation of biofuels (gaseous form) is, however, a bit complicated as it demands special dispersion and framework. Liquid biofuel must stay in liquid form at all temperature ranges as they have high combustion values in order to reduce energy loss and transport costs. Liquid biofuels like butanol have a high heat of combustion, and therefore, it finds its application in aeroplanes. All biofuels must be easy to store and must have tolerant ignition temperatures and vapor pressures (Arshad et al. 2018).

3.4 Biofuel Production from Fruit and Vegetable Wastes (FVW)

Disposal of FVWs is one of the biggest challenges as the majority of them are disposed of in the landfills on a daily basis. The biochemical disintegration of FVWs generates foul smells and sometimes unhealthy by-products. Many developing countries have adopted the system of "use less" and "waste less" in order to minimize the problems generated by FVWs. Although disposal of FVWs in the landfills is one of the economic ways, it is not eco-friendly, and therefore, better food management practices are still in demand. The most common technologies that are being used today include (a) compost formation from FVWs, (b) utilization of FVWs in animal feed preparation, and (c) biogas production. These technologies basically generate gaseous biofuels. The food valorization methods currently produce liquid biofuels from FVWs (Luque and Clark 2013). The green catalytic methods have given a new direction to the liquid biofuel production as a unique combination of chemical enzyme catalytic technique is used for the valorization of FVWs. FVW valorization can be conducted either by a multistep-chemocatalytic method or chemo-enzymatic step or by multistep-enzyme catalysis. These reactions are promising as desired products can be obtained without separation and clarification of intermediate products (Karmee et al. 2010). Therefore, researchers all over the world are trying to transform FVWs into liquid biofuels; for example, crude hydrolysates of carbohydrates, lipids, and amino acids can be obtained by dual enzymatic catalysis of bakery products. These hydrolysates can be then transformed by bio- and chemo-catalytic techniques into biodiesel and bioethanol. The pyrolysis of FVWs can also produce bio-oil (Fig. 3.5) (Karmee and Lin 2014).

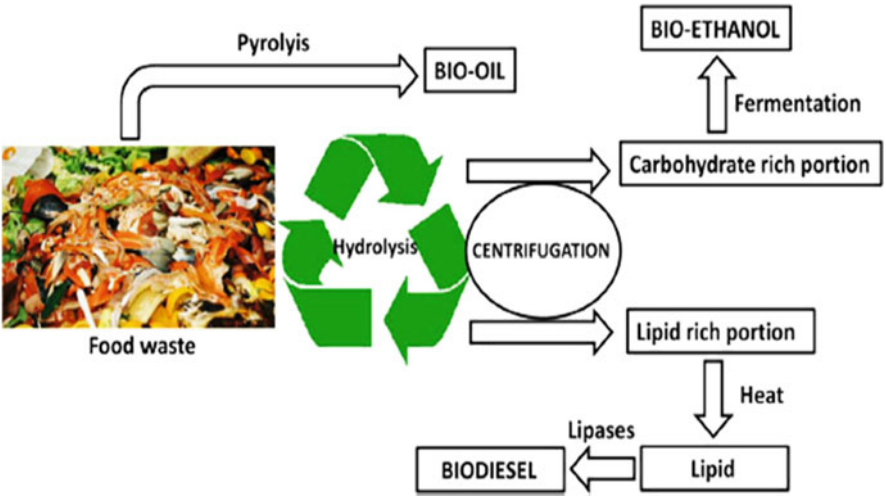

Fig. 3.5 Bio-oil, bioethanol, and biodiesel production from food waste by chemical and biocatalytic methods. (Adapted from Karmee 2016)

3.4.1 Bioethanol

Earlier, ethanol was produced either by fermentation or by chemical processes. Chemically, ethanol was produced by reacting ethylene under higher pressure and temperature. However, the chemical method is quick in comparison to fermentation, but the latter is more advantageous as it requires less developed framework and food and feed products can be utilized (Elshahed 2010). In the last 10 years, however, bioethanol has also been produced by conventional methodologies. Bioethanol of the first generation was produced from grains and sugarcane crops resulting not only in raised prices of the crops but also higher demand of fertile soil and inexpensive labor costs (Bensah and Mensah 2013). To overcome these shortcomings, second-generation bioethanol was produced from agricultural and forestry products, woody biomass, energy crops, and other wastes (Mohr and Raman 2013). Utilization of these materials resulted in elevated CO_2 generation; the enzymatic and physico-chemical treatments caused negative environmental problems (Robak and Balcerek 2018). To avoid these problems, researchers started to work on the production of third-generation bioethanol from algae. Ethanol being a renewable source of energy can be produced from agricultural livestock and fermentation of sugar by microbes, but still, more eco-friendly methods for its production are needed to avoid environmental issues (Thatoi et al. 2014). Marine bacteria and algae can serve as alternative sources of bioethanol as they have a fast growth rate and little or very low lignin content, are highly productive, and do not need tillable lands (Greetham et al. 2018). However, it has already been known that yeast and algae have the potential of producing ethanol by saccharification and hydrolysis, but few marine bacteria have also been discovered to have the same property. *Zymomonas mobilis* and *Saccharomyces cerevisiae* have been used for industrial ethanol production for decades (Yang et al. 2016; Leksawasdi et al. 2001). *Z. mobilis* has been found to be superior than *S. cerevisiae* in producing less biomass and has some advantages, for example, higher uptake of substrates, higher ethanol tolerance, brief generation time, cost-effectiveness, higher production, and lower maintenance needs (Kim and Dale 2004) (Fig. 3.6). Sarkar et al. (2019) discovered a new marine bacterium that can produce ethanol with suitable substrates. Bioethanol is the dominant biofuel in the market as it can be mixed with gasoline and it has higher content of oxygen (35%) in comparison to other biofuels. Higher oxygen content enables better hydrocarbon combustion, reduced carbon monoxide (CO), and reduction of other dangerous hydrocarbon emissions (Gebregergs et al. 2016).

Bioethanol is safe as it has little side effects on the environment, produced via sugar fermentation by microbial activity, and can be used as a gasoline substitute (bioethanol-gasoline mixture has a higher octane number) (Owen 1991). Despite its disadvantages (lower vapor pressure, less energy density, and corrosive nature), bioethanol is a sustainable energy source due to its many positive properties (Bhuvaneswari and Sivakumar 2020). The global bioethanol production has shown a tremendous increase in the last 10 years, and a major proportion of it has been contributed by the USA and Brazil. Corn and sugarcane crops serve as the

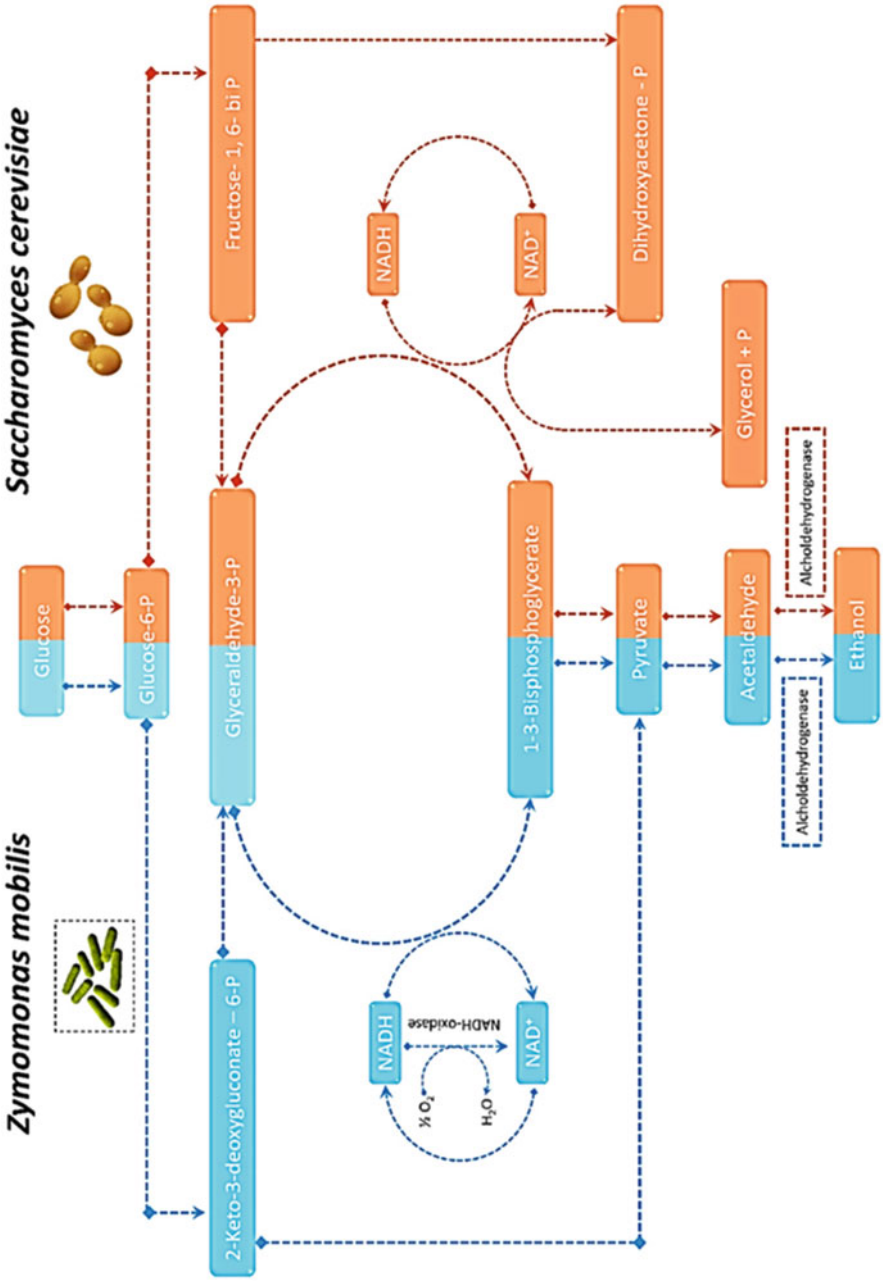

Fig. 3.6 Metabolic pathway of bioethanol production by *Z. mobilis* and *S. cerevisiae*. (Adapted from Yukesh Kannah et al. 2020)

major substrates in large-scale bioethanol production, making it expensive (Canilha et al. 2012). Recently, kitchen wastes, food wastes, bakery leftovers, and banana and potato peels have been used for the production of bioethanol (Sulaiman et al. 2014). Before the enzymatic hydrolysis step, pretreatment of FW or FVW is essential, and generally, these wastes are autoclaved in order to avoid contamination by microbial activity. Degradation of food or FVW by thermal pretreatment is avoided. FW or FVWs then undergo saccharification or hydrolysis after the pretreatment step (Wong and Sanggari 2014). Starch hydrolysis is carried out by a mixture of enzymes, namely, glucoamylase and α- and β-amylases, which convert the food or FVWs into hydrolysates. The hydrolysate then undergoes fermentation, and pure bioethanol is obtained after distillation (final step). *S. cerevisiae* H058 strain converts FVW hydrolysate into ethanol where *S. cerevisiae* K35 converts instant noodle waste into bioethanol by saccharification and fermentation (Yan et al. 2013; Yang et al. 2014). Potato peels are rich in carbohydrates, but their hydrolysis by acid and enzymes followed by fermentation by *S. cerevisiae* var. *bayanus* resulted in significant bioethanol yield. South Korean FW contains about 65% of carbohydrates, and its hydrolysis by enzymes and fermentation by *S. cerevisiae* gave good yields of ethanol. Banana waste can also be used for ethanol production (Snehal and Gaurav 2017). Bello et al. (2014) used pervaporation with hollow membrane to separate ethanol from wastes of banana. Household food waste when subjected to saccharification or liquefaction resulted in a high ethanol yield. Researchers all over the globe have claimed that vegetable waste is rich in carbohydrate biomass and can serve as a potential substrate for the generation of sustainable energy (Matsakas et al. 2014). Biofuel production from FVWs is gaining attention in many developing countries. Sulaiman et al. (2014) generated biodiesel and bioethanol and other valuable by-products from a halal biorefinery in Malaysia. Vegetable wastes can originate in different forms like raw, cooked, edible, or inedible and can be generated during the production, harvesting, and storage processes. Majority of the vegetable wastes enter the landfills and therefore generate foul smells, methane, and harmful leachate (Graf and Koehler 2000). Vegetable waste after microbial digestion can produce bioethanol, and it is rich in lignocellulose. Promon (2015) suggested that vegetable waste being rich in lignocellulose can be degraded into glucose and D-xylose. Vegetable waste is rich in carbohydrates, amino acids, phosphates, and lipids, and these components can be used for bioethanol production (Koppram et al. 2014). Lignocellulose is composed of cellulose, hemicellulose, and lignin, while vegetable wastes are mainly composed of cellulose and hemicellulose components of lignocellulose which can be hydrolyzed into sugar that serves as perfect substrate for microbes (bacteria, yeast of fungi) to produce ethanol by fermentation (Bhadana and Chauhan 2016) (Fig. 3.7).

3.4.1.1 From Fruit Waste by Marine Bacterial Strain *Citrobacter* sp. E4

Sarkar et al. (2019) used kitchen, fruit, garden, and paper wastes as substrates for the production of bioethanol. They collected these wastes and chopped into small

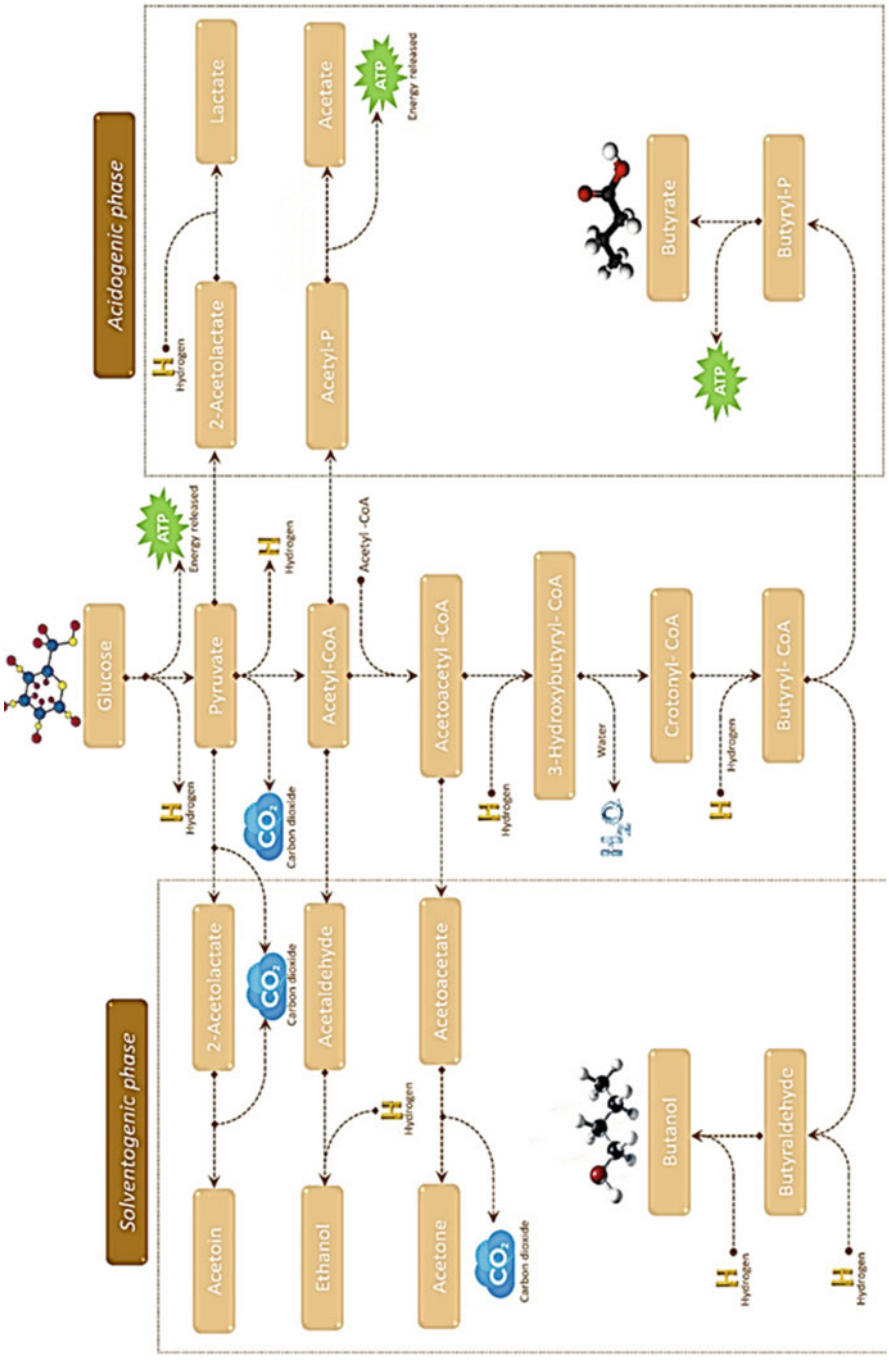

Fig. 3.7 Metabolic pathway of conversion of glucose into bioethanol. (Adapted from Yukesh Kannah et al. 2020)

chunks followed by drying for 2 days. After 2 days, the wastes were ground into fine powder, and their sugar and protein contents were determined (Chang and Zhang 2017). Ten marine bacterial strains (E1–E10) (ethanol resistant) were tested for their substrate utilization and fermentation efficiencies. Incidence of fermentation was detected by formation of bubbles in the Durham tube. They reported that all the marine bacterial strains preferred fruit and kitchen wastes in comparison to the paper waste. Garden waste also showed significantly higher fermentation activity. Strain E3 followed by E8 showed the highest bubble size with the garden waste. Most of the selected bacterial strains showed positive bubble formation activity with slight differences in their substrate selections. For instance, E2 formed larger bubbles with fruit waste, while E1, E2, and E4 strains preferred kitchen waste. E9 had the shortest time duration of bubble formation for fruit waste, while E1 and E8 had shorter durations for kitchen waste. The bacterial strains have the potential to efficiently utilize sugar from the waste samples as the marine bacterial strains have the enzyme cellulase (Shanmugapriya et al. 2012). E8 strain appeared to be the most ethanol tolerant, while E6 strain was least tolerant. E3 preferred fruit waste and appeared to be the most promising strain for ethanol production by efficient fermentation. Ethanol-tolerant strains are not suitable for ethanol production. The lactic acid and acetic acid bacteria are ethanol tolerant and therefore are not suitable for ethanol production (Bartowsky and Henschke 2008). These microbes rather use ethanol for the production of acetic acid; for instance, *Z. mobilis* (ethanol tolerant) can ferment only sucrose to ethanol but not other carbohydrate substrates. E4 strain gave higher yields of ethanol from fruit waste as confirmed by HPLC analysis.

3.4.1.2 From Citrus Peels and Wastes

Many researchers have studied the potential of citrus in ethanol production. Widmer et al. (2010) have explored the outcome of pretreatment on ethanol production from orange peels by sugar hydrolysis followed by fermentation and found considerably higher ethanol yields (Martín et al. 2010). Zhou et al. (2008) tried the approach of ethanol production from citrus peel waste (CPW) by D-limonene recovery. This approach has four steps, namely, removal and recovery of D-limonene by pretreatment, ethanol production by simultaneous saccharification and fermentation (SSF), ethanol removal by distillation, and usage of residual matters in animal feed and by-products. It turned out that fermentation of citrus waste (CW) can produce both ethanol and D-limonene. Citrus comes in the category of most bountiful crops all over the world just like bananas (Marín et al. 2007), and due to poor storage conditions, transport, and sale, most of the fruits are turned into wastes without being consumed. Orange juice wastes, namely, pulp, orange peels, fibers, and tissues, are rich in sugars, celluloses, hemicelluloses, pectins, and proteins (Dahmoune et al. 2013). These wastes result in clogging if dumped in floatation tanks, while landfilling results in environmental contamination, but their sugar-rich nature makes them useful for bioethanol production (Cypriano et al. 2018). *Citrus limetta* (mosambi) and *C. sinensis* (sweet oranges) fruit wastes are rich in glucose with a

little lignin content and are therefore more suitable for the production of bioethanol in comparison to lignin-rich fruits (Girish et al. 2014). The leftover citrus peels and fruits are excellent raw ingredients for the production of bioethanol (second generation). Yeast strain *Candida parapsilosis* NRRL Y-12969 along with other yeast strains excellently carries out the fermentation of citrus wastes as this strain can ferment pentoses present in citrus wastes and therefore increases ethanol yield (Umamaheswari et al. 2010; Swain and Krishnan 2015). CWs and CPWs are gathered, washed, and dried either in the sun or in an oven, and then the dried wastes are ground followed by addition of distilled water to them. This feedstock is then sterilized and cooled before mixing it with enzymes (cellulases, hemicellulases, and pectinases) to make hydrolysates. These hydrolysates are subjected to fermentation either by yeast monoculture or by coculture of *S. cerevisiae* and *C. parapsilosis* under aerobic and anaerobic conditions resulting in bioethanol production that is distilled (Girish et al. 2014). The tropical and subtropical regions of the world are the major cultivators of citrus fruits due to suitable soil and climatic conditions, and among them are oranges, limes, lemons, mandarins, and grapefruits, which are valuable commercially (Hayat et al. 2010). Citrus processing industries account for juice and essential oil production (De Castro 2014). A higher fraction of oranges is utilized as a valuable by-product, and the remaining fraction is converted into wastes composed of seeds, peels, and fibers (Oberoi et al. 2010). CW is chiefly composed of pulp and peel derived from juice industries and discarded fruits, which does not fit the criteria of entering the food chain. The major portion of CWs is composed of citrus peels that are utilized for the production of value-added products like flavonoids and phenolic acids (Ruiz and Flotats 2014). CWs account for 30% of the overall citrus fruits and are suitable for biofuel production. Bio-fermentation of vegetables and fruits as well as ethylene hydration by catalysis produces ethanol. Raw materials needed for ethanol production by fermentation are either starches (potatoes, corn) or sugars (sugar beets, fruits) or cellulosic materials (wood or agricultural residues) (Choi et al. 2013). Sugars can be directly transformed into ethanol, while starches first undergo hydrolysis before undergoing fermentation. Mineral acids convert cellulose into sugars. CWs are composed of fructose, glucose, sucrose, cellulose, pectin, hemicellulose, lignin, limonene, and proteins (Balasubramanian et al. 2011). Choi et al. (2015) described a method for CPW fermentation along with other fruit wastes like apple pomace and banana peel for bioethanol production by yeast and found elevated bioethanol yields. The popping pretreatment of mandarin (*Citrus unshiu*) peel waste with reduced D-limonene content showed higher yield efficiency and ethanol productivity (Hayashida et al. 1982). An integrated process was used by Pourbafrani et al. (2010) for the production of ethanol, limonene, biogas, and pectin where they hydrolyzed the CWs by acid treatment followed by sugar fermentation by baker's yeast. The anaerobic digestion of hydrolyzed CWs produced biogas (Taherzadeh and Karimi 2007). There are two CW biorefineries known for the production of ethanol. The larger one produces ethanol, limonene, and biomethane, while the smaller one produces limonene, digestate, and biomethane (Lohrasbi et al. 2010). In the acid treatment technique, CWs are blended with fixed amounts of sulfuric acid and water, and then this

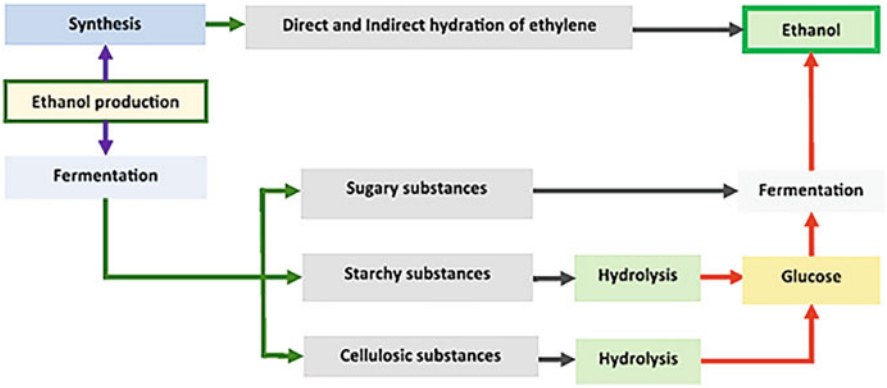

Fig. 3.8 Methods of ethanol production. (Adapted from Taghizadeh-Alisaraei et al. 2017a)

mixture is transferred to a hydrolysis reactor for hydrolysis where evaporation of limonene takes place. The hydrolyzed matter is filtered to get rid of solid materials (Jain and Chaurasia 2014). The sugary content is transformed into the fermenter for fermentation to produce ethanol (beer). This ethanol (beer) undergoes distillation and ethanol recovery (Devi et al. 2016). Solid wastes from filtration and leftovers of the distillation units are transferred to the anaerobic digester for methane production. Small amount of methane is lost in the boiler during steam production, which is used in the hydrolysis reactor and distillation column (Fig. 3.8). After extraction of juice from citrus fruits, the remaining CW is rich in lignocellulose, which can be fermented to produce bioethanol (Will et al. 2000). The saccharification and fermentation of CWs, pineapple peels, and banana peels by *Aspergillus niger* and *S. cerevisiae* produce bioethanol (Wu et al. 2015). Khandaker et al. (2020) found that optimum pH and temperature conditions affect the efficiency of banana peel fermentation. Higher glucose contents of orange and pineapple peels result in higher ethanol yields. Rotten peels of fruits and vegetables are generated in higher amounts in the world, and their disposal in the environment not only affects the health of the environment but also affects the food chain (Itelima et al. 2013). Bioethanol production from fruits and vegetable wastes not only enhances microbial activity but also enriches the soil with humus content (Khandaker et al. 2018).

3.4.1.3 From Pineapple Wastes

Pineapple waste can be recycled in order to obtain value-added products useful for the production of animal feeds as well as alternative industries, for example, production of bioethanol (Prasad et al. 2007). Pineapple waste is a rich source of sugars (glucose, fructose, and sucrose) and other essential nutrients (Hossain et al. 2008). The transformation of pineapple waste into value-added products like bioethanol not only makes the environment waste-free but also supports the process

of recycling. Hossain and Fazliny (2010) explored the total percentage of bioethanol produced from pineapple (*Ananas comosus*) wastes after yeast (*S. cerevisiae*) fermentation. They used rotten pineapples processed under different pH ranges and found that lower pH (pH 4) ranges result in higher bioethanol yields. Higher pH (pH 6) reduces bioethanol production. Higher ethanol production has been obtained at lower pH when leftovers of pineapple juice were used as a substrate; however, no significant effect of pH (3–6) was observed when pineapple effluent was used as a substrate (Muttamara et al. 1994). Pineapple cannery effluents when used as substrate also showed considerable ethanol yield at lower pH ranges (Prior and Potgieter 1981). Total soluble solid (TSS) also shows significant variation before and after the fermentation process just like pH range; for instance, at lower pH (4), TSS undergoes declination. Bioethanol yield increases when the concentration of yeast in the fermentation process is increased. Before fermentation, the TSS values were higher, but after fermentation, they undergo declination. Rotten pineapple wastes when fermented with yeast (*S. cerevisiae*) show maximum ethanol yield at 30 °C, which declines as temperature is lowered, suggesting that 30 °C is the optimal temperature range for the efficient functioning of *S. cerevisiae* (Mohd Azhar et al. 2017). Similar results were reported by Williams (2009) who obtained higher ethanol yields at 30 °C and 27 °C with lower pH ranges from the pineapple juices, as at lower temperatures, the metabolic activity of yeast is slow, which reduces the substrate utilization and rate of product diffusion, thereby affecting ethanol yield. Gil et al. (2018) tested sweet pineapple (*Ananas comosus*) peel and core wastes for ethanol production. They separated pineapple peels and core with the help of a pineapple cutter, which were grounded into a solid pineapple waste for checking the characteristics of the obtained waste, and found that an enough number of sugars and proteolytic enzymes are present for efficient fermentation. Pressing of the solid waste can result in the production of liquor with about 60% alcohol content. Studies conducted on the determination of optimum conditions for saccharification and fermentation of different pineapple wastes suggest that with increasing temperature, the yield of fermentable sugars also undergoes increase (Roda et al. 2016). Similarly, a decrease in TSS is observed with higher pH, suggesting that consecutive saccharification and fermentation (CSF) process can be achieved at pH 6 and 40 °C. Thermal treatment is an essential step before carrying out fermentation as acetic bacteria can grow if the medium is not sterilized during the early hours of fermentation. *S. bayanus* CECT 1926 appears to be the suitable strain for both saccharification and fermentation (SF) and CSF processes as it can effectively work at higher temperature (40 °C) and pH ranges (pH 6) by maintaining relatively higher colony-forming units (CFU). Declining TSS also slows down the growth of microbes due to nutrient depletion. However, in the saccharification step of the CSF process, TSS increases with the onset of fermentation without affecting ethanol yield (Demirbas 2008). In comparison to direct fermentation (DF), CSF shows faster microbial growth with rapid depletion of TSS within the first 24 h of the start of fermentation. This can be due to higher concentration of sugars in the media causing *S. cerevisiae* to slow down their activity when plenty of substrate is available. Similar correlation has been observed between TSS and CFU in the SSF process where elevated

ethanol yields were obtained. It has been suggested that SSF increases ethanol yield by rapid sugar transformation into ethanol by hydrolysis, which inhibits yeast activity due to glucose abundance (Sánchez and Cardona 2008). Both fermentation and saccharification processes curtail pH of the medium depending upon the species of microbes. CSF does not give better ethanol yield in comparison to DF, while SSF shows an increase in ethanol yield. In general, all the fermentable sugars are devoured in DF with a small residual fraction of sugars obtained at the end of saccharification due to the activity of hydrolytic enzymes of the medium. Global energy demand of the second generation of bioethanol through wastes is increasing on a daily basis. Pineapple wastes, namely, peels and shoots, have been utilized as raw materials for bioethanol production in the last decade (Cardoso et al. 2013). Enzymatic hydrolysis and acid pretreatment of the pineapple wastes initiate enhanced fermentation of the sugar content by yeast species. Hydrolysis followed by fermentation, then distillation, and finally dehydration are the key steps leading to production of bioethanol. Higher sugar content of pineapple wastes results in higher yields of bioethanol (Hossain and Fazliny 2010). Before the production of ethanol, pineapple wastes are gathered, washed, chopped, and dried in the oven for 2 days. They are then ground into powder and then finally mixed with distilled water. Acid pretreatment (usually sulfuric acid) of this mixture is then carried out before autoclaving (Del Campo et al. 2006). The autoclaved mixture is allowed to cool down, and enzymes (hemicellulases, pectinases, and cellulases) are added to it to form hydrolysate. Yeast is inoculated in the hydrolysate, and the whole material is then allowed to undergo aerobic fermentation with appropriate mixing. At the end of fermentation, the final product is distilled and dehydrated to collect ethanol (Conesa et al. 2018).

3.4.1.4 From Banana and Mango Wastes

Banana is one of the most crucial fruit crops grown all over the world with major cultivation in Asia and America followed by Australia. A considerable fraction of bananas is wasted or rotten or disposed of during harvesting and transportation (Reddy and Reddy 2005). These uneaten or rotten bananas enter the landfills resulting in contamination of the environment. However, these banana wastes can be utilized in second-generation bioethanol production as they are rich in essential sugars and nutrients needed for fermentation (Jahid et al. 2018). Banana wastes being cheap can serve as an alternative to replace the agricultural crops that were used to produce first-generation bioethanol (Guerrero et al. 2018). Banana peels and banana wastes (rotten ones) serve as substrates for yeast, *S. cerevisiae*, that converts substrates into ethanol. *S. cerevisiae* is a suitable microbe for fermentation as it can thrive well at higher sugar levels and therefore efficiently produce ethanol and carbon dioxide. Acid pretreatment of the substrate eases the hydrolysis of lignin, making the way open to the yeast to devour sugars leading to bioethanol production (Hossain et al. 2011). Banana wastes are not only rich in glucose but also in other carbohydrates that can be converted into sugars by the enzymatic activity of

hemicellulases, cellulases, and pectinases. Banana wastes are first collected and then washed and cut into small pieces before sun drying. The sun-dried chunks are then crushed into a fine powder before mixing with distilled water. The mixture is then treated with sulfuric acid followed by autoclaving. The gathered matter is allowed to cool down, and then enzymes like hemicellulases, cellulases, and pectinases are added to form the enzyme hydrolysate. This mixture is incubated for an hour at 50 °C (Wu et al. 2016). The enzyme hydrolysate is then inoculated with the culture of yeast, and anaerobic incubation starts for 3–7 days at 35 °C and at a pH between 5 and 5.5 with continuous shaking. At regular intervals, aliquots are tested to detect the concentration of bioethanol. Under these regulated conditions, water content of the substrate plays an essential role as the higher the water content, the higher the yield of bioethanol. The produced bioethanol is separated by the process of distillation (Bello et al. 2014). Toxic metals like Cr, Mo, and Pb are present in trace amounts in the produced bioethanol, while Mg, Ca, Mn, and P are present in higher amounts (Hossain et al. 2011). The relatively low amounts of toxic elements make bioethanol suitable to be used as a fuel. Therefore, banana wastes appear to be promising for bioethanol production. Mango (*Mangifera indica*) is an eminently short-lived fruit that is easily rotten right after being plucked till reaching the customers. Tropical nations are the major producers of mangoes. The major fraction of the mango fruit is composed of pulp followed by peel and kernel. The sugar content of mangoes lies between 18 and 20%, and therefore, mango wastes can serve as substrates for the production of bioethanol. The fermentation of mango wastes is effectively carried out by two yeast species, namely, *S. cerevisiae* and *Kluyveromyces marxianus* (Buenrostro-Figueroa et al. 2018). Fruit processing industries generate significantly higher amounts of fruit wastes that can be used for the production of value-added products by the activity of microbes. Fruit peels serve as excellent sources for the production of bioactive compounds. About 30–50% of mango and 20–30% of banana peels are discarded, which contaminates the environment (Gashaw and Getachew 2014). Valuable materials can be produced from these peels, for instance, microbial enzymes that are important at the industrial scale, ethanol, vinegar, wine, methane gas, and animal feeds (Zhang et al. 2005). Arumugam and Manikandan (2011) studied the consequence of acid pretreatment, saccharification, and ethanol production from mango and banana fruit biomass when inoculated with yeast. Their results indicated that bioethanol yield was variable among the fruit samples where yield was highest in the mixed pulp samples followed by banana pulp; however, yield was lowest in mango pulp. Acid pretreatment of enzymatic hydrolysate of mixed fruit pulps (mango and banana) followed by yeast fermentation not only showed higher ethanol yield but also showed elevated fermentation efficiency. Sirkar et al. (2008) also observed that ethanol yield was a little higher in banana peels than in mango peels. Fruit pulp hydrolysates treated with liquid hot water treatment (LHW) and dilute acid pretreatment (DAP) without enzymatic hydrolysis showed poor bioethanol yields in comparison to the normal process of fermentation. Hammond et al. (1996) obtained reduced ethanol yield from ripened banana pulp in the absence of enzymatic hydrolysis. Fermentation study conducted by Joshi et al. (2001) with banana peels along with yeast indicated

significant yields of bioethanol. Fermentation studies of Onwuka et al. (1997) showed higher alcohol yield and fermentable sugar content in the peels of plantain and banana. Ethanol productivity was highest in the mixed pulp mixture of mango and banana wastes, while it was lowest in mango peels. A moderate increase was observed when ethanol productivity increased followed by increased yeast growth and ethanol yield during the fermentation of mixed fruit pulps. Yeast growth was maximum at 48 h in the case of mixed fruit pulps, while it was maximum at 42 h in the case of fruit peels. Earlier reports have suggested that for acid pretreated enzyme hydrolysate, 24-h incubation is enough for maximum bioethanol yield (Sharma et al. 2004). The rapid conversion of sugars to ethanol by yeast can undergo declination when the availability of the substrate is decreased. Akin-Osanaiye et al. (2005) reported that ethanol production from agro-wastes is dependent upon the amount of yeast added. Declination in ethanol production after 48 h in mixed fruit pulps and 42 h in fruit peels can be attributed to the fact that either the availability of the substrate to yeast for fermentation decreases or the number of viable yeast cells decreases or enzyme denaturation occurs. The reduced ethanol yield in the case of mango fruit wastes can be due to the deterring effect of elevated polyphenol content or insufficient amount of fermentable sugar after saccharification; however, significant bioethanol yields were obtained from mango pulps after fermentation by yeast. Bioethanol production from ripened fruit pulps of banana, orange, sapota, and papaya was investigated by Azad and Yesmin (2019). They found that banana not only contains the highest sugar content in comparison to other fruits but also has sufficiently higher bioethanol yield. Sapota produces the lowest amount of ethanol. Production of bioethanol from four banana varieties, namely, champa, bitchikola, sabri, and sagor, was tested by Kumar et al. (2015). They found that sagor has the highest sugar content than the other three varieties and produces a significantly higher amount of bioethanol with efficient reduction in total sugar content after fermentation. Bitchikola variety produced the lowest amount of ethanol. Grapes, apple, banana, and papaya wastes processed for fermentation at 30 °C and pH 5.4 produced considerably higher fractions of bioethanol (Janani et al. 2013). Ethanol yield from jackfruit juice was also satisfactory (Kumoro et al. 2012). Banana (*Musa acuminata*) waste is rich in sugar monomers, and the best temperature with highest ethanol yield has been reported at 35 °C with *S. cerevisiae* (Chandel et al. 2007). Lower ethanol yields have been observed at lower temperatures (23 °C) as at lower temperatures, metabolic activity of yeast is inefficient to carry out sugar fermentation. Shaking does not influence bioethanol yield. Water content shows a profound effect on ethanol yield; for example, fermented banana mash when treated with sufficiently higher volume of water resulted in higher ethanol production. The fermented banana mash without enzymatic saccharification resulted in lower bioethanol yield in comparison to the banana mash supplemented with enzymes (pectinases and cellulases) (Uçkun et al. 2015). Banana mash treated with pectinase, however, resulted in higher yields of bioethanol, and this can be attributed to the fact that pectin disintegration by pectinase results in diminution of water retaining capacity, and therefore, more water is released, which is beneficial for ethanol production. Heat treatment is an essential step for efficient enzymatic

saccharification as it reduces the risk of bacterial contamination and also enhances the conversion of sugar into alcohol (Cheirsilp and Umsakul 2008). Heat treatment of banana mash for a shorter duration results in complete enzymatic saccharification. Pectinase treatment of banana mash at a pH of 5 and an incubation temperature of 40 °C enhances bioethanol yield. Pectinase also resulted in maximum hydrolysis of banana mash wastes. Many studies have reported that enzymatic saccharification results in higher bioethanol yields from vegetable products and fruit juices (Leng 2008). The pure banana juice is very viscous, turbid, and grayish in color and settles down during storage, so it must be processed enzymatically in order to get a potable juice (Singh et al. 2012). After enzyme addition, banana mash is usually incubated in a water bath for the banana juice extraction as this step increases the yield of juice, adds flavor to it, and improves the color also. Heat treatment inactivates the enzymes present in the juice before yeast addition (Lee et al. 2006). The fermented banana mash shows high viscosity values. Pure ethanol has the lowest viscosity value. Enzyme addition to the fermented banana mash reduces viscosity values and therefore facilitates liquefaction of nonsoluble polysaccharides present in the banana cell walls. Medium dilution is also an essential step as it reduces the osmotic pressure. Bioethanol production from fermented banana mash with different yeast concentrations and pH treatments showed variations in the number of elements present. The highest value of silicon was obtained from bioethanol produced from fermented banana mash treated with yeast, while the lowest value of silicon was obtained from banana mash made from rotten banana fruit. Elements, namely, zinc (Zn), calcium (Ca), and magnesium (Mg), showed dominance with different yeast concentrations and different pH ranges during processing of fresh and rotten banana fruits. Zinc (Zn), magnesium (Mg), calcium (Ca), and iron (Fe) are present in bioethanol, and even if they are found in higher amounts, they are not harmful, but lead (Pb) was not observed in the bioethanol produced (Ghobadian et al. 2008). Saint' Pierre et al. (2005) analyzed the trace elements of bioethanol using the ET-ICP-MS (electrothermal vaporization-inductively coupled plasma-mass spectrometry) method and found positive results. Oliveira et al. (2002) used the ETAAS (electrothermal atomic absorption spectrometry) method and were also successful in the determination of the amount of trace elements present in bioethanol. Bioethanol obtained as the final product can be utilized in the operation of petrol engines.

3.4.1.5 From Potato Peels

Potato products are rather more popular among the processed foods. The major proportion of the potato tuber is composed of starch (carbohydrate) followed by a small fraction of protein (Puttongsiri et al. 2012). Major losses in the potato crops are related to the peeling methods of potato. The potato peels can be converted into value-added products instead of being disposed of, for instance, alcohol production from potato wastes. The by-products produced during the processing of potato granules can serve as excellent substrates for bioethanol production (Kawa-Rygielska et al. 2012). Production of ethanol from starchy substrates undergoes

liquefaction followed by saccharification and finally fermentation (Pervez et al. 2014). Ado et al. (2009) found synergistic metabolic cooperation between *S. cerevisiae* and *A. niger* in a starchy medium where enhanced ethanol yield was obtained. The solid residue collected after ethanol production was found to be rich in essential nutrients and could possibly serve as biomanure. This residue when mixed with soil improved the texture of the soil humus and therefore was suitable for the improvement of plant growth (Bhattacharyya and Banerjee 2007). Generally, the nitrogen deficiency of soil is replenished with chemical fertilizers that not only deteriorate the soil quality and texture but are also harmful to the animals of the food chain. Addition of blue green algae to the soil not only overcomes nitrogen deficiency but also replenishes the soil with essential nutrients necessary for plant growth. The blue green algae have been reported to produce plant hormones (gibberellin, auxin), amino acids, and vitamins essential for plant growth and development (Ananya and Ahmad 2014). Biomanure administration to soil is a cost-effective method of increasing soil fertility. Chintagunta et al. (2016) investigated the effect of *S. cerevisiae* and *A. niger* coculture on bioethanol production from potato mash and peels. They reported that both potato mash and potato peel wastes are rich sources of starch followed by cellulose along with minor fermentable sugars and little protein content. Khawla et al. (2014) found differences in the protein and starch content of potato peels, which can be attributed to different climatic conditions and potato harvesting methods. Both potato mash and peels when inoculated with the coculture of *S. cerevisiae* and *A. niger* showed significant ethanol generation within 24 h. Arapoglou et al. (2010) also investigated the production of ethanol from potato peel wastes by saccharification followed by fermentation with *S. cerevisiae*. Fermentation of corn cobs with *A. niger* and *S. cerevisiae* also generated good ethanol yields. The total ethanol yield and average productivity from potato peels were found to be lower than those from mash wastes. Significantly similar rates of starch hydrolysis were obtained for both potato peel and mash wastes. It turned out that *A. niger* produces the enzyme amylase that enhances starch conversion to reduced sugars. These reduced sugars are then utilized by *S. cerevisiae* for ethanol production (Satish 2010). Solid state fermentation is an efficient tool for amylase production *A. niger* as it completes starch hydrolysis within shorter duration. The potato mash wastes and peels after ethanol production can be mixed together where they serve as substrates for the NPK microbes that enhance soil fertility (Suganthi et al. 2011). Chintagunta et al. (2016) studied the effect of different soil microbes (*Anabaena variabilis*, *A. lipoferum*, *A. fertilissima*, *A. chroococcum*, *Chromocyphella muscicola*, *Fischerella muscicola*, and *Nostoc muscorum*) on NPK content when treated with potato mash waste and potato peel biomanure. They found the highest NPK content with *A. variabilis* in comparison to other soil microbes. The vegetative cells of *Anabaena* PCC 7120 replenish heterocysts with glutamate that is converted to glutamine along with other amino acids, and these amino acids are used by vegetative cells for fixing nitrogen (Kumar et al. 2010). Phosphorus-solubilizing cyanobacteria convert inorganic phosphorus from the substrate to soluble phosphorus by organic acids. Some gram-negative phosphorus-solubilizing bacteria also have the same potential. *Bacillus* species can solubilize

soil potassium. *F. muscicola*, *Azospirillum lipoferum*, and *A. fertilissima* mixed with potato waste residue showed increased the NPK content of the soil. *Nostoc* produces nostocyclamide (anticyanobacterial metabolite) that can have an effect on *Anabaena* morphology; similarly, the pigment nostocine A produced by *N. spongiaeforme* can inhibit the growth of many cyanobacterial species (Maheep 2014). *F. muscicola* produces a secondary metabolite, fischerellin A (FsA), which inhibits the electron flow in photosystem II in some cyanobacteria. Studies have indicated that nitrogen fixation by cyanobacteria is not only helpful in rice cultivation but also increases soil fertility (Gantar et al. 2008). Biomanure obtained from potato wastes can enrich the soils with sufficient nitrogen content. Azad and Yesmin (2019) determined bioethanol production from agri-products, namely, sweet potato, potato, pumpkin, carrot, and corn, as well as from ripened fruits, for example, papaya, banana, sapota, and orange. They found that among the agri-products, sweet potato had the highest sugar content, indicating higher bioethanol production. Pumpkins being less sweet had the lowest bioethanol yield. Bioethanol generation from starchy vegetables (sweet potato) by sequential batch fermentation showed lower yields due to higher content of fermentable sugars (Hadiyanto et al. 2013). Red potatoes also gave significantly higher yields of bioethanol in Nepal (Joshi 2014). Buratti et al. (2008) reported higher bioethanol production from mashed corns. Water removal from ethanol is a risky step especially when agri-products are the substrates. They also found a solution to overcome this issue. They suggested that pumping the fermented mash through a multi-column distillation system can easily remove water from ethanol.

3.4.1.6 From Pistachio Wastes

At the moment, Iran is the biggest producer of pistachios, but the wastes generated and their disposal after the removal of pistachio seeds are a major problem in Iran (Açikalin et al. 2012). The shells of pistachios can be transformed into biofuels for energy generation using different methods (Peters 2011). At the industrial scale, the biomass obtained from pistachios can be directly converted into energy. The indirect approach of transformation includes conversion into bioethanol, biodiesel, bio-oil, and biogas (Sharma et al. 2015). Bioethanol is produced by fermentation of sugar, cellulose, and starch and can be used as a fuel to run engines (Sequeira et al. 2007). Thermochemical and biochemical methods can also be implied in the utilization of the pistachio biomass. Anaerobic or aerobic digestion and alcoholic fermentation are the common biochemical methods used in practice (Ahmed et al. 2015). Gasification and pyrolysis are the commonly used thermochemical methods (Morales et al. 2014). Pistachios are also used after shell removal as they are utilized in the preparation of bioactive products (Tomaino et al. 2010). The green pistachio shells are rich in antioxidants as well as fats, proteins, vitamins, and minerals (Mohammadi Moghaddam et al. 2009). Disposal of pistachio shells is a threat to the environment as it contaminates the soil and produces methane that is not healthy to the environment. The pistachio shells are rich in cellulose followed by lignin and have traces of

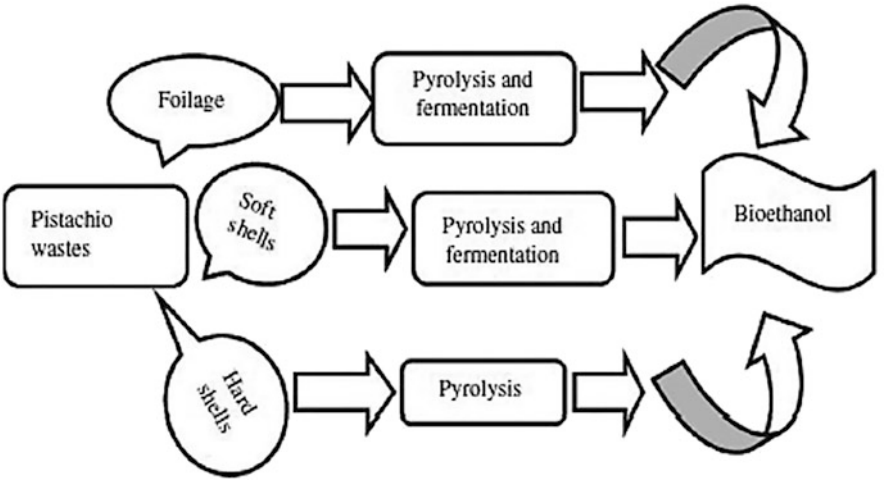

Fig. 3.9 Bioethanol production from pistachio wastes. (Adapted from Bhuvaneswari and Sivakumar 2020)

hydrogen, oxygen, carbon, and nitrogen (Soleimani and Kaghazchi 2014). The shell wastes have high moisture content, while the wood from pistachio trees and empty shells have low moisture content. The biochemical and thermochemical processes can be utilized for the transformation of pistachio wastes (empty and green shells as well as the wood) for the production of biofuels (Ebrahimi Meymand et al. 2013). Fermentation, pyrolysis, and digestion methods can be utilized for the transformation of pistachio wastes into biofuels. Taghizadeh-Alisaraei et al. (2017b) investigated the efficiency of pistachio shells by fermentation and pyrolysis for bioethanol and biogas production and obtained good yields (Fig. 3.9).

3.4.1.7 Factors Affecting Bioethanol Production

Several factors have been known to affect the yield of bioethanol. Different types of FWs produce different proportions of bioethanol (Table 3.1).

(a) Temperature

It has been observed that *S. cerevisiae* cells increase exponentially right after the start of incubation and then they enter the stationary phase after prolonged hours of incubation at all functional temperatures (Torija et al. 2003). Researchers have found that fermentation time decreases if temperature keeps on increasing. If temperature is high, *S. cerevisiae* cells stop growing, and therefore, bioethanol yield is affected as higher temperature interferes with the amount of solvent and soluble substances needed for *S. cerevisiae* growth and results in the accumulation of toxins. Similarly, lower temperature also results in slow growth of the cells due to lower ethanol tolerance (Lin et al. 2012).

Table 3.1 Different types of FW and their yields after hydrolysis (Adapted from Yukesh Kannah et al. 2020)

S. No.	Substrate	Hydrolysis type	Hydrolyzing agent	Hydrolyzing condition	Hydrolysis yield	References
1.	Food waste	Mixed enzyme	Carbohydrase + protease	Temperature: 35 °C	Fermentable sugar: 0.63 g/g	Kim et al. (2011)
				Mixing: 150 rpm		
				pH: 4.5		
				Time: 12 h		
2.	Kitchen waste	Sequence mixed enzyme	α-Amylase	pH: 5.5	Fermentable sugar: 64.7 g/L	Uncu and Cekmecelioglu (2011)
				Time: 1 h		
				Temperature: 95 °C		
				Mixing: 150 rpm		
			Glucoamylase	pH: 5.5		
				Time: 6 h		
				Temperature: 60 °C		
				Mixing: 150 rpm		
3.	Food waste	Fungal mash	*Aspergillus awamori* and *Aspergillus oryzae*	pH: 4.0–4.5	Glucose yield: 143 g/L	Pleissner et al. (2014)
				Time: 24 h		
				Dosage: 8.5 g/100 g		
				FW		
				Temperature: 60 °C		

4.	Kitchen waste	Enzyme	Glucoamylase	pH: 5	Conversion efficiency: 79%	Hafid et al. (2015)
				Time: 6 h		
				Dosage: 85 U/mL	Fermentable sugar: 0.79 g/g	
				Temperature: 60 °C		
5.	Food waste	Fungal mash	*A. awamori* and *A. oryzae*	pH: 4.0–4.5	Glucose yield: 127 g/L	Uçkun Kiran and Liu (2015)
				Time: 24 h		
				Dosage: 7.7 g/L		
				Temperature: 60 °C		
6.	Kitchen waste	Enzyme	Glucoamylase	pH: 5	Conversion efficiency: 59.37%	Hafid et al. (2016)
				Time: 10 h		
				Dosage: 70 U/mL	Fermentable sugar: 62.62 g/L	
				Temperature: 60 °C		
7.	Food waste	Acid	Hydrochloric acid	Time: 1 h	Conversion efficiency: 42.4%	Hafid et al. (2017)
				Dosage: 1.5% (v/v)	Fermentable sugar: 50.5 g/L	
				Temperature: 90 °C		
8.	Food waste	Enzyme	Glucoamylase	pH: 5.0	Conversion efficiency: 50.6%	Hafid et al. (2017)
				Time: 6 h		

(continued)

Table 3.1 (continued)

S. No.	Substrate	Hydrolysis type	Hydrolyzing agent	Hydrolyzing condition	Hydrolysis yield	References
				Dosage: 85 U/mL	Fermentable sugar: 60.32 g/L	
				Temperature: 55–60 °C		
9.	Food waste	Sequential acid-enzyme hydrolysis	Hydrochloric acid + glucoamylase	Time: 1 h	Conversion efficiency: 86.8%	Hafid et al. (2017)
				Dosage: 1.5% (v/v)	Fermentable sugar: 103.4 g/L	
				Temperature: 90 °C		
				pH: 5.0		
				Time: 6 h		
				Dosage: 85 U/mL		
				Temperature: 55–60 °C		
10.	Soaking assisted and thermal pretreated cassava peel waste	Sequence mixed enzyme	α-Amylase	pH: 7	Conversion efficiency: 78.66%	Aruwajoye et al. (2019)
				Time: 60 min		
				Temperature: 90 °C		
			Amyloglucosidase	pH: 4.5	Fermentable sugar: 0.58 g/g	
				Time: 24 h		
				Temperature: 60 °C		
			Cellulase	pH: 5.5		
				Time: 120 min		
				Temperature: 55 °C		

(b) Feedstock concentration

Feedstocks are the essential components required for microbial growth during fermentation. If the concentration of feedstock is high, hydrolysis is fastens as more active sites of enzymes are available to bind with the substrate. Lower concentration of the substrate and fixed enzyme number lower bioethanol yield (Cheng et al. 2009). Lower binding of the substrate with the active site of enzyme results in little ethanol yield. Increasing the concentration of feedstock enhances the production of bioethanol. However, too long exposure of higher feedstock concentration can also alter bioethanol yield (Triwahyuni et al. 2015).

(c) pH

pH has an important role in fermentation. Higher bioethanol production has been observed if the medium is acidic with balanced pH. At a mildly acidic pH, the permeability of cells to certain nutrients is affected (Ritslaid et al. 2010). Studies have shown that *S. cerevisiae* prefers the pH range of 2.75–4.25 for its growth and endurance. For production of ethanol by fermentation, a pH range of 4.0–4.25 is the best. If the pH declines below 4, however, ethanol yield is not very much affected, but a longer incubation is needed. Ethanol yield declines if pH rises above 5 (Zabed et al. 2014).

(d) Fermentation time

Fermentation time influences microbial growth rate. If fermentation time is shorter than fermentation, it will not be effective as microbes will not be able to reach their maximum growth. Similarly, longer fermentation times also affect the growth of *S. cerevisiae* cells due to higher ethanol concentration in the broth. But lower temperature and longer fermentation times also result in lower ethanol production (Zabed et al. 2014). Agitation rate controls the entrance of nutrients into the yeast cells from the fermentation broth and therefore regulates expulsion of ethanol out from the cells into the broth. The higher the rate of agitation, the higher the yield of ethanol. It triggers sugar uptake and therefore elevated ethanol yield. In general, for yeast cells, the agitation rate is 150–200 rpm. The use of excessive agitation rate not only affects the metabolic activity of the yeast cells but also alters smooth ethanol production. The concentration of inoculum affects sugar yield and ethanol consumption (Laopaiboon et al. 2017). If the cell number increases from 1×10^4 to 1×10^7 cells/ml, higher yield of ethanol is obtained. However, if inoculum concentration reaches 10^7 or 10^8, no considerable difference appears on ethanol yield. Usually at higher concentration of inoculum, curtailment of the fermentation time is reported as during this stage, the yeast cells are multiplying rapidly (Zabed et al. 2016).

3.5 Conclusion

In the last few years, the amount of FW as well as FVW has undergone a tremendous increase, and their disposal has caused serious environmental threats. The common approaches utilized for the disposal of FVWs include incineration, feed for animals,

compost formation, or dumping in the landfills. But disposal of FVWs in the landfills has caused serious issues, for example, emission of GHGs, increase in the spread of disease-causing pathogens, and soil, water, as well as air pollution. Sustainable food management practices are needed to solve this issue, and one such option is valorization of FVWs into biofuels. Tremendous research has been done and still ongoing on the potential of FVWs for biofuel production. Researchers have found that FVWs can be transformed into biofuels, namely, bioethanol, biohydrogen, biodiesel, and biogas. FVWs are easily available, abundant, and rich in carbohydrates and are biodegradable; therefore, they can replace the fastly depleting fossil fuels. Hydrolysis of FVWs converts sugars into biofuels by the activity of microbes under anaerobic conditions. These biofuels can be used for cooking, electricity, and energy production. However, although biofuel production from FVW has a lot of advantages, still it has not reached the industrial and commercial scale of utilization, but researches are ongoing all over the globe.

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Chapter 4
From Fruit and Vegetable Waste to Biofuel Production: Part II

Navodita Maurice

Abstract Food waste (FW) disposal is one of the biggest challenges in the present era although many food management practices are being utilized ranging from conventional to nonconventional methods. The conventional methods of FW disposal include burning, landfilling, compositing, and preparation of animal feeds. Dumping of FWs into landfills and burning are not healthy approaches as they pollute the air, water, and soil. FWs are rich sources of carbohydrates, lipids, proteins, as well as lignin in varied proportions. Food valorization in an effective and sustainable manner can be done by transforming FWs into biofuels. FWs can be processed through multistep-chemocatalytic, chemo-enzymatic, and multistep-enzymatic reactions to produce biofuels. Researchers all over the globe are utilizing different types of FWs as substrates for the production of biofuels. Fruit and vegetable wastes (FVWs) are collected from markets, hotels, households, juice centers, etc., and are used as feedstocks for fermentation (anaerobic digestion) to produce biofuels. The commonly produced biofuels from FVWs are bioethanol, biohydrogen, biogas, biodiesel, and bio-oils.

Keywords Fruit and vegetable wastes (FVWs) · Biofuels · Biohydrogen · Biodiesel · Biogas

Abbreviations

CCW	Crude cheese whey
COD	Chemical oxygen demand
FSC	Food supply chain
FVW	Fruit and vegetable waste
FW	Food waste
FWS	Fruit waste slurry

N. Maurice (✉)
Prophyl Ltd., Mohács, Hungary

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GHG Greenhouse gas
HRT Hydraulic retention time
KVW Kitchen vegetable waste
OLR Organic loading rate
SM Swine manure
TS Total solid
VFA Volatile fatty acids
VS Volatile solids

4.1 Introduction

Food safety and proper waste management strategies are the major challenges of the twenty-first century as the majority of the food wastes (FW) are dumped in the landfills. The residues produced by the food supply chain (FSC) are rich in valuable nutrients, but their disposal is a threat to the environment (Gustavsson et al. 2011). FWs can be classified into two categories depending upon their origin: vegetable and animal wastes. Vegetable wastes originate from roots, cereals, pulses, fruits and vegetables, oil crops, and tubers, while animal wastes are derived from meat, fisheries and seafood, and dairy industries (Galanakis 2012). Both plant and animal wastes are rich in carbohydrates, lipids, bioactive compounds, and proteins (Kosseva 2009). Reduction of the amount of the degenerated FWs in order to extract valuable materials can increase the capacity of FSC and therefore can improve food security practices. Fruit and vegetable wastes (FVWs) can serve as alternatives in order to produce value-added products as they are cheap and easily available (Fig. 4.1). The food management practices use chemical or biological conversion of organic matter of the FWs into simpler molecules (H_2O, CO_2, CH_4, NH_3, H_2S, H_2, and CO). These food management practices sometimes are a threat to the environment as they release greenhouse gases (GHG) in the air, polluting the water as well as soil (Papargyropoulou et al. 2014).

Valorization methods appear to be advantageous over the conventional food management practices as they either reduce the waste amount by transforming the FWs into value-added products or incorporate the nutrients into the FSC. FW is the leftover of the food processing industries that is disposed of without being recycled. Food industry is disposing of an innumerous proportion of FWs every day, and according to the estimates, one-third of the food produced for human consumption is thrown away without being consumed (Gustavsson et al. 2013). In developed countries, FWs originate from the consumers who buy a lot of food items and discard them without eating. In developing nations, improper harvesting methods, storage places, and packing and processing methods also dispose of a lot of FWs (Zorya et al. 2011). FWs not only result in currency loss but they also affect the climate as food production uses a lot of materials, for example, seeds, water, energy, labor, fertilizers, and pesticides. The prohibition of FW and feeding the unexpired

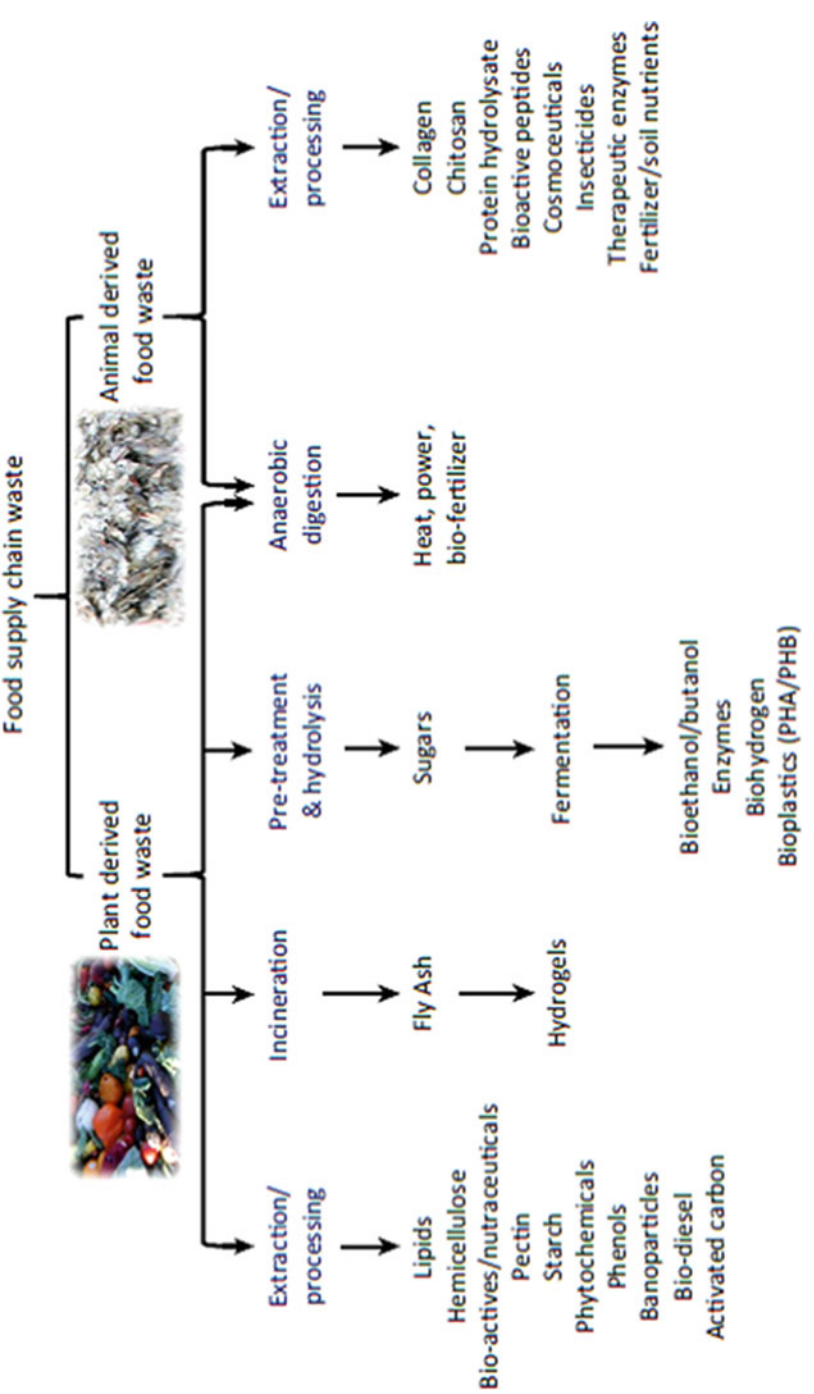

Fig. 4.1 Materials derived from the FSC. (Adapted from Ravindran and Jaiswal 2016)

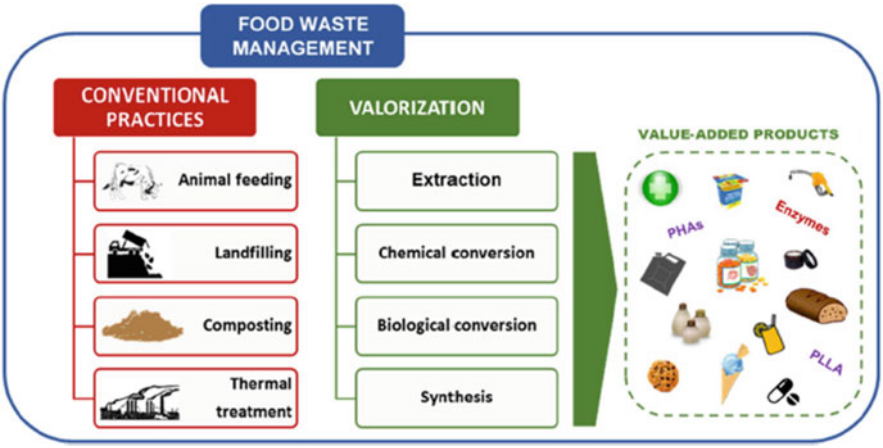

Fig. 4.2 FW management practices. (Adapted from Esparza et al. 2020)

Table 4.1 Advantages and disadvantages of FW transformation into liquid biofuels

	Advantages	Disadvantages	Comments
Conversion to liquid biofuels	• Sustainable utilization of food wastes. • An alternative to fossil fuel. • Energy security and less dependent on politically unstable middle east countries. • Space problems in metropolitan areas can be circumvanted. • Small biofuel plants can be operated near food parks.	• High operational cost. • No cost-effective methods are available so far; especially for mixed food waste. • Advanced and economical valorization methods need to be developed to deal with diverse nature of food wastes. • Most of the ongoing research are in preliminary stages.	• Most of the methods are chemical and biochemical processes. • Technology for mixed food waste valorization is rather complicated. • Catalytic cascade reactions can be tried as an alternative technology. • For extraction of lipids green solvents such as $scCO_2$ can be explored.

Adapted from Karmee (2016)

food to the hungry can solve food wastage to an extent (Lin et al. 2013). However, by 2050, food production is expected to rise to 60% in order to feed the population of the whole world. The protein and sugar content of the FWs can be broken down into easily fermentable sugars as well as amino acids (Pleissner et al. 2014). Microbes can utilize FWs as substrate and therefore can transform them into value-added products like biofuels, essential chemicals, enzymes, and materials (Zhang et al. 2013a, b; He et al. 2012). Transformation of FW biomass into biofuels can be used in the generation of electricity as well as in the preparation of animal feeds (Lin et al. 2013). Valorization of FWs into biofuels can deter human dependence on nonrenewable fossil fuels and crude oils. FWs can be transformed into biofuels, for instance, bioethanol, biohydrogen, biodiesel, bio-oil, and biogas (Tuck et al. 2012) (Fig. 4.2, Table 4.1).

4.2 Biohydrogen

Hydrogen has been accepted as a clear, sustainable, and encouraging fuel in the future. Hydrogen production from waste matter by biological methods is promising as it favors production of bioenergy. Production of biohydrogen is a microbe-governed process where bacteria participate in the production of hydrogen with the help of enzymes, for instance, nitrogenase and hydrogenase, either by photo or dark fermentation. Biohydrogen production can be divided into four grades: (1) water biophotolysis with algae or cyanobacteria in the presence of sunlight, (2) organic compound photodecomposition by photosynthetic bacteria and light, (3) hydrogen production by fermentation and transformation of feedstocks rich in carbohydrates along with essential products like alcohols and acids by the activity of anaerobic bacteria, and (4) combination of photo and dark fermentation (Kothari et al. 2012). Among these methods, dark fermentation appears to be a more promising technology that can be used at the commercial scale as no external light and energy supplementation are needed and it is rather cost-effective (Sreela et al. 2011). During dark fermentation, glucose is transformed into acetic acid as well as hydrogen in the 1:2 ratio. This method is also useful in the integration and practical application of not only biohydrogen production but can be implied in the microbial fuel cell preparations (Kim et al. 2011a). Majority of the studies on biohydrogen production using waste biomass have been conducted using the dark fermentation method (Yasin et al. 2011). The major ingredient of municipal solid waste is composed of FW (Sreela-or et al. 2011). FW consists of not only cooked food but also raw food that is disposed of before or during food processing. FW is rich in moisture content, salinity, and volatile solids, and disintegration of these components contaminates not only the soil but also the groundwater as well as emits GHGs (Lee and Chung 2010). According to the report by Zhang et al. (2007), FW is the major component of waste stream in the USA; similarly, the disposal of expired food items doubled in Malaysia in the last 3 years where 50% of the FW is derived from the kitchens. Bioenergy production from FW has gained much popularity in the last decade especially in the form of biohydrogen production (Yasin et al. 2011). Different types of wastes obtained from the food processing industries, for instance, cheese whey, tofu residue, apple pomace, rice slurry, etc., have been tried for the production of biohydrogen (Doi et al. 2010). Municipal solid waste (FVW), jackfruit peel, and wheat starch also have potential use for biohydrogen production. Since FWs are rich in cellulose, carbohydrates, hemicelluloses, and fats, different metabolic routes are followed for the production of biohydrogen whose details are not so clear yet (Vijayaraghavan et al. 2006). Production of biohydrogen from carbohydrate-rich substrates follows the route of acido- and acetogenesis, but these processes are susceptible to environmental factors like temperature, pH, hydrogen partial pressure, volatile fatty acids (VFA), food waste concentration, and inoculum source. The expanding demand of energy all over the world is a big challenge in the present era, and biohydrogen production from sustainable resources of energy like FVWs and wastes from food processing industries can serve as tools to conquer this

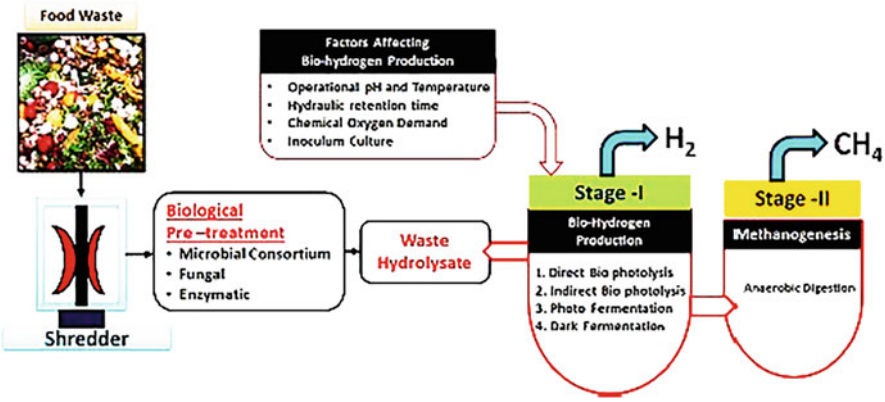

Fig. 4.3 Transformation of food waste into biohydrogen. (Adapted from Bhurat et al. 2020)

challenge and can also lessen the dependence on depleting fossil fuels. FWs and wastes from food processing industries were tested for the production of biohydrogen by Yasin et al. (2013), and they found a number of physiochemical parameters that affect biohydrogen production. Anaerobic decomposition of FW is appropriate for the production of energy as it has a higher feedstock concentration, high carbon-to-nitrogen (C/N) ratio, and good moisture content. For the production of biohydrogen with a properly designed and operational anaerobic digestion system, the physicochemical properties of FWs are crucial. The production and yield of biohydrogen are very much dependent upon the FW pretreatment, pH, low hydrogen partial pressure, and temperature (Kim et al. 2009). Other factors that affect the production of biohydrogen include nutrient content, volatile solid content, and particle size (Zhang et al. 2007) (Fig. 4.3). Researchers all over the world have investigated the effect of feedstock composition and varieties of wastes generated by cafés and their effect on biohydrogen production. Generally, to achieve a suitable concentration of feedstocks, the feedstock substrates are ground with water, which enhances decomposition and therefore affects biohydrogen yield. Ismail et al. (2009) found that controlling the chemical oxygen demand (COD) maximum biohydrogen yield can be obtained; however, Mohan et al. (2009) reported that for efficient production of biohydrogen by anaerobic fermentation, higher C/N ratios are important but C/N ratios higher than 20 decrease the yield. So FW C/N ratios must be between 20 and 21 for higher biohydrogen yields. Researchers have investigated the production of biohydrogen from FWs through different methods, for example, manure and compost in batch, mixed cultures from anaerobic sludge, and semicontinuous as well as continuous modes. Microbes present in FWs along with higher carbon content make them suitable to be used as a substrate for biohydrogen production by anaerobic fermentation. Till now, utilization of pure culture inoculum for biohydrogen production from FW has not been reported so far. Jo et al. (2007) isolated *Clostridium tyrobutyricum* JM1 (single strain) while testing the

biohydrogen yield of FW by anaerobic fermentation, while other researchers tried to achieve higher biohydrogen yields via microbial activity by mixing FW with sludge (Zhu et al. 2008) or usage of landfill matter so as to enhance the protein content and balance the C/N ratio (Mohan et al. 2009). The growth and metabolism of hydrogen-producing bacteria can be increased by the addition of sludge digested anaerobically (Kim and Lee 2010). Kim et al. (2011d) tested the effect of nonsterile FW on biohydrogen production without the addition of inoculum and found that yields were much lower in comparison to the FW pretreated with heat as heat treatment enhanced the activity of hydrogen-consuming bacteria. Elbeshbishy et al. (2011a, b) tested the effect of sonication of FW with heat treatment but without inoculum and found that heat treatment is a necessary factor in enhancing the yield of biohydrogen, while others tested the effect of protein, carbohydrate, cellulose, and lipid concentrations on biohydrogen production. Danko et al. (2008) tested the effect of cellulose, carbohydrate, lipid, and protein ratio when mixed with cabbage, chicken breast, potato flakes, and pork lard and observed that hydrogen yield can be affected by this ratio when granular sludge is added. Yasin et al. (2011) tested the effect of fiber, carbohydrate, and protein ratio by mixing restaurant waste and municipal solid FW with fish, vegetable, and rice and obtained good yields of biohydrogen.

Various types of waste products are released from food processing industries, for instance, cheese, cereals, tofu, potato peels, etc., along with many liters of wastewater rich in starch as well as sugars, and usage of these wastes for the production of biohydrogen can help these industries as they can use biohydrogen as a source of electricity (Van-Ginkel et al. 2005). Mixed fruit peels, pineapple wastes, fully ripened fruit peels, and apple pomaces have no market value, but they are rich in sugars and can serve as substrates for biohydrogen production. Kim et al. (2011a, b) obtained good biohydrogen yields from fully ripened fruit wastes and tofu-processing wastes where the latter needed acid and heat pretreatments in order to increase the content of soluble carbohydrates in the feedstock. Cheese whey wastes are rich in proteins, and cereal wastewaters are rich sources of sugar (glucose), and their utilization can also result in higher yields of biohydrogen (Castelló et al. 2009). *Clostridium saccharoperbutylacetonicum* singly can convert crude cheese whey waste into biohydrogen (Ferchichi et al. 2005). Fermentation carried out by clostridial cells can also produce biohydrogen. Agro-residues as well as wastewaters from food processing industries serve as suitable substrates for hydrogen production by clostridial fermentation, and this method appears to be not only cost-effective but also sustainable (Sivagurunathan and Lin 2019). Fruit wastes are biodegradable and are good sources of proteins, carbohydrates, minerals, and amino acids that are the key components of clostridial growth in order to conduct fermentation leading to hydrogen production (Abubackar et al. 2019). Fruit wastes are available throughout the year as a major proportion is wasted without being consumed due to several reasons, for example, poor harvesting, transport, and storage facilities (Sagar et al. 2018). Dumping of fruit wastes in the landfills is not suitable for the environment due to several side effects; therefore, fermentation of these wastes by clostridial fermentation not only solves the problem but also generates biohydrogen that is a renewable source of energy (Mandavgane et al. 2017). Very few clostridial species,

for instance, *C. pasteurianum* MTCC116 as well as a mixed culture of *C. baratii* and *C. perfringens*, have been investigated for their potential use for biohydrogen production by using fruit wastes. It is clear that fruit wastes can serve as a substrate for clostridial cells to conduct fermentation; therefore, there is a need for searching other strains to enhance biohydrogen production. *Clostridium* strain BOH3 produces saccharolytic enzymes (xylanase, amylase, and cellulase) naturally and therefore can ferment agro-residues (sesame oil cakes and rice bran) leading to butanol and hydrogen production (Turhal et al. 2019). Biohydrogen production from fruit waste fermentation by *Clostridium* strain BOH3 has been confirmed by Mahato et al. (2020). Wet digestion of fruit wastes (mixed fruits and melon wastes) by clostridial fermentation results in higher biohydrogen yields (Amekan et al. 2018). Suspending fruit waste in an anaerobic basal medium (ABM) when processed for sterilization by autoclaving in order to increase sugar content resulted in enhanced *Clostridium* strain BOH3 growth that in turn not only increased the rate of fermentation but also gave a higher yield of biohydrogen. Enhancing the concentration of fruit wastes up to a certain extent not only increases the total solid (TS) but also increases cell density of *Clostridium* strain BOH3 resulting in higher biohydrogen production; however, after this extent, a drop in cell growth and biohydrogen yield has been noticed. It can be attributed to the fact that increasing the concentration of total solids can cause stress as well as reduces the amount of water needed for the metabolic activity of the clostridial cells; therefore, cellular growth as well as hydrogen yield is affected (Cheng and Zhu 2016). *Clostridium* strain BOH3 after fermentation not only produces biohydrogen but also produces butyric and acetic acids. Studies have shown that *Clostridium* strain BOH3 also produces other saccharolytic enzymes (cellulase, amylase, xylanase, and pectinase) during the fermentation of mixed fruit wastes, which enhances not only biohydrogen production but also butyric acid, acetic acid, acetone, ethanol, and butanol production. Fruit waste slurry (FWS) when processed by direct fermentation (DF) results in higher biohydrogen yields in comparison to fruit waste hydrolysates collected from microwave irradiation (MWH) and autoclaving. FWS when hydrolyzed by enzyme cocktails (containing a mixture of cellulase, amylase, xylanase, and pectinase) is able to extract macro- and micronutrients from the substrates much better than other pretreatments. FWS processed by DF not only increases the growth of *Clostridium* strain BOH3 but also enhances the catalytic activity of [FeFe]-hydrogenase enzyme that produces hydrogen after the metabolic activity of clostridial cells. Studies indicate that supplementing the media with iron (Fe^{2+}) enhances the activity of hydrogenase that oxidizes reduced ferredoxin resulting in increased hydrogen production by *Clostridia* (Dada et al. 2013). FWS carried out for enzymatic hydrolysis releases a higher sugar content in the growth medium, favoring enhanced cellular growth of *Clostridium* strain BOH3 and therefore resulting in higher biohydrogen yield. Microbes grown in slurry fermentation can experience stress at a certain level, and therefore, their physiological nature is different from microbes grown in submerged fermentation. FWS processed for slurry fermentation when supplemented with macro- and micronutrients released from the enzymatic activity of *Clostridium* strain BOH3 results in higher biohydrogen yield in comparison to the DF of FWS

(Mthethwa et al. 2018). It has already been reported that the C/N ratio of feedstocks plays an important role in biohydrogen production by dark fermentation and biohydrogen production reduces when the C/N ratio of the feedstocks increases above 30 (Sethupathy et al. 2019). The C/N ratio of fruit waste below 30 favors the growth of *Clostridium* strain BOH3 and therefore also affects biohydrogen production. Till now, very few Clostridial strains (*C. paraputrificum* M-21, *C. pasteurianum* MTCC116, and *C. thermocellum* ATCC 27405) have been investigated for biohydrogen production from a mixed medium supplemented with fruit wastes, sugarcane bagasse, and chitinous wastes as feedstocks. It has been reported that *C. pasteurianum* MTCC116 strain is incapable of producing saccharolytic enzymes and therefore produces very little biohydrogen from fruit wastes. However, *C. thermocellum* ATCC 27405 and *C. paraputrificum* M-21 can excrete saccharolytic enzymes, namely, cellulase and chitinase, and therefore can produce good levels of biohydrogen. Clostridial strains ATCC 27405 and M-21, just like strain BOH3, are able to produce saccharolytic enzymes and therefore can result in biohydrogen production. The consolidated bioprocessing (CBP) of fruit wastes produces higher yields of biohydrogen than chitinous wastes and sugarcane bagasse; however, strain BOH3 gives high yield of biohydrogen from fruit and chitinous wastes processed by CBP. This suggests that *Clostridium* strain BOH3 can directly convert fruit waste into bioenergy that can be used on a commercial scale (Cheng and Zhu 2016).

Dark fermentation favors better biohydrogen yield as it can make use of sustainable substrates (agro-industrial wastes and wastewaters). Majority of the studies have been conducted by mono-digestion of vinasses, FWs, soybean oil extraction residues, water hyacinth, wastewater from cheese processing industries, and mushroom wastes. The results obtained from these studies have shown that biohydrogen yield can be decreased or stopped due to the microbial communities consuming hydrogen or producing methane or due to nutrient imbalance or deficient buffer capacity of the wastewaters and FWs (Chuang et al. 2011). The solution to this problem is co-digestion of the wastes, which can replenish the media with the deficient nutrients and can result in complete fermentation. An effective C/N ratio can improve the buffer capacity, and also co-digestion inhibits the effect of negative factors that affect biohydrogen yield (Wang et al. 2011). Advances have been made in the co-digestion methods for effective production of biohydrogen, for example, utilization of cow manure-waste milk, kitchen wastewaters, municipal food wastes, waste glycerol sludge, cassava stillage sludge, food waste sewage sludge, pressed mud sewage, and rice straw sewage sludge. Crude cheese whey (CCW) (liquid waste) is produced from cheese processing industries and is rich in proteins, lactose, and other essential nutrients. Similarly, FVWs are produced from markets as well as industries, which are suitable sustainable feedstocks (Prazeres et al. 2012). Both CCW and FVW being rich in organic as well as biodegradable compounds can serve as sources of substrates for co-digestion for biohydrogen production. Since both of them are produced in bulk and are easily available, they are cheap feedstocks, and utilization of nonsterilized feedstocks modifies the inoculated microflora. Studies have reported that mixing a small fraction of feedstocks during co-digestion modifies

bacterial populations; however, more studies are needed to strengthen this fact (Wang et al. 2013a, b, c). Gomez-Romero et al. (2014) tested the biohydrogen producing efficiency of CCW and FVW by co-digestion by studying the effects of microbial distribution, C/N ratios, and co-substrates. They found that CCW as a lone substrate showed lower biohydrogen yield and lower C/N ratio resulted in a second lag phase. This can be due to change in the growth conditions after the initial phase of biohydrogen production (Kim et al. 2009). Increase in the acidity of the medium also affects the glycolytic enzymes of the microbes and therefore inhibits microbial growth, which in turn affects biohydrogen yield. CCW has lower buffer capacity that can also increase the acidity of the medium resulting in enzymatic activity inhibition (Perna et al. 2012). It has also been reported that utilization of sludge as the only feedstock can result in lower biohydrogen yields due to its highly proteinaceous nature (Zhu et al. 2008). Supplementing CCW with FVW enhances biohydrogen production in comparison to FVW or CCW alone, suggesting that the absence of proteins in the FVW is an essential factor for enhanced biohydrogen yield. Higher C/N ratios can result from higher acidity of the medium that can alter biohydrogen production (Mohanakrishna et al. 2010). Enhancing FVW concentration while mixing with CCW increases the lag phase of biohydrogen generation as more cellulosic material is available to the microbes for hydrolysis. Cellulose hydrolysis can become a limiting factor in some anaerobic digestion methods. Zhu et al. (2008) reported enhanced biohydrogen yield when municipal food waste was mixed with sewage sludge. Tenca et al. (2011) obtained higher volumetric hydrogen production rate (VHPR) when FVW was mixed with swine manure (SM), suggesting that the alkalinity of the medium and the ratio of total VFA and alkalinity have a correlation with substrate concentration and biohydrogen yield. Lower C/N ratios also affect the yield of biohydrogen as higher protein content of the medium inhibits the yield, suggesting a need for a cellulosic material. Radjaram and Saravanane (2011) obtained a higher yield of biohydrogen by co-digestion of sewage and pressed mud in an up flow anaerobic sludge blanket (UASB) reactor. Differences in the yield of biohydrogen can be due to properties of substrates, populations of microbes, pH conditions, pretreatments, and conditions of anaerobic co-digestion (Gomez-Romero et al. 2014). Studies suggest that the initial utilization of easily degradable feedstocks allows faster metabolic activity of the microbes in comparison to complex substrates that can inhibit biohydrogen yield. The hydrolytic enzymes (cellulases, amylases, lipases, and proteases) produced by microbes degrade and solubilize the complex macromolecules into sugars, glycerol, long-chain fatty acids, and amino acids to enhance cellular transport (Parawira et al. 2005). Usually in the early phases of fermentation, an increase in lactic and acetic acids has been observed, but as the concentration of butyric acid increases, their concentration decreases. This suggests that during the early hours of CWW fermentation, lactic acid is produced as the end product of lactose; similarly, for FVWs, acetate is the end product of fermentation. Lactic acid and acetate are utilized by *Clostridium* for triggering its metabolic activities in order to produce biohydrogen and butyrate. The assimilation of easily digestible carbohydrates initiates cellular growth by producing lactate and acetate as they are needed for ATP production (acetate-butyrate) and redox balance (lactate).

Acetate and lactate along with the utilization of complex substrates present in CCW and FVW in turn enhance the production of biohydrogen. When the concentration of butyrate and acetate reaches maximum, higher biohydrogen yields are obtained, suggesting a substrate competition between the hydrogen-producing and lactic acid bacterial species (Bando et al. 2013). When CCW is converted into lactic acid by mixed inoculum and when fruit kitchen wastes are fermented to lactate and then degraded by *Clostridium thermolacticum*, it resulted in higher biohydrogen yields (Azbar et al. 2009). *Lactobacillus bifermentans* and *Clostridium tyrobutyricum* can utilize acetate and lactate, suggesting that increase in biohydrogen production and butyrate levels is related to acetate and lactate consumption. Biohydrogen generation from lactate requires acetate as without acetate, microbial cells must produce acetate first by their metabolic reactions (Grause et al. 2012).

Several organic substrates, namely, starch, carbohydrates, potato-processing wastewaters, sugar beets, and brewery and cheese whey wastes, have been tested for biohydrogen generation by acidogenic fermentation under different conditions (thermophilic, hyperthermophilic, and mesophilic), and variations in yields have been observed (Venetsaneas et al. 2009). Fermentation equilibrium can be interrupted by a variety of operating and biochemical parameters. When biodegradable feedstocks are used, the production of VFA by acidogenic bacteria can alter biohydrogen production by changing buffer capacity and pH, which play an important role in the metabolic pathways of microbes. The optimal pH for fermentation lies between 5 and 6 as it avoids solventogenesis as well as methanogenesis and in turn enhances biohydrogen production. pH values less than 4.5 severely affect hydrogenase enzyme activity directing the metabolic pathways of microbes in the direction of other pathways; similarly, weak or neutral pH also directs microbial pathway in the direction of homoacetogenesis and methanogenesis when hydrogen is consumed (Wu et al. 2010). Therefore, optimal pH ranges are maintained by the exogenous administration of acids. Livestock manure is rich in nutrients and alkali resources necessary for cellular growth, and therefore, it appears to be an ideal feedstock for the fermentation process when mixed with easily degradable carbohydrates. Livestock waste can serve as a sustainable feedstock for biohydrogen generation in the animal industry areas. Pig slurries can also serve as a source of renewable feedstocks suitable for biohydrogen production as they are abundantly available in pig farms, but very little studies have been conducted with this feedstock. Utilization of pig slurry as the only substrate results in lower biohydrogen yields under hyperthermophilic and mesophilic conditions (Wagner et al. 2009). Higher biohydrogen yields were obtained when swine manure was mixed with glucose (an easily hydrolyzable substance). Management of FVWs can increase biohydrogen yields in being cheap, easily available, and rich in sugar content. Batch experiments executed under mesophilic temperatures with different types of FVWs, namely, composite mixture of vegetables, cabbage-carrot pulp, lettuce leftovers, potato peels, jackfruit peels, and sweet lime peels, resulted in higher biohydrogen yields (Zhu et al. 2009a, b). Tenca et al. (2011) investigated the effect of swine manure (co-substrate) on biohydrogen yield under thermophilic conditions by supplementing it with FVWs. They tried to maximize the yield of biohydrogen by

stabilizing the manure's buffer capacity without the addition of any exogenous alkali and found very little or no biohydrogen yield. They observed that swine manure when mixed with a poor carbohydrate material resulted in higher methane yield rather than biohydrogen as the growth of methanogenic bacteria was increased. Higher proportion of FVW than swine manure also resulted in lower biohydrogen yield as the pH of the medium dropped drastically. FVW (40%) when mixed with swine manure (60%) resulted in higher biohydrogen production, suggesting that there was an optimal harmony between the carbohydrate fraction of FVW and alkali ratio of swine manure. Majority of the studies conducted on biohydrogen production by dark fermentation have focused on the influence of substrate type and concentration or temperature (Khan et al. 2018). In the last few years, the effect of trace elements of biohydrogen generation has been conducted, and it has been reported that trace elements can affect biohydrogen yields (Yang and Shen 2006). Every trace element plays an important role in the metabolic activity of microbes and thereby affects biohydrogen production; for instance, Fe^{+2} and Ni^{+2} are the essential parts of hydrogenases (active site). Hydrogenases can be categorized into [Ni-Fe], [Ni-Fe-Se], or [Fe]. Similarly, Mg^{+2} participates as ATP transport and is also the key component of various enzymes. Zn^{+2} and Mn^{+2} are also important as they affect the survival and growth of cells and thereby affect the production of biohydrogen (Mohan and Srikanth 2012). Optimization studies by statistical, classical, and mathematical methods can determine the requirement of trace elements needed during fermentation. Every method has pros and cons where classical ones are expensive and take a lot of time while statistical ones are efficient, effective, and economical. Performing ANOVA methods can optimize biohydrogen fermentation (Singh et al. 2017). Keskin et al. (2018) studied for the first time the effect of 11 trace elements (Ni, Zn, Fe, B, Mn, Mo, Cu, Se, W, Al, and Co) on biohydrogen yield of FVWs. The FVWs were composed of parsleys, cucumbers, lettuces, zucchinis, lemons, portulacas, watercress, dill, green peppers, potatoes, cabbages, green beans, tomatoes, cauliflowers, eggplants, and red peppers. They determined the organic matter content as well as the COD of these FVWs and found that all of them are biodegradable with lower Al and Na levels. They determined biohydrogen yield as biochemical hydrogen potential (BHP) and reported that FVWs can be used as suitable substrates for energy production as higher biohydrogen yields were obtained. Lin and Cheng (2006) obtained good biohydrogen yields when xylose was used as a substrate in the batch reactors. Okamoto et al. (2000) also got similar results when cabbage and carrot wastes were used in the batch reactor fermentation studies. Mohan et al. (2009) obtained higher biohydrogen yields when they tested mixed vegetable wastes. Similar results were obtained by Dong et al. (2011) for potato and lettuce wastes and starch. Biohydrogen level directly affects microbial diversity in the inoculum; for example, under thermophilic conditions, the yield as well as microbial diversity is higher. VFA (butyric acid) concentration also affects the yield of biohydrogen; for example, an increase in butyric acid concentration decreases biohydrogen yield. Addition of trace elements brings a significant change in the production of biohydrogen by showing a two to three times increase. Not only the concentration of trace elements but their type also affects biohydrogen

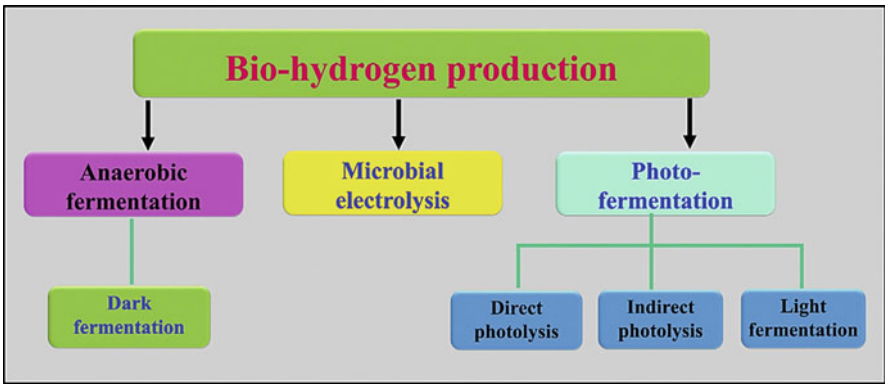

Fig. 4.4 Biological processes involved in biohydrogen production. (Adapted from Saratale et al. 2019)

production, and most of the substrate-related optimization studies have been done with trace elements, namely, Zn, Cu, Fe, and Ni. Alshiyab et al. (2008) investigated the influence of trace elements, namely, Cu, Fe, and Mg, independently on the production of biohydrogen by *Clostridium* in a closed-loop reactor and obtained significant yields; however, yields were higher when Zn and Cu were used as trace elements with glucose as a substrate. Yang and Shen (2006) suggested that when starch was mixed with Fe, considerable yields were obtained. Trace elements Zn, Co, Ni, and Fe significantly affect biohydrogen yield when FVWs are used as substrates. Photofermentation, biophotolysis, and dark fermentation are cost-effective and eco-friendly methods. Anaerobic or dark fermentation appears to be an encouraging method for biohydrogen production as it results in higher yields, requires less energy, is highly feasible, and can use renewable feedstocks (wastes and wastewaters) (Argun et al. 2017) (Fig. 4.4).

Glucose by dark fermentation produces hydrogen either by butyrate or acetate pathways. A number of factors affect biohydrogen production, for instance, inoculum pretreatment, temperature, reactor configuration, and pH (Kanchanasutaa et al. 2016). Archaea and other bacterial domains are the champions in hydrogen production due to their thermophilic nature, which has several benefits like higher rate of biohydrogen production, lower media viscosity, enhanced hydrolysis of substrates, and less contamination level (Pradhan et al. 2015). Bacteria belonging to the order Thermotogales are ideal for industrial biotransformation of waste materials as they have higher biohydrogen yields and can efficiently ferment carbohydrate-loaded substrates (Cappelletti et al. 2012). The feedstock utilization, density of biomass, as well as biohydrogen yield of *Thermotoga neapolitana* is higher on pure carbohydrate substrates than *Miscanthus* hydrolysate. This can be due to the fact that addition of exogenous nutrients to the medium can bring out efficient fermentation. Biohydrogen yields of *T. neapolitana* from carrot pulp hydrolysate were also high (de Vrije et al. 2010). In the last few years, *T. maritima* has also been reported to have the potential of producing biohydrogen from a variety of carbohydrate

substrates as this species excretes a series of hydrolytic enzymes, namely, invertase, xylanase, and cellulase, that are not only thermostable but also hydrolyze carbohydrate polymeric chain into simple monomers (Boileau et al. 2016). Saidi et al. (2018) investigated the effect of *T. maritima* culture of biohydrogen production from FVWs by addition of seawater as a source of inorganic elements and obtained significantly higher yields. Studies have reported that the growth of *T. maritima* depends on yeast extract, glucose, dissolved hydrogen concentration, as well as thiosulfate, and it can utilize both simple carbohydrates and complex polysaccharides (Boileau et al. 2016). The end products of *T. maritima* fermentation are hydrogen, acetate, carbon dioxide, and traces of lactic acid. *T. maritima* gives higher yield of biohydrogen in comparison to *T. neapolitana* when grown on carrot pulp in the bioreactor as the latter fails to grow. *T. maritima* can lower the redox potential of the growth medium as long as glucose source is available (Lakhal et al. 2011). Photofermentation is carried out by photosynthetic microbes that demand light as a source of energy for the disintegration of simple organic materials (monosaccharides or VFAs) to biohydrogen (Escamilla-Alvarado et al. 2012). However, dark fermentation produces biohydrogen by the degradation of complex organic molecules into hydrogen, volatile organic acids, simple sugars and alcohols, so it is more preferred over photofermentation as it does not require light. Hydrogen is the end product of acido- and acetogenesis steps of dark fermentation where anaerobic bacteria (*Clostridium* spp., *Ruminococcus* spp., *Thermoanaerobacterium* spp., *Enterobacter* spp., or *Bacillus* spp.) are involved (Zagrodnik and Laniecki 2015). For dark fermentation, substrates (FVW or agri-residues) derived from plants are preferred as their major component is lignocellulose and the pretreatment step eases its degradation (Sindhu et al. 2016). An excellent substrate must be rich in carbohydrate content, renewable, easily available, and cheap and must need little pretreatment. Such ideal substrates are FVW, sugar beet pulp (SBP), and corn silage (CS). CS is a suitable substrate for biohydrogen production but is susceptible to molds (*Aspergillus*, *Penicillium*, *Gibberella*, and *Fusarium*) on exposure to air (Łukajtis et al. 2018). Cieciura-Włoch et al. (2020) studied dark fermentation of FVW, CS, and SBP under different conditions resulting in biohydrogen production and reported an increase in the population of methanogens during fermentation. Wang et al. (2011) reported that lower organic loading rate (OLR) supports biohydrogen and methane production during dark fermentation even if pH is low. Methanogens use hydrogen for the production of methane, while acetic acid bacteria (Acetobacteraceae) generate acetic acid from sugars without hydrogen production. Batch testing carried out with higher concentration of inoculum along with FVW, kitchen wastes (KW), and plant and animal fats produces higher biohydrogen yield as a variety of substrates are available to microbes. KW also serves as a suitable substrate as it is composed of fruits, vegetables, plant and animal fats, fish, etc. Biohydrogen yields from CS are, however, lower than FVW and SBP (Cieciura-Włoch and Borowski 2019). Vijayaraghavan and Desa (2006) tested the biohydrogen producing efficiency of wastewaters containing palm oil mill effluents as well as brewery wastewaters and obtained higher yields from the latter. Rice winery wastewater also produces higher biohydrogen yield due to higher starch content. Biohydrogen generation by

C. thermolacticum from dairy waste (Collet et al. 2004) and food waste co-digestion with sewage (Kim et al. 2004) was significantly higher. Longer hydraulic retention time (HRT) and addition of nutrients to the medium favor higher biohydrogen production (Van-Ginkel et al. 2005). Biohydrogen yields obtained with glucose are highest in comparison to sucrose and xylose (Lin and Cheng 2006). Jackfruit peel wastes (Vijayaraghavan et al. 2006) and palm oil mill effluents (Vijayaraghavan and Desa 2006) processed by anaerobic fermentation resulted in considerably higher biohydrogen yields.

Hydrogen at present is produced from fossil fuels by chemical methods, gasification, or photo/dark fermentation (Trane et al. 2012). Hydrogen production by thermochemical treatment of wastes can be an alternative for replacing fossil fuels but has some limitations (Balat 2010). Pyrolysis brings out solid biomass decomposition at very high temperatures. A huge variety of wastes, for example, olive husks, cassava plantation residues, rice husks, forest biomass, and agri-residues, have been degraded by pyrolysis by using different catalysts (K_2CO_3, ZSM-5, dolomite, and commercial $NiMo/Al_2O_3$) (Bakar and Titiloye 2013). Kitchen vegetable waste (KVW) in the presence of silica gel and silica sand as catalyst when processed for thermogravimetric analysis (TGA) showed that biohydrogen yield is directly related to the pyrolysis time, suggesting that greater the pyrolysis time, the greater the yield (Agarwal et al. 2013). Qinglan et al. (2010) reported that hydrogen yield is also related to pyrolysis temperature as the higher the temperature, the higher the yield. KVW pyrolysis catalyzed by silica gel resulted in significant biohydrogen yield as the yield was directly proportional to the pyrolysis temperature. Chen et al. (2003) also observed similar results when sawdust and rice straw pyrolysis was conducted with Cr_2O_3 as a catalyst, indicating that catalyst can affect the yield. Researchers have suggested that co-digesting the sterilized organic waste with inoculum results in better yields as a balanced C/N ratio is obtained and good synergistic association is developed within the microbes. Co-digestion not only regulates the amount of volatile organic acids in the medium but also limits gas production during biohydrogen generation. Higher butyric acid concentration limits the growth of many hydrogen-producing bacterial strains (Angeriz-Campoy et al. 2015). Facultative and obligate anaerobic bacteria inoculated on organic wastes can improve biohydrogen yields as these bacteria can hydrolyze complex sugars into monomers on which hydrogen-producing bacteria can act easily (Mthethwa et al. 2019). Acid pretreatment can, however, kill hydrogen-producing bacteria and therefore can affect the total yield (Prabakar et al. 2019). Hernández et al. (2014) tested coffee mucilage mixed with pig manure for biohydrogen production and obtained higher yields along with other products like acetic and butyric acids. Cano (2015) investigated biohydrogen production from urban organic wastes and obtained considerably higher yields. Cárdenas et al. (2019) crushed FVW and mixed them with fresh coffee mucilage and transferred it to a bioreactor along with some agricultural lime and obtained significantly higher yields. Biohydrogen production was found to improve when FW was mixed with organic municipal solid waste (Angeriz-Campoy et al. 2015). Tawfik and El-Qelish (2014) reported that biohydrogen yields are doubled by the co-digestion process. Hydrogen yield from the municipal organic

solid waste can undergo declination due to increased ammonium concentrations. pH is an important factor in biohydrogen production by fermentation process as lower pH can damage microbial plasma membrane and can inhibit hydrogenase enzyme activity.

4.2.1 Factors Influencing Biohydrogen Production

Successful biohydrogen production is dependent upon the environmental conditions as when optimal conditions are available, hydrogen-producing bacteria achieve maximum growth, which in turn increases the yield of biohydrogen (Kim et al. 2008). A number of physiochemical factors affect biohydrogen yield.

(a) Pretreatment

Hydrogen-producing bacteria can reach their maximum growth only if the population of hydrogen-consuming bacteria is suppressed as it will lead to higher biohydrogen yield. These hydrogen-producing bacteria can thrive well in extreme conditions of excessive heat or pH (Valdez-Vazquez and Poggi-Varaldo 2009). Mixed inoculum contains not only hydrogen-producing bacteria but also methane- and acid-producing bacterial strains, and therefore, pretreatment is necessary in order to eliminate hydrogen-consuming bacterial strains (Kim et al. 2009). Yasin et al. 2011 reported higher populations of *Clostridium* sp. and *Caloramator australicus* after heat treating the FW. Without pretreatment, populations of methane and acid-producing bacteria have been reported to increase. Heat pretreatment either by autoclaving or boiling is a cheap and simple method in comparison to chemical treatments (Danko et al. 2008). Argun and Kargi (2009) reported that enhancing the time period of heat treatment efficiently removes methane- and acid-producing bacterial strains.

(b) pH

pH also influences the production of biohydrogen from FW by affecting metabolic pathways, enzymatic activity, and biohydrogen generation (Zhu et al. 2009a, b). pH is crucial as it can limit the growth of methane-producing bacteria (Pan et al. 2019). Methanogens and acetogens are active at a pH range of 6.3–7.8, which is not apt for hydrogen-producing bacteria. These hydrogen-producing bacteria require a pH range of 5–6 in order to grow, and if pH is higher than this range, their growth is suppressed resulting in lower biohydrogen production (Kim et al. 2011d). Yasin et al. (2011) obtained higher biohydrogen yields at a pH of 7.0. Lower pH ranges not only suppress the growth of hydrogen-producing bacteria but also affect degradation of substrates. Many studies have suggested controlling the initial pH at 5–6 if chemical pretreatment is done, but neutral pH has been proved to be best for biohydrogen yield. Kim et al. (2011c, e) reported that there was no biohydrogen production at a pH below 4 and above 8. Very low pH (acidic) can disrupt the cell equilibrium, and

majority of the energy produced by the bacterial strains will be spent in neutralizing the pH instead of biohydrogen production (Zong et al. 2009). The absence of active ATP can affect hydrogenases as well as iron-containing enzymes of hydrogen-producing bacteria. pH higher than 8.5 also affects biohydrogen yield as lag phase becomes longer than normal. Biohydrogen yields have been reported to be higher in the batch fermentation systems in comparison to the continuous systems as the former requires short time intervals for the completion of fermentation process. Biohydrogen yield can also be increased by mixing the inoculum with buffers and alkalis (potassium hydroxide, calcium carbonate, and sodium hydroxide) (Maranon et al. 2012).

(c) Temperature

Biohydrogen yield from FVW and industrial processing wastes is very much influenced by temperature. Maintenance of mesophilic conditions is easy to control at the industrial level as in the majority of cases, production of biohydrogen occurs under these conditions. Mesophilic temperature (30–37 °C) can directly transform FW into biohydrogen. However, thermophilic conditions also result in better biohydrogen yields; for example, Kargi et al. (2012) observed that biohydrogen yield from CCW was higher at thermophilic temperature than at mesophilic temperature due to the production of lactic acid that suppressed the populations of hydrogen-producing bacteria. Chu et al. (2008) reported that mesophilic temperatures are rather suitable for methanogens. Pretreatment by heat or maintenance of thermophilic temperatures can suppress acid-producing bacteria and can enhance the growth of hydrogen-producing bacteria, thereby increasing biohydrogen yield.

(d) Volatile Fatty Acids

Anaerobic degradation of FWs generates end products like butyric acid, acetic acid, propionic acid, and lactic acid. Generally, biohydrogen production is associated with acetic and butyric acid production. If the end products are propionic and lactic acids, no biohydrogen yield has been noticed (Kim et al. 2008). Failure of hydrogen fermentation of FW can enhance the growth of lactic acid bacteria. Heat or pH shock or chemical pretreatments can suppress lactic acid as well as hydrogen-consuming bacteria (Kim et al. 2009). Under extreme environmental conditions, germination of hydrogen-producing-bacteria occurs, while hydrogen-consuming bacteria fail to survive. Fermentation of FWs and food processing wastes under controlled environmental conditions can produce butyrate that inhibits production of lactic and propionic acids (Valdez-Vazquez et al. 2009).

4.3 Biodiesel

Biofuels being eco-friendly and renewable have gained much attention in the last decade as they can replace fossil fuels (Murugesan et al. 2009). They are sometimes referred to as green energy sources. Green energy can be defined as the form of

energy without any environmental threat and is renewable. It can be implied for both industrial and non-industrial processes. Green energy is a promise of the future in being sustainable and stable. Transport industry is the largest producer of GHGs, and this can be minimized by the use of biodiesel. Burning of the conventional fossil fuels releases harmful particulate matter that is not only dangerous to the atmosphere but also harmful to human health, and utilization of biodiesel as a fuel can minimize this risk (Panwar et al. 2011). Biodiesel is either composed of a mono alkyl or ethyl or methyl ester of long-chain fatty acids obtained from sustainable lipids (animal fats or vegetable oils) and can be used as an alternative for conventional fuels to drive diesel engines (Canakci 2007). Vegetable oils can be transformed into fuels for compression-ignition (CI) engines by blending, thermal cracking, micro-emulsification, and transesterification methods. At a commercial scale, the production of biodiesel occurs by vegetable oil transesterification with alcohol. The commonly utilized alcohol is ethanol or methanol as they can easily be generated from biomass and do not cause erosion of the engine (Choudhury and Bose 2008). The sustainable raw components of biodiesel are straight vegetable oils (SVOs) (both evitable and inevitable), waste oils, animal fats/oils, edible oil, and dairy by-products, as well as saturated and unsaturated fatty acids. Vegetable oils are more preferred as they are renewable, easily available, abundant, and cheap as more than 350 species of plants have been known to produce oil. Oil-yielding crops are perennial (some are annual) and can be cultivated in hilly areas as well. The oil-yielding potential of peanut, rapeseed, soybean, olive, sunflower seed, linseed, and palm is well known, but now, there is a shift from annual to perennial species (*Jatropha*, palm) (Bart et al. 2010). At present, biodiesel is produced mainly from soybean oil (SBO), sunflower seed oil (SNO), palm oil (PMO), and rapeseed oil (RSO) along with new high oleic sunflower oil (HOSNO). Other sources of biodiesel from plants include soybean oil, canola oil, palm oil, rapeseed oil, and sunflower oil, while animal sources are sheep tallow, beef, and cooking and poultry oils. Biodiesel production has also been tried with coconut, copra, andiroba (*Carapa guianensis*), groundnut, fish oil, microalgae (*Chlorella vulgaris*), *Jatropha curcas*, almond, camelina (*Camelina sativa*), etc. (Pinto et al. 2005). Vegetable oils in being sustainable have attracted the attention of researchers all over the world with the energy content similar to diesel. Biolipids, for example, waste vegetable oils, virgin vegetable oil feedstocks (microalgae, sunflower, mustard, palm oil), non-edible oils (Jatropha, castor, and neem oils), and animal fats can also be used for biodiesel production (Sharma and Singh 2008). Different developing countries are using different oil-yielding crops for biodiesel production (Srivastava and Verma 2008). Apart from plant and animal sources, some algal strains have also shown the potential of producing biodiesel. Algae can grow in the presence of sunlight and is rich in all essential nutrients (lipids, nucleic acids, carbohydrates, and proteins). Some algal strains have a biomass composed of 40% fatty acids. Some studies have shown that the yield of algal oil is 200 times more in comparison to vegetable oils. Microalgae grow very fast in the presence of sunlight and complete their cycle within a few days; however, oil production varies between species. Some produce about 50% oil by weights. However, algal cultivation for biodiesel production has

Fig. 4.5 Biodiesel production from food wastes. (Adapted from Karmee 2017)

not reached the commercial level, but researchers are working on this issue. Some new varieties of mustard have been discovered that are effective to be used as biodiesel as well as pesticide (Demirbas 2008). Biofuels like biomethanol, biodiesel, biohydrogen, and bioethanol have appeared to be promising for the future generations. A substitute for fossil fuels is fatty acid methyl esters (FAME) (Shrirame et al. 2011). Currently, about 35% of the global energy need is fulfilled by petroleum, but with the expanding population, petroleum deposits are depleting at an alarming rate, which calls the need for sustainable energy sources like biodiesel. The chemical modification of straight vegetable oils (SVO) by transesterification produces biodiesel. Transesterification is the conversion of ethanol or methanol mixed with SVO to fatty acid esters as well as glycerol under the influence of a catalyst (Panwar and Shrirame 2009). At the industrial level, transesterification of animal fats and vegetable oils generates FAME (biodiesel) (Fig. 4.5).

Everyday growing human population as well as industrial growth has increased the need for fats and vegetable oils, and therefore, the production of oil from alternate vegetables has become mandatory, for example, citrus plants. Citrus (Rutaceae) has about 1300 known species and is one of the most important crops grown all over the globe with many benefits. Citrus fruit waste includes seeds, fibers, and peels, and all can be used for the production of value-added by-products. Citrus seeds account for 40–80% oil by weight (Bull and Obunwo 2014). Sarno and Ponticorvo (2020) investigated an electrocatalytic approach composed of a nanocatalyst (Pt, Ir, and Ru trimetallic alloy (PtIrRu)) for biodiesel production from lemon seed wastes. They simply used an electrocatalytic method with a methanolic reaction mixture (aqueous solution) by applying mild voltage and tested the influence of temperature, voltage, methanol/oil ratio, water content, reaction time, and NaCl content on the yield of methyl esters by stability tests. They found temperature has a minute effect on methyl ester conversion rate. They obtained ~90% yield of biodiesel from lemon seeds with PtIrRu as a catalyst at 20 V. Application of higher voltages decreases the yield as other reactions (saponification and hydrogenation) can interfere with the process (Larichev 2008). Increases in the yield of FAME have been observed at a voltage of 5–20 V as increased voltage increases the release of hydroxyl ions that react at methanol surface and thereby promote transesterification. NaCl also promotes increased yield by increasing

methanol/oil molar ratio, but too high NaCl concentration can result in decreased FAME yield (Lotero et al. 2005). FAME yield also increases if electrolysis time is increased. Biodiesel obtained from lemon seed oils is composed of α-linolenic methyl ester. It has been an established fact that biodiesel is a biodegradable, nontoxic, and oxygenated fuel that has the capacity to replace petroleum fuels, but its production by chemical methods has some side effects as these methods are corrosive and toxic (Teo et al. 2014). Homogeneous catalyst increases the price of wastewater produced from reaction mixture (Abdullah et al. 2017), while heterogeneous solid catalyst has some advantages as it is insoluble in esters and therefore can be reused (Wang et al. 2013a, b, c). Green chemistry has emphasized the use of reusable and eco-friendly heterogeneous catalysts, for example, sulfonated carbons and sulfated zirconia (Nakajima and Hara 2012). The utilization of waste matter for the production of alkali and acid catalysts with activated carbon has gained much attention, and researchers have used different types of waste materials (wood ash, coconut shell, palm shell) for this purpose (Konwar et al. 2014). Although the utilization of activated carbon as catalyst offers many advantages as it has a large surface area and pores for effective catalysis, the production of this catalyst requires high wasteful chemical reaction and carbonization temperatures, and therefore, heterogeneous catalysts using CaO are rather more popular as they can be easily synthesized from wastes (waste crab shells, eggshells) (Shankar and Jambulingam 2017). Transesterification in the presence of heterogeneous catalysts results in catalyst deactivation with the passage of time resulting in sintering, poisoning, leaching, and coking. Heterogeneous catalysts must be hydrophobic in order to trigger adsorption of triglycerides and therefore must abstain catalytic site deactivation by polar by-product (water and glycerol) adsorption, and the use of metals as catalyst can solve this problem (Osman et al. 2017). The production of eco-friendly and nontoxic catalysts by Knoevenagel condensation (method for C–C bond formation) by using mesoporous zirconia as well as ion exchange resins has gained much attention (Dewan et al. 2018). Papaya plants are grown in many tropical and subtropical countries, and the fruits are a rich source of vitamins, minerals, and energy. Papaya has been employed in the production of many medicinal products (Kokila et al. 2016). Gohain et al. (2020) for the first time suggested the use of *Carica papaya* stem as heterogeneous biocatalyst as it is cost-effective, nontoxic, recyclable, eco-friendly, renewable, and suitable for Knoevenagel condensation reaction (transesterification). Oil-to-FAME conversion is very much dependent upon alcohol/oil molar ratio as well as oil quality and properties of the catalyst (Meher et al. 2006a, b). Studies have reported that the higher the methanol/oil ratio, the higher the conversion rate. Higher percentage of methanol can, however, inhibit transesterification by the formation of methoxide, which causes reverse transesterification. Gohain et al. (2020) investigated the conversion of waste cooking oil (WO) under the influence of *Carica papaya* stem (CCPS) into methyl esters of waste cooking oil (WME) and found that a MeOH/oil molar ratio of 9:1 is optimum for the process. They suggested that with increasing the amount the catalyst, the number of the active sites (after a certain lapse of time) followed by the conversion rate becomes constant or low as saponification starts to occur (Kumar et al. 2018).

The rate of conversion is usually slow during the first transesterification reaction due to the slower transformation of triglycerides into methanol, but as this reaction increases, the generation of FAME also increases till it enters equilibrium (Sirisomboonchai et al. 2015). The results of nuclear magnetic resonance (NMR) spectroscopy have confirmed that WO is rich in glyceridic protons that disappear in the WME after transesterification. The presence of methoxy carbon of methyl esters in WME confirms the complete transformation of WO into biodiesel (Betiku et al. 2016). Biodiesel production from *Scenedesmus obliquus* (SO) lipid with CCPS as catalyst resulted in significantly higher yield, and the physicochemical characteristics of WME are as per the FAME standards. The lipid substrate for the production of biodiesel can be classified as edible/non-edible oil, waste/recycled oil, and animal wastes. The availability of crude materials, oil content, and plant species along with the period of harvesting are crucial factors for the production of biodiesel. The availability of raw materials depends upon climate, soil texture, and agricultural methods. Traditional feedstock come from oil-yielding plants like sunflower, soybean, rapeseed, and palm that have higher yields due to lower free fatty acid (FFA) content (Baskar and Aiswarya 2016). In the last few years, biodiesel from non-edible-oil-yielding plants (*Jatropha curcas*, *Calophyllum inophyllum*, *Hevea brasiliensis*, *Moringa oleifera*) has gained much attraction (Arumugam et al. 2018).

Ceiba pentandra, commonly known as kapok or silk cotton tree, is a deciduous tree found in many tropical countries. Oil obtained from silk cotton seeds is not only used as a fuel but also used in soaps, paints, etc. (Lim 2012). Many researchers have tested the potential of *Ceiba pentandra* oil (CPO) in the production of biodiesel along with different substrates. Balajii and Niju (2020) inspected biodiesel generation from banana peduncle when used as a substrate by esterification and transesterification processes. Catalysts obtained from biomass are gaining much attention in the last few years. Biomass obtained ash catalyst, for instance, rice husk ash, wood ash, peanut husk ash, *Musa balbisiana* Colla underground stem and peel ash, etc. (Gohain et al. 2017). Banana is widely cultivated all over the world, and although banana peduncle has no commercial value, it is a rich soure of minerals like Na, P, Ca, K, Si, and Mg (Pazmino-Hernandez et al. 2017). Very few studies are available on the potential of banana peduncle in biochar production. Balajii and Niju (2020) collected banana peduncles and prepared a heterogeneous catalyst with CPO for the production of biodiesel. They reported that when concentration of catalyst is increased, FAME yield also increases due to the presence of K_2O in the calcined banana peduncle (CBP) catalyst. Biodiesel production was higher when ash obtained from rice husk was used as a catalyst. Betiku et al. (2016) also obtained higher FAME yields when catalyst concentration was increased as it increased the availability of the active sites, but yield rate decreased when reaction time was increased as the reaction entered the equilibrium phase. The reactants and mineral oxides of CBP attribute to higher FAME yields. If the concentration of the catalyst is increased, yield is decreased due to the increase in the viscosity and poor mixing of the reaction mixture. Pathak et al. (2018) investigated the effect of ash derived from *Musa acuminata* peel as a catalyst and obtained higher yields of biodiesel; however, increasing the methanol/oil molar ratio resulted in decreased FAME yield

as excess alcohol can dilute the concentration of catalyst. Mendonça et al. (2019) reported declination in biodiesel yield when methanol/oil molar ratio was increased with ash as a catalyst. They investigated the influence of reaction time on soybean oil transesterification with tucumã peel as a catalyst and observed that the shorter the reaction time, the higher the FAME yield. *Cola lepidota*, commonly known by the name yellow monkey cola, is an edible wild fruit of central and western African forests with high nutritional value (Ogbu and Umeokechukwu 2014). Studies have reported that this fruit has several bioactive compounds like saponins, polyphenols, anthraquinones, and alkaloids and has other medicinal properties. Obi et al. (2020) investigated the potential of *C. lepidota* oil for biodiesel production with clay as a catalyst by transesterification reaction. During transesterification, hot oil was mixed with butoxide and allowed to stay for 24 h, and then biodiesel was removed from glycerol. Glycerol being heavier collects at the bottom. The yield of biodiesel was significantly good. Viscosity of any fuel is linked to fuel lubricity as fuels with low viscosity offer good lubrication but can result in seepage. However, fuels with high viscosity led to increased exhaust emission, incomplete combustion, and engine chocking (Wang et al. 2006). American petroleum index (API) is crucial for the determination of biodiesel suitability for industrial and domestic purposes. It measures the density of biodiesel in comparison to water; for example, if API is >10, it is lighter in weight and will float on the water surface, but if this value is <10, it is heavy and will sink. Biodiesel obtained by Obi et al. (2020) fits to the API standard values, and it is optimum for regions with extreme climatic conditions. Orange peel waste (OPW) is the by-product of orange juice and has been used as a substrate for the production of valuable products like ethanol and supplement for microbial culture (Santi et al. 2015). OPW is rich in D-limonene (cyclic monoterpene) and inhibits the growth of yeast and also has commercial value as cancer-preventing agent, flavoring agent, etc. (Espina et al. 2011). OPW (limonene-free) can serve as a source of sugar as its aqueous extraction retrieves fructose, sucrose, and glucose, and the product is termed orange peel extract (OPE) (Santi et al. 2015). Carota et al. (2020) tested a liquid medium incorporated with OPE for the lipid production and growth of 18 strains of yeast. OPE as a feedstock has not been tested for the production of lipid by oleaginous yeast strains, but studies have been conducted with filamentous fungi and algae with OPE (Carota et al. 2018). According to studies, recently, OPW is the most widely used wastes on the earth as it finds use in water-based biorefineries by valorization methods (Carota et al. 2017). OPW valorization is conducted for the extraction of D-limonene, pectolytic enzymes, pectin, bioethanol, and citric acid (Espina et al. 2011). Some researchers have tested the potential of OPE for the production of biodiesel and pectinases (Park et al. 2014). Carota et al. (2020) reported that OPE can serve as a substrate in biodiesel production by oleaginous yeast strains by minimal changes in the pH of the medium and incorporation of inorganic nitrogen. OPE supplemented with a cheap and easily available nitrogen source (ammonium sulfate) has a positive effect on the yeast biomass and accumulation of lipids in them (Leiva-Candia et al. 2014). OPE has a low phenol content, of which the major ones are naringenin, naringin, and hesperidin. *Trichosporon fermentans* NRRL Y-1492 and *Cryptococcus curvatus* NRRL

Y-1511 are the only yeast strains that have been reported to have a lipid accumulation of more than 20% of their dry biomass (Karamerou and Webb 2019). Under reduced nitrogen conditions, de novo lipid biosynthesis occurs that can affect lipogenesis as polysaccharides are synthesized in order to provide energy for the metabolic activity of yeast cells (Dourou et al. 2017). Tchakouteu et al. (2015) suggested that for *C. curvatus* NRRL Y-1511, sucrose is not a good choice as good results have never been obtained. *Cryptococcus laurentii* UCD 68-201 and *Rhodosporidium toruloides* NRRL Y-1091 when cultivated on an OPE-supplemented medium resulted in higher lipid accumulation. *Rhodosporidium toruloides* NRRL Y-1091 showed higher lipid accumulation than *Cryptococcus laurentii* UCD 68-201. *R. toruloides* NRRL Y-1091 as well as *R. toruloides* DSM 4444 can rapidly utilize the sugars present in the OPE-supplemented medium and convert into storage lipids. *C. laurentii* UCD 68-201 cultivated on an OPE medium can utilize sucrose and fructose only after glucose depletion, and this strain can use a wide variety of carbon sources (Tsakona et al. 2019). Carota et al. (2017) tested the effect of ricotta cheese whey as a source of lipid on the growth of *C. laurentii* UCD 68-201 and *R. toruloides* NRRL Y-1091 in a stirred tank reactor. Since both strains are known for lipid accumulation, it was found that diminished levels of nitrogen trigger declination of adenosine monophosphate (AMP) concentration within the cells resulting in triacylglycerol accumulation. Decrease in the volume of carbon and nitrogen in the medium results in the enhancement of biomass, but lipid accumulation undergoes reduction. Similar results have also been reported for *R. toruloides* DSM 4444, *Umbelopsis isabelina*, and *Yarrowia lipolytica* (Papanikolaou et al. 2017). It has been reported that carbon deficiency in the growth medium can result in the partial degradation of stored lipids in order to produce energy required for cellular maintenance for synthesis of new lipid precursors in the oleaginous microbes (Dourou et al. 2017). Dourou et al. (2018) have recently reported that molecular pathway manipulation can result in enhanced single cell oil generation in oleaginous microbes. FAME yield of *R. toruloides* and *C. laurentii* indicates that their lipids are composed of 18 and 16 carbon chains of saturated and monosaturated fatty acids (Carota et al. 2017). Long-chain saturated fatty acids and oleic acid are the key components of biodiesel. *R. toruloides* and *C. laurentii* resemble Jatropha (*Jatropha curcas* L.) and *Elaeis guineensis* Jacq. oils in their lipid composition. The production of lipids from oleaginous yeast species is promising, but very few strains have been investigated for this purpose. The use of OPE for lipid production from oleaginous yeasts has certain advantages over other FWs as it does not require complex enzymatic and chemical treatments. The major drawback of OPE is the presence of D-limonene, but the use of OPW without limonene can solve this problem (Santi et al. 2015).

4.3.1 Factors Influencing Biodiesel Production

Several factors are known to influence biodiesel yield, namely, temperature, molar ratio, water content, free fatty acid content, and pressure. With the rise of temperature, the pressure reaction rate also increases. Methyl ester yield is very much affected by the molar ratio of alcohol/vegetable oil as well as by the reaction temperature during the transesterification process.

(a) Molar ratio

Alkyl ester yield has been reported to increase with an increased molar ratio of oil/alcohol. Sahoo et al. (2007) suggested that a vegetable oil/alcohol molar ratio of 9:1 during alkaline esterification reaction and a molar ratio of 6:1 during acid esterification are apt for the production of biodiesel from free fatty acids (FFAs) with polanga seed oil and rubber seed oils as substrates. However, Veljkovic et al. (2006) suggested that a molar ratio of 6:1 during alkaline esterification reaction and a molar ratio of 18:1 during acid esterification process result in higher biodiesel yields. Meher et al. (2006a, b) reported that a molar ratio of 12:1 during alkaline esterification reaction and a molar ratio of 6:1 during acid esterification phase result in higher biodiesel yield. Tiwari et al. (2007) emphasized volume for measurement and reported that the higher the molar ratio, the higher the production of ester.

(b) Temperature

The yield of ester transformation increases with increasing reaction temperature. In the case of alkalis (KOH, NaOH), transesterification process temperature is maintained between 318 and 338 K as higher temperature can cause burning of the alcohol, and therefore, a lower yield of biodiesel will be obtained. Leung and Guo (2006) reported that temperatures exceeding 323 K can negatively affect the yield from neat oil but can positively affect waste oils due to their dense viscosities. Enhancing the reaction temperature close to supercritical temperatures can enhance ester conversion rates.

(c) Water and free fatty acid (FFA) contents

The acidity of vegetable oil must be <1 during the transesterification reaction as acidity >1 can neutralize FFAs. Excess water content can cause frothing and soap formation, and soaps can result in increased viscosity and gel/foam formation, making glycerol separation difficult (Ghadge and Raheman 2005). In the traditional vegetable oil catalytic transesterification, water content plays an important role. In the production of biodiesel and FFAs by conventional transesterification of vegetable oils/fats, water can cast a negative effect by soap formation, which can alter the effect of catalyst and thereby affects catalysis. Kusdiana and Saka (2004) suggested that the substrate used for the synthesis of FFAs must be free of water, while Canakci and Gerpan (1999) emphasized that water decreases transesterification of vegetable oil and therefore affects ester conversion. FFAs and water in the feedstock can form soap, which decreases the yield of alkyl ester as well as affects the efficiency of the catalyst. However, water casts a positive effect on the yield of methyl esters during the

substitution of methanol to supercritical methanol at room temperature, while water has no effect on the activity of lipase (Demirbas 2006).

(d) Catalyst content

It has been found that Cao can increase methyl ester conversion from sunflower oil even if added in trace amounts. Cao is known to increase the speed of transesterification reaction, but too much increase in the amount of CaO casts a very little effect on the yield of methyl ester (Demirbas 2008).

4.4 Biogas

The rate of energy utilization is increasing tremendously every day, and the demand for sustainable energy sources is also increasing as they do not harm the environment. The utilization of FW and municipal waste management programs are the options where energy can be generated from waste materials by microbial activity and biogas or methane can be collected. The production of biogas is a useful technology as it can serve two purposes, namely, generation of biogas that can be used as a source of energy and the remaining organic matter that can be used as fertilizer. Biogas is a sustainable form of energy that can be generated from degrading plant or animal wastes rich in CO_2, methane, H_2, nitrogen, and hydrogen sulfide (Heb 2009). Methane production from the biodegradable organic waste by anaerobic digestion is dependent upon the nature and amount of material used for digestion. Different methods can be employed for methane production from FVWs and cow manure, and the most commonly used ones are single/two-phase digestion, co-digestion, and dry fermentation (Chanakya et al. 2006). Methane yields can be increased by the co-digestion process due to combined coalition between the microbes and medium for fulfilling the need of missing elements. Therefore, FVWs, FWs, and cow manure can be processed by co-digestion for improving biogas production. The reactor for co-digestion demands mesophilic conditions (25–40 °C) in order to decrease heating prices. Although methane yields are good at thermophilic temperatures (55–60 °C), maintenance of the reactors to these high temperatures is not an easy task. Deressa et al. (2015) investigated biogas production from biodegradable wastes by the co-digestion method in a biogas digester and found that moisture content was highest in tomatoes compared to breads, which had the lowest moisture content with a varied number of volatile solids (VS) in all tested wastes. Nand (1994) tested tomato, mango, pineapple, lemon, and orange wastes and found a considerably higher VS ratio. The higher the moisture content in the organic waste, the better the anaerobic digestion process. The FVWs and cow manure have considerably higher percentages of TS, VS, and mixture content (Deressa et al. 2015). FVWs when mixed with cow manure for co-digestion had a pH between 6 and 7.2, which is suitable for biogas production as the microbes were not harmed at this pH range and continuous biogas yield was obtained (Chua et al. 2008). The temperature of the co-digestion process is usually maintained between 26 and 320 °C (mesophilic conditions), which is optimum for biogas production. Biogas

combustibility can be tested by using a Bunsen burner as flame is generated if biogas production starts. Biogas yield is expressed as TS/VS ratio, and it is now evident that FVWs mixed with cow manure result in higher biogas production. Biogas incombustibility can occur in digesters due to the absence of sufficient amounts of methanogenic bacterial strains that produce methane by the conversion of carbon dioxide and acetic acid. The settled solids can result in the formation of scum at the bottom of the digester. Burning of fossil fuels results in the emission of GHGs, which pose a negative impact on the environment, and therefore, renewable sources of energy can help to overcome this issue. The utilization of FVWs, organic fraction of municipal solid waste (OFMSW), sewage sludge, and manure not only produces biogas but also reduces their disposal in the landfills (Di Maria et al. 2015). Anaerobic digestion appears to be a promising technology for biogas production from FVWs as well as OFMSWs although the rate of biogas production is a little lower due to longer time needed for organic matter stabilization and low VS removal efficiency. Anaerobic digestion can be affected by VFA accumulation and long-chain fatty acids that inhibit the activity of methanogens (Borowski 2015). The higher sugar content of FVWs results in faster biomass acidification, which inhibits the activity of methanogens (Scano et al. 2014). FVW and OFMSW processed together for co-digestion cannot only stabilize anaerobic digestion process but can also enhance biogas generation. Different types of substrates mixed for co-digestion balance the C/N ratio, increase the buffering capacity of the digester, and therefore increase biogas yield and biodegradation of the organic biomass. The end product of co-digestion can be directly used as a fertilizer. OFMSW has been considered as a suitable substrate for biogas production both alone and in combination with other wastes, for instance, fat, activated sludge and rice straw, FWs, sewage sludge, and oil and grease waste (Abudi et al. 2016). Pavi et al. (2017) tested FVW and OFMSW co-digestion with bio-digestive sludge under mesophilic temperatures for biogas production. They reported that both FVW and OFMSW can be completely transformed during anaerobic digestion and C/N ratio is variable with the type of feedstock selected. Co-digestion increases the alkalinity and therefore controls the acidification process when FVW undergoes digestion. High pH, inoculum properties, and alkalinity are the important components that maintain the stability of the anaerobic digestion. Biodegradation of FVW by anaerobic digestion occurs at a faster rate in comparison to OFMSW as the former is richer in sugar content (Di Maria et al. 2014). Biogas yield for FVW/OFMSW was reported to be higher by co-digestion in comparison to FVW or OFMSW alone (Pavi et al. 2017). FVW and OFMSW co-digestion produced higher content of methane in comparison to these substrates alone as microbes are able to get a balanced supply of nutrients from these wastes (Huang et al. 2016). Borowski (2015) reported that the methane content in the biogas produced by the co-digestion of OFMSW/sewage sludge was higher than OFMSW mono-digestion. Lin et al. (2011) obtained higher methane content in the biogas produced by the co-digestion of FVW/FW in the ratio of 1:1.

In the anaerobic digestion process, composite organic biomass is broken down by microbes without oxygen into CO_2, methane, and ammonia with other gases in trace amounts. During FVW degradation by anaerobic digestion, acidification takes place

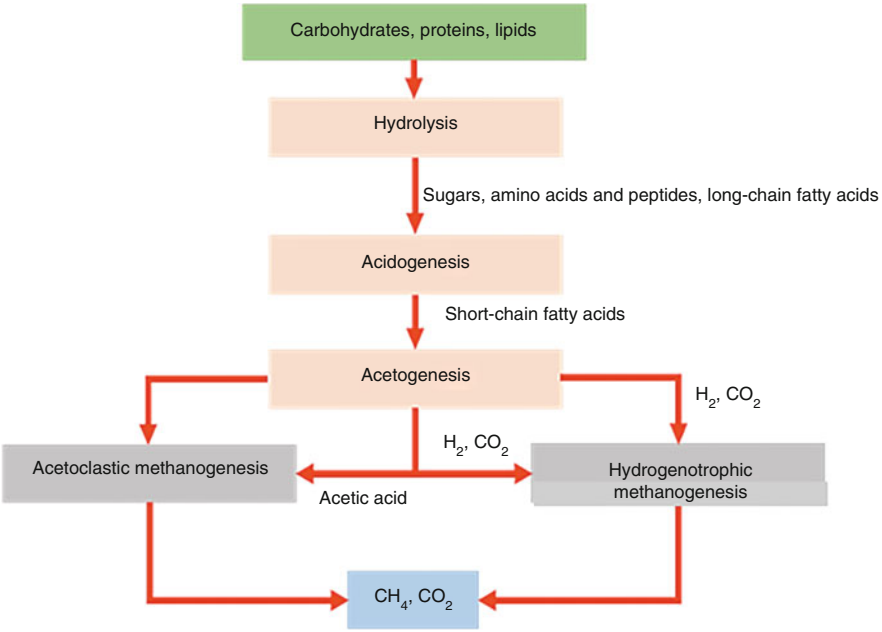

Fig. 4.6 Reactions of anaerobic digestion. (Adapted from Taghizadeh et al. 2017)

much earlier as the pH decreases resulting in the production of VFA, which reduces the efficiency of methanogens (Fig. 4.6). Therefore, methanogenesis determines the rate of reaction in the anaerobic digestion of FVWs as methanogens have longer doubling times. Banana wastes are easily available and are rich in moisture content and therefore can be used in bioconversion processes (Bardiya et al. 1996). Many studies have reported the potential of banana wastes as suitable feedstocks for biogas production by anaerobic digestion; the residues serve as fertilizer (El-Mashad and Zhang 2010). Majority of FVWs are rich in easily disintegrable organic matter, VS, but have less TS content, and they hydrolyze faster resulting in the formation of acids, and thus, the pH of the medium lowers, which inhibits the activity of methanogens. Generally, all organic wastes have sufficient amounts of nutrients that furnish the growth of methanogens aiding in the production of biogas (Khan et al. 2013). Many studies have reported that mixing of the organic content of FVW with cow dung leads to satisfactory yield of biogas as biogas contents are solely dependent upon the selected feedstock. Biogas is composed of methane (major component, 50–70%), CO_2 (30–40%), nitrogen, hydrogen, and hydrogen sulfide (Rahmat et al. 2014). Methane yield is very much dependent upon the C/N ratio of the feedstock. Abebe (2017) reported that FVWs alone can also serve as a feedstock for producing biogas without cow manure and human/animal waste as they contain a balanced C/N ratio that is efficient enough for the methanogens to produce biogas by anaerobic digestion. Biogas production increases with increased anaerobic digestion

but slows down as the amount of residue is increased. Methane content in the biogas also increases with the onset of anaerobic digestion, but it decreases as the residues start to accumulate in the digester where CO_2 is the major content of the biogas. Biogas is also referred to as green fuel as it can be used for electricity production, as a fuel, and for cooking. Generally, it is produced by the anaerobic degradation of organic materials (dead animals, plants, feces, and wastes from kitchens).

Some countries use biogas for heating purposes as well as for transport, and it can be converted to natural gas by cleaning to meet fuel standards. Coker et al. (2008) conducted a study on the amount and type of FW produced by Yoruba households, Ibadan (Nigeria), and found that 62% of the FW are composed of prepared or processed food items. They suggested that instead of dumping the FWs in the landfills, they can be utilized in the conversion of methane (biogas) that can serve as fuel. Due to its higher moisture content, FW serves as a disease carrier and also generates foul odors that can be minimized by its conversion to biogas. Two types of clean gases that serve as a source of energy can be procured from FWs, namely, methane and hydrogen, which can be used to run vehicles (Schnepf 2007). OjikutuAbimbola and Osokoya Olumide (2014) explored different types of FWs for the production of biogas by the co-digestion method. The FWs selected by them were yam peels, fish leftovers, and orange and plantain peels processed by anaerobic digestion in batches. Their results showed that FW kind does not significantly affect the yield of biogas. They found that mixing of the FWs resulted in highest biogas production, and the yield increased in the first 5 days before it slowed down. Wastes from water spinach and banana are being released from the markets every day, and they are dumped in the landfills or sometimes fed to animals. Their disposal in the landfills is a serious threat to the environment as it pollutes soil, water, as well as air, but efficient treatment methods can overcome this issue (Scano et al. 2014). The biodegradation of water spinach and banana wastes in the landfills results in the emission of GHGs and leachate due to higher content of organic compounds (Zhu et al. 2010). Water hyacinth (*Eichhornia crassipes*) is an aquatic weed with a higher growth rate, but its excessive growth is harmful to the aquatic environment. Recently, some reports have indicated that biomass of water hyacinth can serve as a raw material for biofuel production due to its higher hemicellulose percentage. Hydrolysis of water hyacinth results in the production of carbon dioxide and methane, but some studies reported that methane yield is still not high enough to be used at the industrial level and therefore, more research is needed (Rittmann et al. 2008). Soeprijanto et al. (2021) investigated the efficiency of banana peel, water spinach, and water hyacinth biomass for the production of biogas by anaerobic co-digestion and anaerobic digestion methods. They fed both mono and co-digested feedstocks into the anaerobic digester and measured biogas generation. Their results indicated that biogas production by the mono-digestion of feedstock increased up to 5 days and then became stationary while biogas yield from the co-digestion of the mixed feedstocks remained stable between 5 and 24 days with fluctuations in the yields due to microbial activity. Patil et al. (2010) also obtained higher biogas yield from water hyacinth under mesophilic conditions. Gómez et al. (2006) investigated the co-digestion of FVW and primary sludge (PS) at different

OLRs and mixing conditions and reported that biogas production was satisfactory, while Habiba et al. (2009) reported that activated sludge and FVW co-digestion improves biogas production. Inoculum has an important role in anaerobic digestion reactors as usage of highly active inoculum can result in higher biogas yield. Saad et al. (2019) studied the effect of different kinds of inoculum on hydrogen and biogas production and found that elevated yields were obtained with aeration tank sludge. Dennis (2015) tested the inoculum composed of cow manure mixed with rumen fluid by anaerobic digestion for biogas production and reported that increasing the concentration of inoculum results in the increase of biogas production. Hidalgo and Martín-Marroquín (2014) used the inoculum composed of vegetable oil waste and pig manure and codigested it with the leftovers of hotels, restaurants, and catering (HORECA) and found higher yields of biogas. Li et al. (2011) tested the inoculum composed of dairy and swine manure, corn stover, and municipal sludge by anaerobic digestion and obtained higher biogas yields from swine manure. Forster-Carneiro et al. (2007) tested biogas production from municipal solid wastes by anaerobic digestion under thermophilic temperatures with six different types of inoculum (corn silage, rice hulls, digested sludge, swine excrement, swine excrement mixed with sludge, and cattle excrement) and found that biogas production was higher when digested sludge was considered as the inoculum. Elsayed et al. (2020) investigated the anaerobic digestion of FVW-PS by co-digesting them at different ratios for biogas production and found that biogas yield was higher from PS alone than FVW as the former is more easily degraded than the latter. The cumulative methane yields (CMYs) from both PS and FVW under mesophilic temperature were continuous for a period of 30 days. Nansubuga et al. (2015) reported that methane yields are higher if PS and FVWs are co-digested in an equal ratio, while Heo et al. (2003) suggested that methane yields are higher if FW and activated sludge are mixed in equal ratios. Koch et al. (2016) reported that FW co-digested with raw sludge results in higher methane yield than the sludge alone as a nutritional balance is maintained in the reactor. FVW co-digestion with PS results in increased methane yield as microbial activity increases due to the highly biodegradable nature of FVW. Similar reports have been obtained by many researchers; for example, Pan et al. (2019) reported that FW-sewage sludge (SS) in equal ratio results in higher methane content in biogas, while Jugal Sukhesh and Venkateswara Rao (2019) reported that dairy manure and rice straw co-digestion resulted in higher methane yields. But methane yield is always not higher with co-digestion; for instance, Liu et al. (2009) reported that FW-green waste co-digestion resulted in lower yields in comparison to the mono-digestion of feedstocks as there was imbalance between the C/N ratio and essential nutrients resulting in VS reduction as well as yield of methane. Several studies have tested anaerobic tridigestion that seems to be superior to co-digestion in providing more balanced nutrients, improves disposal capacity, and also reduces the cost (Korai et al. 2018). Zahan et al. (2018) tested the biogas production by tridigestion of FW, wheat straw, and chicken litter and obtained higher yields. Li et al. (2020) evaluated the effect of mono-, co-, and tridigestion on the production of methane from FVW, FW, and KW and found that the three digestion processes became active within a short period of time as all the three types of wastes are rich in

sugars that can be easily converted. Biogas yield in the FW mono-digestion decreased after the second day due to pH changes (acidic) leading to the accumulation of VFA that inhibits production of biogas, but adjusting the pH to alkaline levels increased the biogas yield again. The acidic phase was not observed in the tridigestion of FW/KW/FVW, suggesting that this method has higher buffering capacity in comparison to mono- and co-digestion processes. Wang et al. (2013a, b, c) investigated the effect of tridigestion on biogas production from chicken manure, rice stalk, and cow manure and obtained higher yields of biogas in comparison to the mono and co-digestion methods. Lee et al. (2019) also obtained higher methane yields from the tridigestion of FW, activated sludge, and yard waste mixtures, suggesting that feedstock components, mixing ratio, and reaction processes affect biogas yield. The higher methane content by tridigestion can be attributed to the fact that sufficiently higher methanogenic activity occurs in the digesters resulting in complete conversion of VFA to methane (Mu et al. 2020). Kim et al. (2019) reported that increasing the concentration of FW results in higher methane yield. Tang et al. (2020) tested the efficiency of anaerobic digestion by taking vegetable wastes as feedstocks and factors affecting anaerobic digestion. They observed that in the beginning, however, the yield of biogas was high but methane content was low but later methane yield was found to increase. At the onset of fermentation, methane yield was low due to hydrolysis of the vegetable wastes, but later the fermentation process entered the acidification stage that increased the methane yield. dos Santos et al. (2020) evaluated the efficiency of passion fruit peel (PP), cashew, and orange bagasse (OB) for biogas production by anaerobic digestion with anaerobic sludge (industrial sludge (IS) and sewage sludge (SS)) as inoculum. They obtained lowest yields from CB-IS-SS, while higher yields were obtained from OB-IS as well as OB-SS, and this can be attributed to the fact that OB has a higher lignin and hemicellulose content and a higher C/N ratio that enable efficient hydrolysis resulting in higher methane yield. Ruiz and Flotats (2016) obtained higher methane yield from orange peel pretreated with bovine manure under mesophilic conditions. Similar results were obtained by Martín et al. (2010) who pretreated the orange peel with industrial sludge under mesophilic temperatures as well as by Carvalho et al. (2017) who pretreated the orange peel with SS and obtained higher yields. Pretreatment decreases D-limonene (essential oil) concentration, which is toxic to methanogenic Archaea. Zhao et al. (2016) obtained higher methane yields from PP mixed with SS under mesophilic temperatures in the batch system. Similar results were obtained by Prabhudessai et al. (2013) who treated CB with SS under mesophilic temperatures by taking an inoculum composed of bovine manure, sludge from a UASB reactor, and goat ruminal fluid. Fruit and vegetable harvesting waste (FVHW) includes stems, leaves, nonconsumable vegetables, and fruits that have little lignocellulose and do not require complex pretreatment before fermentation. Günerhan et al. (2020) suggested that combining chemical-driven thermal pretreatment with anaerobic digestion can improve biogas production from FVHW. They dried the FVHW in the sun and then ground them into powder before pretreating them with hydrochloric acid (HCl) and sodium hydroxide (NaOH) before processing for anaerobic digestion under mesophilic conditions. They found that

thermal pretreatment without chemicals resulted in highest methane yields while lowest yield was obtained in the case of FVHW pretreated with NaOH. It can be predicted that increasing the proportion of NaOH decreases the yield of methane due to disintegration of the produced sugar molecules and increase in the number of Na^+ ions (Song et al. 2019). Similar results were obtained with HCl pretreatments, and lower methane yields were obtained with increasing HCl concentrations. Song et al. (2014) tested the effect of four different acidic (H_2O_2, H_2SO_4, CH_3COOH, and HCl) and three alkaline ($Ca(OH)_2$, $NH_3 \cdot H_2O$, and NaOH) pretreatments on the production of methane by corn straw and reported that both pretreatments have a positive effect on methane yield as they initiate solubilization of organic matter and therefore increase the availability of active sites of enzymes. Mozhiarasi et al. (2020) explored the potential of fruit market wastes (FRWs), flower market wastes (FLWs), vegetable market wastes (EVWs), extruded flower market wastes (EFLWs), vegetable market wastes (VWs), and extruded fruit market wastes (EFRWs) for methane generation. They found that methane yield was highest for EFRW, suggesting the presence of high biodegradable organic matter that can be fastly hydrolyzed and converted into methane. FLW also resulted in higher methane yields in comparison to other extruded wastes due to higher dry matter content; therefore, it can be concluded that extrusion plays a role in increasing methane yields as well as reduces the digestion time. Suhartini et al. (2020) investigated the biogas production from fruit-based agro-industrial wastes (jackfruit straw, banana, apple, pineapple, and orange peelings) and agricultural crop residues (maize and rice straw, oil palm empty fruit bunches (OPEFB), vegetable waste, and coffee husk). They chopped and then ground all the raw materials before transferring for anaerobic digestion under mesophilic conditions and reported that fruit-based agro-industrial wastes produce higher proportions of biogas in comparison to the agricultural crop residues. For the fruit-based agro-industrial wastes, biogas yield moderately increased for 15 days before entering the stationary phase, while for the agricultural crop residues, the yield was rather slow. Both the two types of selected wastes have a higher proportion of lignin, which positively affects methane content in biogas. Zheng et al. (2013) also reported that apple, orange, and banana peels have the potential of producing biogas under mesophilic conditions by anaerobic digestion. Ahmed et al. (2018) suggested that the physicochemical properties of substrate can cast an effect on the metabolism as well as performance of microbes in the anaerobic digestion process, and this can affect biogas production.

4.5 Conclusion

FWs are composed of vegetables, fruits, dairy products, starches, sugars, brewery waste, meats, and grain flours. They are rich sources of proteins, carbohydrates (cellulose, hemicellulose, and starch), lipids, organic acids, and lignin. The disposal of FWs is a major challenge in the present era. They are either incinerated, composted, burned, or dumped in the landfills. The disposal of FWs in the landfills

is not a healthy practice as it results in the emission of GHGs as well contamination of soil and groundwater. Researchers have figured out that FWs can be directly converted into value-added products like biofuels. Biofuels are sustainable sources of energy as they are produced from the organic biomass by the activity of microorganisms. Fruits as well as vegetable wastes can undergo fermentation by microorganisms and produce different types of biofuels, for example, bioethanol, biohydrogen, biogas, and biodiesel. These generated biofuels can serve as sources of energy and can be used as a means of transport, cooking as well as a source of bioenergy. Further research is needed as the production of biofuels from FWs is still at a preliminary stage, but it is very probable that in the future, biofuels will be able to replace conventional fossil fuels.

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Chapter 5
Recent Advances in Biogas Production from Food Waste

Gaurav Kumar Pandit, Ritesh Kumar Tiwari, Shanvi, Veer Singh, and Meenakshi Singh

Abstract Energy is very significant for the holistic development of any country, and with the growing urbanization and industrialization, there's a rapid rise in the burning of fossil fuel. But the burning of fossil fuels leads to a lot of greenhouse gas (GHG) emissions in the atmosphere. It tends to increase global warming, which ultimately affects human health and the whole ecosystem. Besides, fossil fuels are non-renewable sources of energy. This compels us to think of an alternative sustainable energy source that can meet the energy demands and provide environmental protection and nutritional recovery to a greater extent. Natural resources like biogas are renewable sources of energy that can be used instead of fossil fuel burning. Biogas has the attributes of sustainability, environmental protection and nutritional recovery. Anaerobic digestion produces biogas through various organic wastes like food wastes, animal dung, agricultural wastes and sludge. CO_2 and CH_4 made in the anaerobic process are upgraded to biomethane used for various purposes like production of electricity, cooking fuel or transportation fuel. This chapter relies on the production of biogas from food wastes and the recent advances made in the process with an appropriate consideration given to other related aspects.

Keywords Fossil fuels · Greenhouse gas (GHG) · Organic wastes · Food wastes · Biogas · Anaerobic digestion · Co-digestion · Circular economy

5.1 Introduction

Energy forms a significant factor in contributing to the holistic development of any country (Barnes et al. 2011; Stern 2011; Singh et al. 2021a). With the increase of population and urbanization, the need for energy is also increasing exponentially

G. K. Pandit · R. K. Tiwari · Shanvi · M. Singh (✉)
Department of Botany, Patna University, Patna, Bihar, India

V. Singh
School of Biochemical Engineering, IIT (BHU), Varanasi, Uttar Pradesh, India

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(Mazur 1994; Graham 2009). The burning of fossil fuels poses a significant threat to the environment and human health (Kataki et al. 2017; Singh et al. 2021b). It is considered the primary contributor to global warming. According to a US Energy Information Administration report, about 76% of the US greenhouse gas emissions rooted from fossil fuel burning in 2016. Besides, fossil fuels present non-renewable energy sources. So, an alternative must be looked and explored into the full potential to meet the energy demands so that the adverse effects on the environment can also be curtailed (Singh et al. 2021c, d).

Apart from the energy need, one other parameter increasing exponentially with the increasing industrialization and urbanization is the tonnes of waste generated by both developed and developing countries annually. These wastes are of various types ranging from household organic wastes to e-wastes. When dumped into landfills or undergo combustion, these wastes contribute to the emission of potent hazardous gases leading to catastrophic climatic changes. Municipal solid wastes, consisting mainly of household wastes, may leach out into the aquatic bodies and cause water pollution. Thus, besides the energy need, the waste management strategy of any country also decides the fate of its socio-economic position (Singh et al. 2020a, b, c, d; Chaturvedi et al. 2020).

Hence, the alternative energy source should meet the energy needs while reducing the harmful effects on the environment and ensuring efficient waste management. In a nutshell, the energy source should promote a circular economy (Singh et al. 2016, 2021b).

Renewable energy sources are good alternatives to be switched upon (Koberg and Gedanken 2012; Singh et al. 2020e), and biogas is one of the potential and efficient alternatives. The easy availability of biomass makes it a very potential renewable energy source that can significantly meet the world's energy demand (Perea-Moreno et al. 2019). The anaerobic digestion of organic matter produces biogas with the help of microorganisms. Various organic substrates may act as the raw material for biogas production, such as food waste, manure and plant material (Singh et al. 2017, 2020f; Yadav et al. 2019).

5.2 Food Waste

There is no definite terminology for food loss and waste (Abdelradi 2018). Different researchers and institutions perceive it in different ways and accordingly put forth their definitions. According to FAO (Food and Agriculture Organization), "food waste is simply the discarding of food that was once fit for human consumption but left to spoil or the one which has expired" (FAO 2013). Food loss is another term that is sometimes used interchangeably with food waste. But these two terms differ slightly in their literal meanings. FAO defines food loss as "the decrease in quality or dry matter of the food that was once produced for human consumption". Food loss and food waste are collaboratively denoted as FLW. There are other definitions of the FLW also as per the perception of different institutions from time to time. The

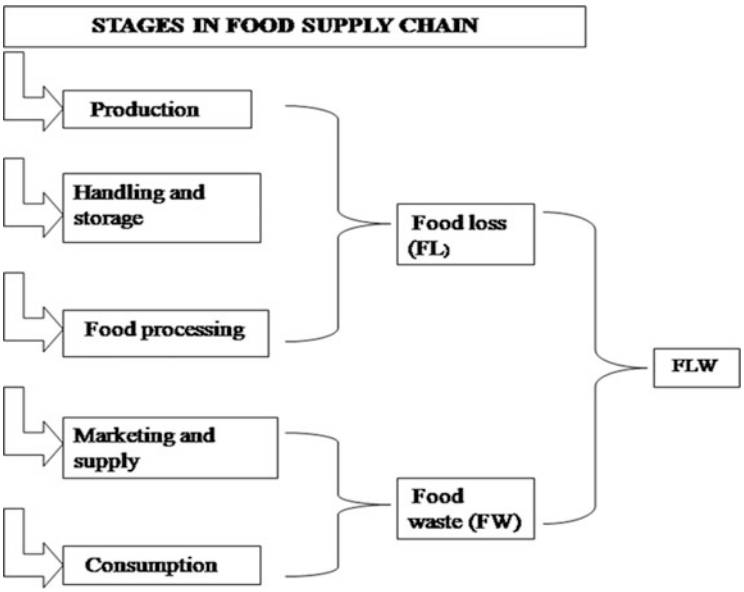

Fig. 5.1 Framework of FLW in the food supply chain

wasted food can then be disposed or recovered afterwards. The disposal strategies encompass composting, anaerobic digestion, bioenergy production, incineration, cogeneration, ploughing of crops and not harvesting and disposal to landfills, sewer or discarded to sea (Singh and Mishra 2020; Östergren et al. 2014). Thus, FLW broadly can be understood as the deterioration of the food quality or quantity once considered for consumption by a human. The dissimilarity arises from the consideration of extrinsic factors and the interrelation between the terms "food waste" and "food loss".

According to FAO, loss of food mainly takes place during the initial first three phases of the food supply chain, whereas food waste is more or less related to the final stage of consumption. Some institutions also relate all the deterioration in the food quantity or quality to food waste and completely obliterate the concept of food loss. The food waste generated through food chain is represented in Fig. 5.1.

5.2.1 Composition of Food Wastes

The exact composition varies with the type of food waste and its constituents. They mainly comprise lipids, proteins, carbohydrates and traces of inorganic compounds (Paritosh et al. 2017).

5.2.2 *Impacts of Food Waste Accumulation and Disposal*

According to FAO, 1/third, 1.3 billion tons of food material is wasted or lost annually across the world (Gustavsson et al. 2011; Ishangulyyev et al. 2019). The accumulation of these wasted food materials has severe adverse impacts. FLW seriously impacts economics, poverty and natural resources. Nutritional insecurity is also greatly affected as FLW decreases food availability for human consumption (Abiad and Meho 2018; Lipinski et al. 2013; Capone et al. 2014).

5.2.2.1 Environmental Impacts

- On the whole, the animals, plants and ecosystem are affected by food production and simultaneous wastage.
- Food processing amounts to a more significant input of energy and materials.
- Non-seasonal and the imported foods add to transportation and energy use. This puts pressure on the environment.
- FLW cause loss of water, land and energy sources as the food supply chain requires all these factors.
- Land quality is also directly affected by food production and supply, leading to soil erosion, nutrient depletion, desertification and deforestation.
- The carbon footprint of the food wastes suggests that almost 3.3 billion tonnes of carbon dioxide are accumulated annually to atmospheric greenhouse gas (GHG) (Paritosh et al. 2017).
- Incineration of food wastes and dumping this food waste into the open area are conventional techniques for their disposal as municipal solid wastes (Liu et al. 2015). This can cause various environmental and health complications (Rushton 2003; Capson-Tojo et al. 2016; Harrad and Harrison 1996; Zagozewski et al. 2011).
- When food wastes are disposed of in landfills, a large amount of GHG and methane are also produced, which is 23–25 times as much as that of the potential of carbon dioxide in bringing about global warming (Kleerebezem et al. 2015; Parry et al. 2007).
- Leachates from these landfills can substantially cause contamination of ground-water (Gupta and Arora 2016).
- Dioxins are released when food wastes containing high moisture content are incinerated (Katami et al. 2004; Shibamoto et al. 2007). This can also cause several environmental issues.
- The economic value of the substrates also gets reduced due to poor nutrient recovery because of incineration. Thus, proper waste management strategies need to be devised (Ma et al. 2008).
- All these factors can lead to catastrophic climatic alterations, which may have profound health implications.

5.2.3 Waste Management Strategies for Food Wastes

Food waste imposes a threat to social, economic, environmental and aesthetical aspects of the country. Considering the range of harm it can cause, it becomes imperative to resort to strategies for the efficient management of food wastes and design ways in which its generation can be reduced.

Let us look at some of the approaches for the management of food wastes (Dung et al. 2014):

(a) Reduction at source—This is the most preferable and conventional strategy to manage food wastes. Reduction of the food waste at the onset itself is encouraged so that the FLW can be reduced. Several approaches can be adopted to promote the removal of food wastes at the source. Some of them are;

- Publicizing—Various organizations can create awareness about and promote food waste reduction (FWR) by various campaigns on their social media platforms, websites or notice boards, etc.
- Various outreach programmes—Institutions can devise their outreach programmes and recruit Food Wastage Reduction Ambassadors (FWRA).

(b) Reuse—It involves the redistribution of uneaten or unsold foodstuffs. In this approach, various concerned organizations and institutions encourage the reuse of suitable and healthy food but because of any reason being considered for discarding. The concerned institutions (various public organizations) can donate their surplus food to food distribution agencies or points.

(c) Recycle—The food waste which cannot be avoided should be treated for recycling. For this, segregation of the food waste from the non-food waste items should be done at the generation point itself. Composting of the food wastes with the help of worms is also being practised in households and industrial sites to form vermicompost.

(d) Recovery of energy—The food wastes which cannot be processed by recycling should be then valorized for energy generation via destruction to energy plants. Several innovative techniques are being explored for bioenergy generation and bio-resource recovery with a circular economy approach. Anaerobic digestion of food wastes to produce biogas is gaining popularity for its multipurpose use as cooking fuel or in the generation of electricity, and a lot of advancements have been made in the technology (Lee et al. 2020). Co-digestion is also being explored (Cecchi and Cavinato 2019). The overall process of waste management and its application in bioenergy production are shown in Fig. 5.2.

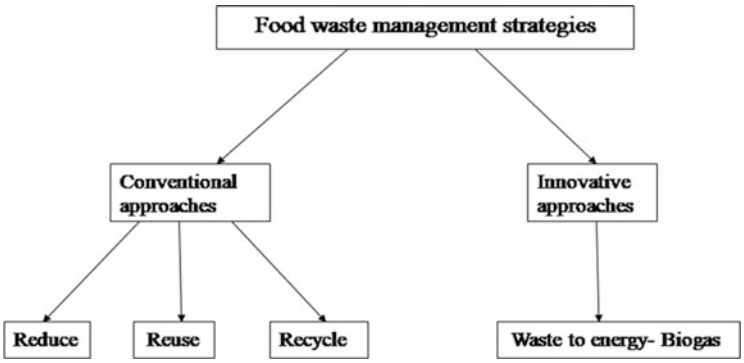

Fig. 5.2 Waste management strategies (conventional and innovative)

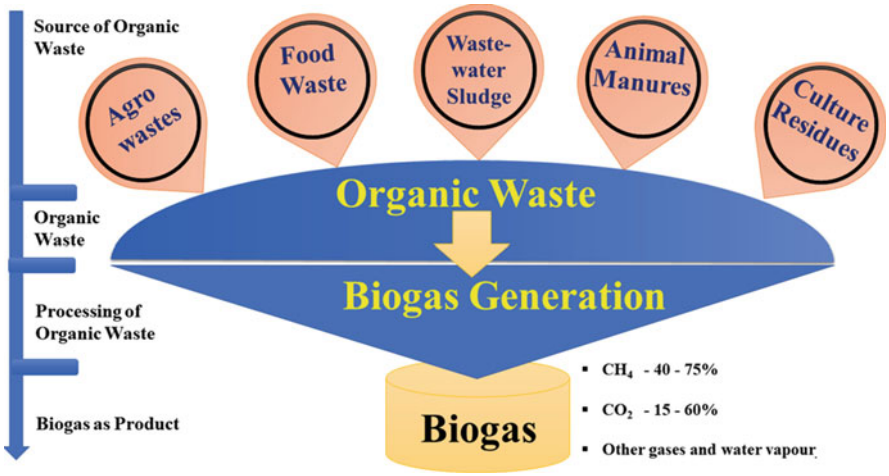

Fig. 5.3 Different sources of organic wastes for biogas generation

5.3 Biogas

Nowadays, the demand for a renewable, clean energy source is also increasing proportionally to protect the environment. Biogas is one of the renewable and clean energy sources that can circumvent both these issues and provide us with better and environment-friendly energy. It is produced by anaerobically digesting biomass.

Different sources of organic wastes are utilized as substrates for biogas generation (Agarwal et al. 2005; Bhatt and Tao 2020; BETO 2017; Mohanty et al. 2021). The sources for biogas generation are shown in Fig. 5.3.

Biogas mainly contains CH_4 and CO_2. The percentage of methane in untreated biogas may vary from 40 to 75% and carbon dioxide from 15 to 60% by volume. The

Table 5.1 Typical composition of biogas and their concentration (sources: Adebayo et al. 2015)

S/no	Typical composition of biogas	Formula	Concentration v/v
1	Methane	CH_4	40–75%
2	Carbon dioxide	CO_2	15–60%
3	Hydrogen sulphide	H_2S	0–5000 ppm
4	Nitrogen	N_2	0–5%
5	Hydrogen	H_2	Trace
6	Moisture	H_2O	1–5%
7	Carbon monoxide	CO	0–3%
8	Oxygen	O_2	<2%
9	Other trace gases		<2%

remainder of the gas mainly consists of CO_2 along with other gases and water vapour (Bhatt and Tao 2020).

It can be used as fuel or can be treated or upgraded to form biomethane, which is also known as renewable natural gas. This upgradation of biogas to biomethane allows transportation over long distances, which can be then utilized for transportation fuel and various industrial uses (Bhatt and Tao 2020; Kleerebezem et al. 2015). Microbes play a very crucial role in biogas production from bio-wastes. They feed on biomass and release energy via the process of anaerobic digestion. These anaerobic bacteria can be found naturally in water bodies such as lakes and swamps, soils and in the gut of animals and humans (Table 5.1).

Formation and collection of biogases can be achieved through municipal solid wastes in landfills, or the product can be achieved in anaerobic digesters under controlled conditions. The biogas is utilized for diverse purposes, and the digestate (left after completion of anaerobic digestion), which is nutrient-rich, can be applied as fertilizers.

5.3.1 Driving Forces for Biogas Production

There are many drivers for the production of biogas. Let us look at some of the prominent driving forces among them (Hasan et al. 2020):

(a) Social drivers:

- Renewable energy market potential.
- Recognition of the benefits of green energy politically.
- Increased awareness among end users about bioenergy.
- Possible reduction in emission of greenhouse gases (GHG).

(b) Technical drivers:

- Efficient waste utilization methods.
- Variety of feedstocks available for biogas production.

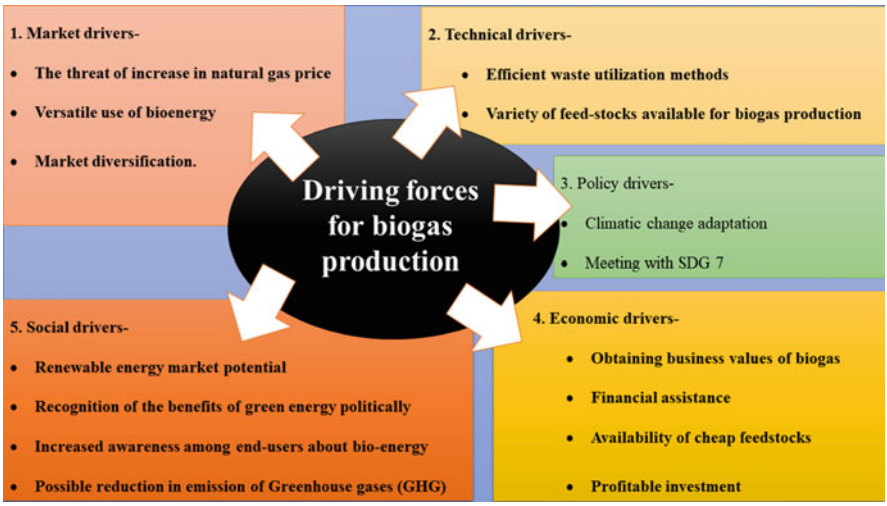

Fig. 5.4 Various driving forces for biogas production

(c) Economic drivers:

- Obtaining business values from biogas projects.
- Financial assistance.
- Availability of cheap feedstocks for energy generation.
- Profitable investment.

(d) Policy drivers:

- Climatic change adaptation.
- Meeting with SDG 7.

(e) Market drivers:

- The threat of increase in natural gas price.
- Versatile use of bioenergy.
- Market diversification.

The above-mentioned driving forces are much important for biogas production. These driving forces provide the raw materials for production and enhance productivity. The important driving forces are shown in Fig. 5.4.

5.3.2 Biogas Production from Food Waste: The Process

The substrate for biogas production can be various organic waste types (food wastes, agro-wastes, manure, etc.). Biogas represents renewable and clean energy which also promotes circular economy by recovering organic nutrients during production

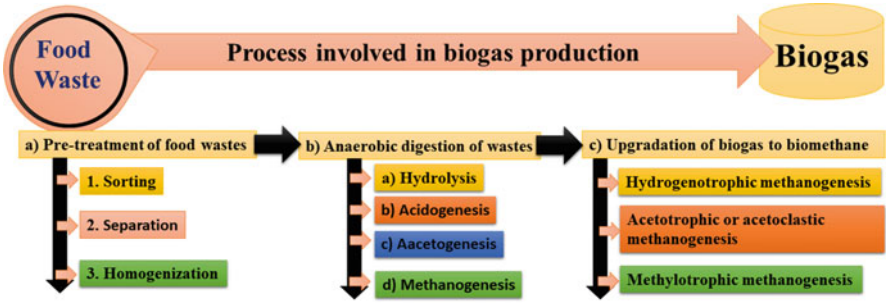

Fig. 5.5 Process involved in biogas production

(Mohanty et al. 2021; Ramos-Suarez et al. 2019; Mateescu and Dima 2020; Martinov et al. 2020; Kumar et al. 2020).

The process of biogas production can majorly be divided into the following sequential stages:

(a) Pretreatment of food wastes.
(b) Anaerobic digestion of wastes.
(c) Upgradation of biogas to biomethane.
(d) Recovery of digestate (leftover after the completion of anaerobic digestion).

The process involved in the biogas production is mentioned in Fig. 5.5.

5.3.2.1 Pretreatment of Food Waste

Generally, domestic food wastes do not require pretreatments other than the reduction of particle size. The pretreatment, if needed for the facilitation of microbial decomposition downstream. The types of pretreatment typically used are (Banks et al. 2018):

- *Sorting*—Removal of non-biodegradable and inert materials from the food wastes is involved in this process. If not sorted at the source, these materials can negatively impact the downstream process or the quality of digestate.
- *Separation*—Mechanical separation can be applied further in the process for the removal of any contaminant. The design of the separation technique to be followed depends on the type of food wastes, its nutritional composition and the collection of historical data if any.
- *Homogenization* is a critical process as it promotes degradation and prevents settling or layer formation inside the digester. It involves size reduction and then conversion to a slurry of bio-wastes for easy mixing and pumping.

Pretreatment Techniques

Ultrasonication (at commercial scale) and mechanical pretreatments (e.g. using the microwave, high-pressure disruption) and electrokinetic (at laboratory scale mainly or using prototypes at the small sale) are intended to improve hydrolysis during anaerobic digestion (Eskicioglu et al. 2008; Pazos et al. 2009; Tyagi and Lo 2011; Tyagi et al. 2014).

Chemicals use, alkali, acidic, ozonation (Neumann et al. 2016; Le et al. 2019), advanced oxidation processes application (Martínez-Huitle and Ferro 2006; Feki et al. 2020) and biological approaches (Zhen et al. 2017; Xu and Dai 2020; Park et al. 2018) or the combination of these techniques is used to increase solubilization effectivity (Sevillano et al. 2021).

5.3.2.2 Anaerobic Digestion

Once the pretreatment is done, the feedstock is fed into the anaerobic digester to decompose and produce biogas. And then, biogas is stored in the tank. The biogas is rich in methane and can be upgraded further depending upon the end use, transport fuel, electricity, etc.

It is piped through the unit of desulphurization for the reduction of sulphur content. The organic matter that is left after digestion, called digestate, is extracted and may be processed through pasteurization, followed by composting or dry and wet solid separation. This digestate is nutritionally rich and can be used as fertilizers (Goswami et al. 2016; Korbag et al. 2020; Shah et al. 2017; Speece 1983). The stages involved in anaerobic digestion are represented as:

(a) *Hydrolysis*: Breakdown of organic matter (e.g. carbohydrates, proteins and fats) into simplest form (e.g. glucose, amino acids and fatty acids, respectively) takes place in this process. Various hydrolytic and cellulolytic bacteria are involved. *Acetovibrio*, *Bacteroidetes* and *Cellulolyticus* are some of the examples.
(b) *Acidogenesis*: Breakdown of simplest organic molecules like amino acids, glucose and fatty acids to the alcohols and volatile fatty acids. H_2S, ammonia and CO_2 are released as by-products. The consortium of microbes that may be used in this stage is the widest. Various acidogenic bacteria such as *Peptococcus* and *Campylobacter* and *fungi* may participate in this stage.
(c) *Acetogenesis*: Alcohols, fatty acids and some amino acids are oxidized into simpler forms. Acetate and carbon dioxide are primarily produced in addition to hydrogen gas. Syntrophy is observed as close cooperation between microbes involved in oxidation in this stage, and methanogens active in the following intermediate stage are required. Examples of microbes that can carry out acetogenesis are *Syntrophomonas wolfeii, Syntrophobacter wolinii*, *Clostridium*, etc.
(d) *Methanogenesis*: It is the final and rate-limiting step of anaerobic digestion (AD) which leads to the production of methane (primary product), hydrogen,

CO_2 and other gases (in small amount). At least six different substrates can be utilized through six different pathways to produce methane gas by methanogens.

The six substrates are (Slonczewski and Foster 2014):

1. Carbon dioxide.
2. Methanol.
3. Formic acid.
4. Methylamine.
5. Dimethyl sulphate.
6. Acetic acid.

The most common pathway used is reducing CO_2/H_2, leading to conversion of CO_2 to CH_4 gas.

The three biochemical pathways of methanogenesis:

1. Hydrogenotrophic methanogenesis: Hydrogenotrophic methanogens such as Methanobacterium arbophilicum involved in these pathways. The reaction of biogas production is shown as (5.1)

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \tag{5.1}$$

2. Acetotrophic or acetoclastic methanogenesis: Acetoclastic methanogens such as *Methanosarcina barkeri* are involved in the pathways. The chemical reaction of methane production is shown as (5.2)

$$4CH_3COOH \rightarrow 4CO_2 + 4CH_4 \tag{5.2}$$

3. Methylotrophic methanogenesis: The chemical reaction of biogas production is shown as (5.3)

$$4CH_3OH + 6H_2 \rightarrow 3CH_4 + 2H_2O \tag{5.3}$$

The techniques involved in the biogas production are summarized in Fig. 5.6.

5.3.2.3 Factors Affecting Biogas Production

Since various microbial consortia are associated with different biochemical steps involved in the anaerobic biogas production. It is evident that many factors influence microbial growth and so the whole process of anaerobic digestion. It would ultimately affect the biogas yield. Some of the significant factors are (Goswami et al. 2016; Korbag et al. 2020).

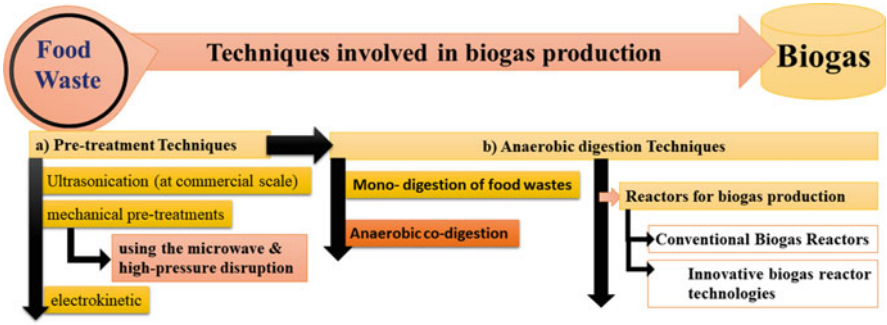

Fig. 5.6 Techniques involved in biogas production

- *Temperature*: The activities of anaerobes involved in the decomposition of organic wastes to biogas are temperature-dependent. It has been found that the three ranges of temperature are mainly used for the process of anaerobic digestion. Mesophilic range, 25–40 °C; psychrophilic range, <20 °C; and thermophilic range, 45–60 °C.

  It has been observed in previous studies that the anaerobes are most active in the thermophilic and mesophilic range (Kumar 2012).
- *pH*: The activity of the hydrolytic enzymes and the whole process of anaerobic biodegradation are affected by pH. The pH range 6.5–8.2 is efficient for methanogenesis, whereas hydrolysis at pH 5.5 and pH 6.5 for acidogenesis (Lee et al. 2009).
- *C/N ratio*: Range between 20:1and 30:1are found to be ideal for anaerobic digestion of organic wastes, with 30:1 being the optimal operational ratio (Korbag et al. 2020). In practicality. However, feedstocks have either higher or lower C/N percentage, and co-digestion improves the balance (Goswami et al. 2016).

5.3.2.4 Anaerobic Digestion Systems

Mono-Digestion of Food Wastes

The food wastes serve as suitable digestion substrates since they are highly degradable and have a high methane potential. However, after prolonged operation, methane production was inhibited, and there was also an increase in volatile fatty acids. Sometimes fall in the digester pH and digestion process failure were also observed in extreme conditions. This finding could be related mainly to the high concentration of nitrogen in the food wastes. Nitrogen is decomposed to ammonia which is crucial for growing anaerobes, but a high concentration is believed to be inhibitory. Also, the acetoclastic methanogens are found to be more sensible towards ammonia. Thus, the replacement of acetoclastic methanogenesis by hydrogenotrophic methanogenesis provides a potential solution to this problem with mono-digesters (Khalid et al. 2011; Mathew et al. 2015; Lv et al. 2013; Banks et al. 2018).

Anaerobic Co-Digestion and Enrichment of the Biogas Production

Anaerobic co-digestion of food wastes techniques is gaining importance due to their multiple advantages. Animal slurry and sewage sludge generally have high moisture content and low potential to produce methane. So the performance of anaerobic digestion depends mainly on the hydraulic retention time and not on the organic loading rate (OLR). Thus, the digesters can have the extra loading capacity to be applied, given that the retention time is not reduced significantly. Co-digestion with food wastes has a better production rate of biogas and has economic viability besides the capacity to circumvent some complications associated with mono-digestion techniques, such as toxic material existence, nutrient imbalance or recalcitrant substances in the feedstocks (Yentekakis and Goula 2017; Adnan et al. 2019; Banks et al. 2018).

Different factors must be considered for anaerobic co-digestion of food wastes like suitable co-substrates and proper blend ratio to achieve optimally, and disruptive compounds are diluted while increasing the methane production.

Adjustment of the balance of nutrients improved organic matter stabilization, and cost-effectivity can be achieved by adding suitable co-substrates (Kumar and Goel 2009; Kumar et al. 2009; Pattnaik and Reddy 2010; Sevillano et al. 2021; Mata-Alvarez et al. 2014; Hagos et al. 2017). Biogas production can be increased from 25% to 400% (Shah et al. 2015) by co-digesting different feedstocks with animal manure compared with the digestion of mono-substrates. The use of pig manure with glycerol under 250 °C to 400 °C in the ratio of 24/1 compared to the pig manure alone. Almost 400% more biogas was obtained (Rabii et al. 2019; Shah et al. 2015; Meiramkulova et al. 2018).

Higher C/N ratio in feedstocks (>50) such as wheat straws, seaweed, algae, corn stalks, etc. can be digested easily compared with wastes having less C/N ratio, for example, kitchen and food-wastes, pig manure and poultry manure. The nutrient balance is achieved and inhibition leading to instability of the system and decreased biogas production due to unfavourable C/N ratio (Talyan et al. 2008; Ali Shah et al. 2014; Hagos et al. 2017; Alqaralleh et al. 2017; Panpong et al. 2014; Sosnowski et al. 2003).

Sewage sludge as co-substrates to favour and promote organic degradation is extensively evaluated and reported in many works of literature (Zhao and Kugel 1996; Kim et al. 2003; Gomez et al. 2006; Cecchi and Cavinato 2019; Moestedt et al. 2019; Mu et al. 2020).

It is believed that the addition of conductive material like activated carbon, char and graphite provides favourable condition for electron transfer between species. Degradation of proteins and volatile fatty acids is also increased by this addition (Sevillano et al. 2021; Arenas et al. 2020; Lu et al. 2020).

Overall benefits of the anaerobic co-digestion techniques (Rabii et al. 2019):

- Increase in yield of methane per unit of digester volume.
- Synergistic effects on microorganisms.

- Economic viability.
- Balance of nutrients.
- Toxic materials dilution.

5.3.2.5 Advantages of Anaerobic Digestion

According to WBA Global Potential of Biogas, 2019, anaerobic digestion technologies can contribute significantly towards achieving some of the UN Sustainable Development Goals- SDG 2,3,5,6,7,9,11,13 and 15. Some of the benefits of anaerobic digestion of organic wastes. Production of renewable and eco-friendly energy (Sevillano et al. 2021; Rekleitis et al. 2020).

- Mitigation of climatic change.
- Contribution towards a circular economy.
- Improvement of urban air quality.
- Contribution towards food security.
- Improved sanitation and health through better solid waste management.
- Development of economy.
- Job creation.
- Address of the crisis of waste management.

Other benefits of anaerobic digestion:

- Different feedstocks in varying quantities and composition available locally can be used for anaerobic digestion.
- It exhibits flexibility of scale.
- Biogas produced can be used flexibly for multifarious purposes: as cooking fuel, for electricity generation (Lee et al. 2020) and as transportation fuel when upgraded to biomethane (Rogulska et al. 2018; Ardolino et al. 2021).
- The products and by-products of the anaerobic digestion process present multiple streams for revenue.
- It has various advantages over aerobic digestion in energy requirements, sludge production, expense and producing stable digestive besides energy production (Shahid et al. 2020; Lee et al. 2019).

5.4 Reactors for Biogas Production

5.4.1 Conventional Biogas Reactors

These are designed devices so that the degradation of organic wastes by anaerobic microbial consortia is achieved for biogas production. Ideal requirements for a biogas reactor are:

- Gas tight.
- Water tight.
- Protection against UV light, corrosive gases and chemicals.
- Insulation against extreme climatic conditions.

Different types of conventional bioreactors have the common purpose of producing biogas. They differ, however, in the modes of operation, operating temperature, design of reactor or the materials used for the construction of reactors and total solid content.

Steel, bricks, plastics, concrete and stainless steel are materials commonly used to construct bioreactors. Some of the conventional biogas reactors (Patinvoh et al. 2017) are:

- *Fixed-dome reactors*: Consist of a hemispherical or cylindrical chamber constructed permanently underground and construction made by cement and bricks. Inlet and outlet valves of the reactor are connected to mixing and overflow tanks, respectively. Then biogas produced is collected in the gasholder. As the pressure increases due to the continuous production of gas, the digestate is ejected through the outlet and into the overflow tank. Because of the long life span, these types of bioreactors are primarily used in rural households.
- *Floating drum reactors*: Similar to fixed-dome reactors operationally. They possess floating drum for the separation of gas production and collection. Mild steel is used for the construction, and bricks are used to construct the bottom and walls of the reactor. The drum provides gas at constant pressure and is easy to operate.

5.4.2 Innovative Biogas Reactor Technologies

The additional modifications can improve the last three interrelated and strictly biological steps involved in biogas production. Common approaches towards this objective (Postawa et al. 2021) (Table 5.2):

Table 5.2 Type of reactor and digester for anaerobic digestion

1	Conventional biogas reactor technologies	(a) Fixed-dome reactor
		(b) Floating drum reactor
2	Innovative biogas reactor technologies	(a) Two-phase anaerobic digestion (TPAD)
		(b) Autogenerated high-pressure digestion (AHPD)
3	Three broad categories can be used to analyse the biogas industry	(a) Micro-digesters
		(b) Scale digesters
		(c) Medium- to large-scale digesters

- *Two-phase AD (TPAD)*: This approach involves the phase separation at different temperatures with the help of two reactors that's why this approach is commonly called temperature-phased AD. Temperature separation is one of the most common ways to separate phases (Sung and Santha 2003). The temperature in the first tank is nearly 65 °C for hydrolysis, while the lower tank is maintained at a temperature of around 35 °C (Pervin et al. 2013). The pH inside the reactors usually depends on the feedstock and remains neutral in the second stage (NT-TPAD) (Lay et al. 1997).
- *Autogenerated high-pressure digestion (AHPD)*: This is also a two-stage approach that depends on pressure instead of temperature. If there is a delay in the collection of fuel, then pressure is automatically increased due to the production of biogas, and so it can be raised up to 20 bar (Lindeboom et al. 2012). Change in pressure impacts the biological parts of the process directly but affects the final biogas composition. This may be attributed to the fact that there is no proportional change in the Henry's law constants for CH_4 and CO_2 with pressure, 0.0016 mol/L/bar and 0.318 mol/L/bar, respectively (Lindeboom et al. 2011; Wang et al. 2003). Thus, the solubility of carbon dioxide increases more rapidly than methane with pressure, leading to a rise in concentration of CH4 in the biogas, reaching 90% (Lindeboom et al. 2011). The first reactor is operated usually at high pressure and the second reactor at atmospheric pressure, enabling very pure yield in the first step and overall removal of chemical oxygen demand.

5.5 16S rRNA Gene Sequencing of Microbial Consortia for Anaerobic Digestion

In the procedure of anaerobic co-digestion, the microbial community dynamics is greatly affected by environmental factors and various waste streams. Since the microbial community in anaerobic digestion dramatically impacts the effectiveness of biogas production, it becomes a matter of importance to analyse and understand the microbial community involved. However, because of the insufficiency of the metabolic data on the microbes associated with the process, there is very limited information on the consortia of microbes in anaerobic co-digestion. The conventional molecular techniques do not fully understand 16S rRNA gene sequencing offers an efficient alternative to traditional molecular methods. This technique has the potential to identify and compare microbes in the sample. It also serves as an established technique analysing complex communities of microbes or environments that are otherwise difficult to study. Unknown details about how the microbes respond to the enhancement of the digester can be obtained from 16S rRNA gene-based fingerprints (Rabii et al. 2019).

5.6 Biogas Industry: Current Status

Despite having widely recognized applications of biogas technologies worldwide, the biogas industry is still yet to develop to its full potential and is currently in the initial developmental stages. According to a report by the World Biogas Association, three broad categories can be used to analyse the biogas industry:

1. *Micro-digesters*: They are an integral part of waste management, energy security and farming in rural areas of developing countries. Approximately 50 million micro-digesters are being operated around the globe. Of these, 42 million are working in China and 4.9 million in India. Among the Asian countries, China holds 84% of these digesters (Mohanty et al. 2021; Yang et al. 2019). Biogas produced by microscale digesters is mainly utilized as cooking fuel in stoves. 50 million stoves are used cumulatively for cooking purposes by about 126 million people (112 million in China and ten million in India).
2. *Scale digesters*: Electricity generation from biogas is a widely established and accepted technology worldwide. A CHP engine linked to any operating anaerobic digester is commonly used to recover some heat and use. Trigeneration of electricity, heat and cooling according to need is also gaining interest. China has approximately 110,448 biogas-based systems operated with large-scale digesters numbering 6972(2015). India has an estimated 300 MW biogas-based installed capacity.
3. *Medium- to large-scale digesters*: Upgradation of biogas to biomethane is a proven technology. Biomethane may either be utilized as a transportation fuel or introduced to national or local grids. Biogas is upgraded to biomethane by an estimated 700 plants globally.

5.7 Food Waste Digestion: The Potential

According to a report by the World Biogas Association:

- Approximately 1.6 billion tonnes of food are wasted per year, out of which 1.3 billion tonnes is edible and 0.3 billion tonnes inedible.
- 880 to 1100 TWh of energy can be generated if "all available" food (68.5% of food waste captured maximally for anaerobic digestion) waste/loss is collected and recycled through anaerobic digestion. The energy so generated can then be used for electricity and heat. This energy can meet the electricity needs of 112 to 135 million people.
- 85 to 100 bcm of biomethane upgraded from biogas can replace the natural gas consumption of Germany.
- Collection and recycling of "all available" food waste/loss can lead to mitigation in the emission of greenhouse gases equivalent to 510 to 560 metric tonnes CO_2, equal to the emissions of the United Kingdom.

- The digestate leftover is nutrient-enriched, and it can serve as an excellent fertilizer. This organic fertilizer can replace 1.1 Mt calcium, 5.03 Mt nitrogen, 0.13 Mt magnesium, 1.8 Mt potash, 0.58 Mt sulphur, and 0.75 Mt phosphate and return organic carbon to the soil. This can fertilize 53 million hectares of arable land (equivalent to Australia's arable land) by providing nutrients.

5.8 Biogas Production-Economic Perspectives

Anaerobic digestion of wet wastes for the production of biogas is a beneficial and well-established technology. It is a waste-to-energy technology having an emphasis on reducing the emission of greenhouse gas emissions as well, as the number of waste increases alarmingly and non-renewable fossil fuels pose a significant threat to the environment. We need to devise our WTE (waste-to-energy) technologies in such a way so that they can ensure the sustainability of energy by utilizing wastes which are economically viable also. Various factors need to be considered for determining the economic viability of the process (Bhatt and Tao 2020). Some of the factors that should be taken into consideration are:

- Direct capital costs (associated with the biogas reactors).
- Indirect capital costs including the cost of additional piping, which can be approximately 4.5% of Inside Battery Limits (ISBL), and other costs.
- Working capital (5% of fixed capital investment, FCI).
- Operating expenses include import of electricity, water required for the process, heat, and nutrients such as ammonia added to provide them to anaerobic digestion microbes.
- Other operational costs include maintenance and insurance of property, accounting for 3% of the capital costs for plants and 0.7% of the FCI, respectively.

5.8.1 Biogas Economics for Food Wastes

Since food wastes can have various sources, they exhibit a wide range of costs for anaerobic digestion. Food waste may include surplus food that is left after consumption, food that is lost either before or after meal preparation and the food thrown away during various processes such as manufacturing, retail, distribution and food services. They also exhibit wide variability in the VS amount and its conversion to biogas (Bhatt and Tao 2020).

Technological advancements leading to the enhancement of methane content in biogas can cut down the costs for anaerobic digestion and make them economically viable for small-scale plants. Novel pretreatment technologies, including biological, chemical and physical techniques, have been tested to enhance the energy intensity and productivity of biogas (Zhang et al. 2016). Co-digestion of wastes is also

profitable in this regard because they bring about nutrient balance and cut down the negative impacts of the harmful substances on digestion (Colantoni et al. 2017). Optimizing the operational parameters also helps in stabilizing the system and increase in biogas production.

5.8.2 Anaerobic Digestion of Food Wastes and the Circular Economy

Digestion of food wastes mediates an essential role in the circular economy (Banks et al. 2018).

- It circularizes the organic material and nutrients present in organic wastes and reverts them in the form of digestate, which serves as a bio-fertilizer in soil.
- It also improves the sustainability and self-sufficiency of industries by using their effluents to extract energy and use it further for electricity and heat generation.

5.9 Issues Related to Biogas Production

There are various limitational aspects related to biogas production. Some of the issues concerning biogas:

- Biogas contains certain harmful and unwanted substances, which are regarded as biogas pollutants, such as volatile organic compounds, Si, H_2S, siloxanes, NH_3 and CO (Korbag et al. 2020).
- NH_3 and H_2S are considered highly corrosive and toxic, which tend to damage the metal parts and cogeneration or CHP (combined heat and power) unit through the emission of SO_2 from combustion (Angelidaki et al. 2018; Abatzoglou and Boivin 2009; Tomassetti et al. 2019).
- H_2S is hazardous environmental emissions that damage the biogas purification machinery engines by corrosion. The quantity and quality of biogas are also affected by H2S, limiting their use (Farghali et al. 2020; Lar and Xiujin 2009).
- Methane released into the atmosphere could pose adverse environmental effects, causing a penalty for GHG emissions (Bhatt and Tao 2020).
- The mismanagement of consortia of the microbes involved in the anaerobic digestion process could lead to instability of the system and inefficient biogas production (Mao et al. 2015).

5.10 Future Prospects and Conclusion

- The increasing demand for energy and the adverse environmental impacts of fossil fuels are need of on renewable, clean and sustainable energy sources. Biogas from various organic wastes is the potential alternative to non-renewable energy sources.
- There are various types of food wastes also depending on the source and composition. Biogas produced from anaerobic digestion food wastes have multiple applications—they can be used for electricity generation, as cooking fuel, or can be upgraded to biomethane and used as a transportation fuel.
- Co-digestion techniques further tend to increase the yield and efficiency of biogas production systems.
- Extensive research and study should be carried out to design reactors with innovative technologies that can promote enhanced and efficient biogas production and being economically viable at the same time.
- Upgradation of biogas to biomethane possesses almost 100% efficiency levels as compared to just 40% in conversion of biogas to electrical energy (World Biogas Association report, Global Potential of Biogas 2019). This upgraded methane can be utilized for diverse purposes, such as injection into the gas pipes or as a transport fuel. Upgradation to biomethane involves removing carbon dioxide and other pollutants or impurities from biogas, resulting in the increase of methane content up to more than 97%. Membrane technology utilizing gas permeation technique for the separation of O_2, CO_2, and H_2 from CH_4 and N_2 has become the most preferred upgrading technology in recent years. More than 50% of all upgrading plants globally use membrane technology for upgradation of biogas to biomethane.
- Though food wastes hold great potential for biogas production, specific measures need to be implemented and followed to achieve this potential:
  - Awareness on the ill effects of food wastes and ways to prevent them should be raised.
  - Local governments should provide facilities for separate food waste collection to citizens.
  - GHG reduction, which results from anaerobic digestion of food wastes, should be acknowledged and incentivized.
  - Regulations and certifications for the safe trading and utilization of digested should be implemented.
  - In the era of urbanization, when energy demand increases exponentially, biogas production from the generated wastes is a very effective way of managing waste.

Biogas Production from food wastes is very beneficial in environmental protection, nutritional recovery and sustainable approaches. However, further research on the various aspects of biogas production from food wastes should be done to increase the efficiency of the process.

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Chapter 6
Biogas from Kitchen Waste

S. M. Bhatt

Abstract Biogas production is the best renewable technology which has opportunity to convert various biowaste released from agricultural, animal, industrial, and kitchen waste into energy. Biogas development has opportunity not only to improve sanitation but also to reduce air pollution and greenhouse gases.

India's current production of biogas is 2.07 billion m^3/year which should be around 29–48 billion m^3/year.

Anaerobic digestion process has widely been employed for treatment of various organic wastes for conversion into biogas and bio-fertilizer. A complex microbial community is used to degrade various organic compounds into final products such as methane and carbon dioxide, collectively called biogas. This has been explored in detail in the current book chapter based on recycle, reuse, and reduce. Most of the public are now aware and using dustbins as per government guideline. Organic composting is not possible without microbial community.

Keywords Methane · Biogas · Acetate · BioCNG

Abbreviations

AD	Anaerobic digestion
BMP	Biochemical methane potential
HVPD	High-voltage pulse discharge
McoDi	Mesophilic co-digestion of food waste and manure
MDi	Mesophilic digester
OFMSW	Fraction of municipal solid waste
TcoDi	Thermophilic co-digester
VFA	Volatile fatty acid

S. M. Bhatt (✉)
Agriculture Department, Amritsar College of Engineering and technology, Amritsar, Punjab, India

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6.1 Introduction

Food waste includes both precooked and peels of vegetables, left over of the food after eating, waste discharge from food processing industries and restaurants, and mess. FAO estimates food waste generated worldwide is around 1.3 billion ton which has been released from various sources such as vegetable mandi, fruit seller, bakery shop, and dairy and meat products, and these food waste degrades in open air causing so much pollution and inviting airborne infections (Ananno et al. 2021). In the next few 25 years, food waste generation is projected to increase almost more than double due to huge population growth mainly in Asian growth. According to an estimate, there may be rise in waste from 278 million tonnes to 416 million tonnes from 2005 to 2025. In India solid waste generated per year is around 62 million tonnes while 377 million by urban society, out of which 50% is food waste (Ghosh et al. 2018) is generated each year.

The major questions arises how to manage these food waste. In India basically fraction of municipal solid waste (OFMSW) technology is used to manage food waste. In this technology basically fraction of waste generated is segregated and pretreated.

Dissemination of waste does occur in bio-methanation plant where most of kitchen waste and food waste undergoes anaerobic digestion (AD) for production of compressed biomethane for running vehicle in the city (Shanmugam et al. 2019).

It has been noted that due to mismanagement of these biowastes, there is huge loss of nutrients useful for the plant, and biodegradation of waste leads to release of various metals and thereof pollutants in water (Chandra Manna et al. 2018).

Food waste digestion is done under anaerobic condition after proper treatment of organic waste which include shredding of waste into fine particle and then treatment at various stages to yield biogas and fertilizers. The yield depends on KPI.

6.2 Biofuel Classifications

As mentioned in Figs. 6.1, 6.2 and 6.3 based on food source, classification for biofuel into first, second, and third generations can be done. Bioethanol and biodiesel is an example of first-generation biofuel, while ethanol production via biomass such as lignocellulosic biomass is an example of second-generation biofuel, and bioethanol production from algae is an example of third-generation biofuel.

Food wastes like starch and vegetable are often categorized under first-generation biofuel. Bioethanol production with direct bioprocessing can be done after hydrolysis using yeast as microbes.

Naturally, Biogas production occurs of organic materials is digested under anaerobic condition, which needs rightly designed anaerobic biodigester with optimized condition for growth of microbes. Integrated modelling of bioreactor

Fig. 6.1 Different types of kitchen waste

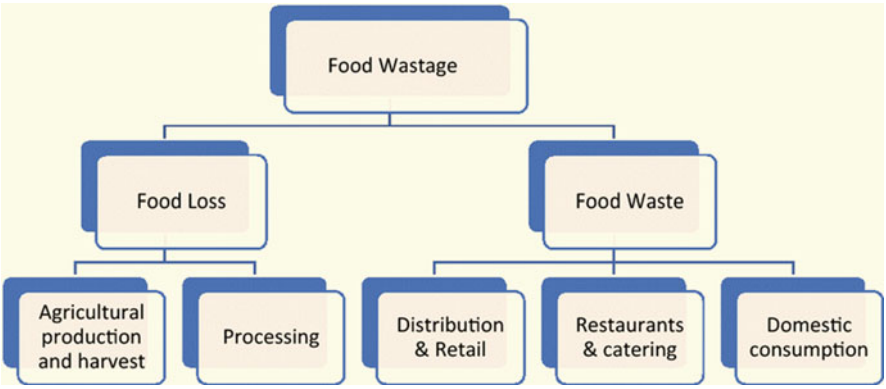

Fig. 6.2 Food waste classifications. (Modified from Lytras et al. (2021). Source: https://en.wikipedia.org/wiki/Food_loss_and_waste)

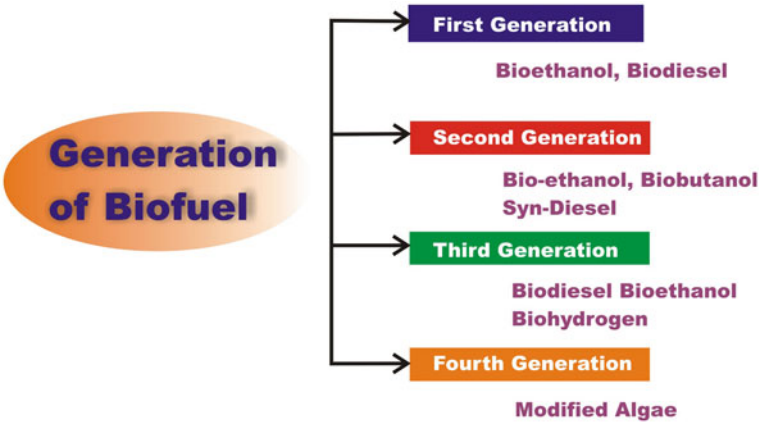

Fig. 6.3 Biofuel generation. (Source: https://en.wikipedia.org/wiki/Food_loss_and_waste)

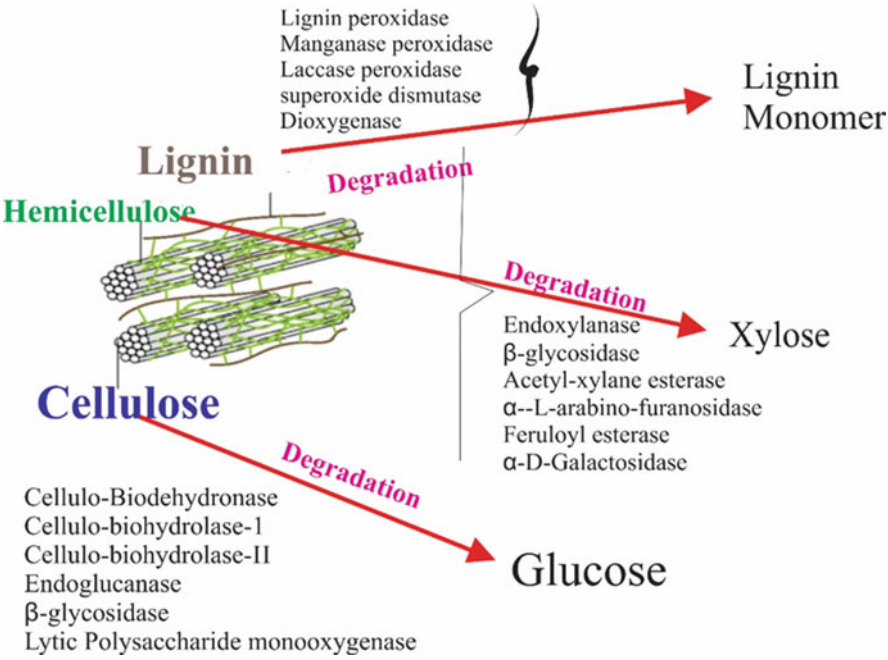

Fig. 6.4 Hydrolysis of lignocellulose various enzymatic steps involved (Champreda et al. 2019)

condition had a dual advantage that besides production of biogas, useful manure is also recovered.

Syngas synthesis gasification method is used to produce *syn*gas where oxygen organic matter gets pyrolysis after combustion. The carbon monoxide helps in converting gas.

Cellulase, Lignase Hemicellulase Pectinase Xylanse enzyme used in lignocellulosic based ethanol also depicted in Fig. 6.4.

6.2.1 Kitchen Waste Composition

Composition analysis of food waste, in many reviews, shows the basic components are carbohydrates, proteins, and lipid. The composition varies: (1) 60–80% moisture, (2) 3–5% ash, (3) 40–60% carbohydrate, (4) 18–30% volatiles, (5) 10–30% protein, (6) 15–40% fat, and (7) 45–65% carbon (Palaniveloo et al. 2020).

Protein-based meals are rich in mostly protein content. With moisture content of 4–7%, wheat meals are high in carbohydrates (range of 88–92%).

6.2.1.1 Biochemical Methane Potential (BMP)

Technique is applied for checking methane production potential with anaerobic biodegradation of wastewater and waste biomass.

Organic substrate degrades and releases methane and carbon. Generally BMP test assay is applied for mixed food waste containing boiled rice, peels of cabbage, and left over of cooked meat which are digested with cellulase as control (since greater rate of production of methane; 472 mL/g VS with total reduction in V Sup to 86%).

Another study conducted over canteen waste when mixed with wheat straw in different ratios in order to increase total methane production. As a result BMP reported was around 0.26 and 0.16 m^3 CH_4/kg-VS, respectively, and we conclude that food waste is easily biodegradable as high VS, while due to lignin the straw is difficult to degrade anaerobically.

Four phases involved in the biogas production such as (1) enzymatic hydrolysis, (2) acidogenesis, (3) acetogenesis, and (4) finally methanogenesis.

Steps in methanogenesis and biogas production:

1. Hydrolysis: These microbes secrete various types of enzymes that hydrolyzes complex food materials into its monomer like glucose fatty acid and amino acids.
2. Then monomer like glucose FA and amino acid get converted to higher volatile fatty acids, into propionic and butyric acids, by hydrogen-producing acetogenic bacteria produced, to H_2, CO_2, and acetic acid.
3. Finally, methanogenic bacteria convert all acetate and others products to CH_4 and CO_2.

   (a) Kitchen waste first collected.
   (b) Shredded into fine particles.
   (c) Substrate hydrolysis.
   (d) Acidogenesis convert hydrolysed substrate into acid which is used by microbes which convert acid into acetate E) ACETATE is used as substrate to methane and CO_2.
   (e) Hydrolytic enzymes (lipases, proteases, cellulases, amylases) are released to convert waste into various types of acids which are being converted into acetic acid.
   (f) Lipases convert lipids to long-chain fatty acids. *Clostridia and the micrococci* known for extracellular lipase production. The long-chain fatty acids produced are further degraded by *p*-oxidation to produce acetyl CoA.
   (g) Proteins are generally hydrolyzed to amino acids by proteases, secreted by various microbes such as *Clostridium*, *Bacteroides*, *Butyrivibrio*, *Fusobacterium*, *Streptococcus*, and *Selenomonas*. The amino acids produced are then degraded to fatty acids such as acetate, propionate, butyrate, and ammonia as found in *Clostridium*, *Peptococcus*, *Selenomonas*, *Campylobacter*, and *Bacteroides*.
   (h) Polysaccharides such as cellulose, starch, and pectin found in the kitchen waste are hydrolyzed by enzyme secreted by the cellulases, amylases, and pectinases. The majority of microbial cellulases are hydrolyzed to produce glucose. While Raw starch present in food waste is converted to glucose by

amylolytic activity of amylase enzyme. Five amylase species need to be active which includes (a) α-amylase (cleaves 1–4 bonds), (b) β-amylase (cleaves 1–4 bonds), (c) amyloglucosidase (cleaves l-4 and l-6 bonds), (d) debranching enzyme (cleaves l-6 bonds), and (e) maltase that acts on maltose-liberating glucose. Pectins are degraded by pectinases, while xylans to produce xylose.

Microbes Required for Hydrolysis

To know the microbes required for hydrolysis of food waste. There are five types of food waste that were investigated in anaerobic digester to produce biogas (Chen et al. 2010). Waste used from soup-processing plant and kitchen waste of fish farm were under experimental analysis.

Anaerobic digestion mostly yield 60% methane and 40% CO_2, and it has been observed and reported that formation of methane is good by using thermophilic microbes such as *Syntrophaceticus schinkii* acetogenic microbes which release acetate and methane but requires hydrogenotrophic methanogens. The diversity of thermophiles analyzed in biogas was *Syntrophaceticus* (38.24%), *Gelria* (23.53%), *Thermogymnomonas*, etc. (Kushkevych et al. 2020) (Tables 6.1 and 6.2).

Table 6.1 Microbes and their reaction and product (Krzysztof Ziemiński 2012)

Reaction Type	Microorganism	Active Genera	Product
Fermentation	Hydrolytic bacteria	*Bacteroides, Lactobacillus, Propionibacterium, Sphingomonas, Sporobacterium, Megasphaera, Bifidobacterium*	Simple sugars, peptides, fatty acids
Acidogenesis	Syntropic bacteria	*Ruminococcus, Paenibacillus, Clostridium*	Volatile fatty acids
Acetogenesis	Acetogenic bacteria	*Desulfovibrio, Aminobacterium, Acidaminococcus*	CH_3COOH
Methanogenesis	Methanogens (Archaea)	*Methanosaeta, Methanolobus, Methanococcoides, Methanohalophilus, Methanosalsus, Methanohalobium, Halomethanococcus, Methanolacinia, Methanogenium, Methanoculleus*	CH_4

Table 6.2 C/N ratio in different wastes

Material	% N	C : N
Animal urine	15–20	1
Cotton stalks	1.7	30
Cow, buffalo manure	1.4–3	15–40
Oat straw, flax straw	1–1.2	50–60
Wheat and rice straw	0.3–0.5	120–150
Sawdust	0.1–0.25	200–500

For enzymatic hydrolysis by *Streptococcus* and *Enterobacter* are the main genera of anaerobic microbes that are responsible for enzymatic hydrolysis and degradations mainly for degradation of polysaccharide into monomer various mesophilic bacteria, under optimal conditions.

Hydrolytic product forms such as acetate, butyrate, propionate, and valerate (volatile FA products), along with isobutyrate some carbon dioxide, NH3, and hydrogen.

Under anaerobic condition, facultative anaerobes require some amount of oxygen and carbon to produce methane. The main substrates used for methane productions are acetate, carbon, and hydrogen.

One study was conducted by co-digestion of kitchen waste/food waste by mixing cow dung/manure during methanogenic production (Zamanzadeh et al. 2017).

In the mesophilic digester, the highest methane yield (480 mL/g VS) was observed when fed with food waste alone. While codigestion of manure yielded more methane (26%) which is sum of individual digestions of manure and food waste. The main volatile fatty acid (VFA) in the mesophilic systems was acetate, averaging 93 and 172 mg/L, respectively.

The main VFAs found in most of the digester were acetate and propionate. The prominent bacteria present and reported were *Firmicutes*, *Thermotogae*, and *Synergistetes* present in the digesters, however, the relative abundance of these phyla were different (see Tables 6.1 and 6.2).

Methanogenesis

Substrate for methane production mostly uses acetate, hydrogen, and carbon dioxide, but VFA like valerate, propionate, butyrate, and isobutyrate are the most relevant and mostly is used by the acetogenic bacteria to convert them into the acetate and hydrogen. As we know More will be the acetate in the media that is reduced finally and changes into the methane.

Methane production is also affected by C/N ratio and it must be more than >19.6 (See Table 6.2). Use of thermophilic microbes is more beneficial as compared to mesophilic microbes as temperature mostly rises beyond optimal level.

Therefore, Methanogens are of two types: (a) acetoclastic methanogens (basically produces methane from acetic acid) and (b) hydrogenotrophic methanogens (hydrogen is used to reduce carbon dioxide) (Fig. 6.5).

Methanogenesis uses CO_2 as a terminal electron acceptor to convert other substrates into methane. Thus, methanogens mostly grow and found in such habitats where electron acceptors are present such as O_2, NO^{3-}, Fe^{3+}, and $SO_4{}^{2-}$ (Berghuis et al. 2019; Kato and Igarashi 2019).

Kitchen waste must be degraded into simple more simple products (H_2, formate CO_2, and acetate) which get converted into ethyl-containing compounds, substrates for most of the methanogens. Thus methane is produced.

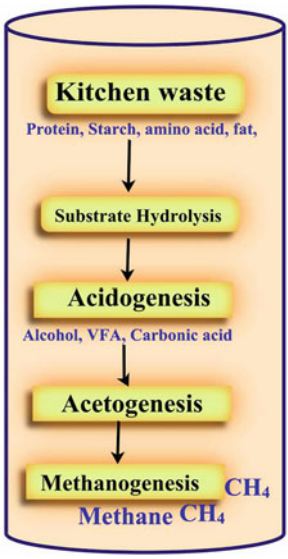

Fig. 6.5 Depicting flow chart in biogas production. To enrich high methane production >70%, it's essential to follow each step since all the steps are connected to each other. The product of one step is a substrate of other steps (Paritosh et al. 2017)

Classification of methanogens based on their substrate:

1. Hydrogenotrophic.
2. Aceticlastic.
3. Methylotrophic.

Hydrogenotrophic methanogens reduce CO_2 to CH_4. Hydrogenotrophic methanogens were found in deep marine sediments, termite hindguts, and human and animal gastrointestinal tracts, which altogether contribute a third of biologically generated methane emissions. There are about 1.5 billion cows on earth, and a cow releases around 200 L of methane per day. Thus, the total methane by is released about 300 billion liters per day or 72 Tg per year (Zhuang et al. 2018).

Aceticlastic methanogens convert acetate into CH_4 and CO_2. Hydrogenotrophic methanogens have the capacity to reduce H_2 to make conducive environment for acetate formation. Aceticlastic methanogens mostly found in anaerobic digesters play important role in methane production.

In the aceticlastic pathway, formation of acetyl-coenzyme leads to oxidation of acetate and CO_2 with ferredoxin as the electron acceptor.

In an anaerobic digester, a consortium of microorganisms exist which are involved in breakdown of organic waste into biogas. Hexose metabolism via the Embden-Meyerhof-Parnas pathway (EMP) utilized by most anaerobic bacteria which convert hexoses and pentoses to C2 and C3 intermediates (with reduced electron carriers (e.g., NADH) produces pyruvate with NADH. The pyruvate and NADH are converted into lactate, propionate, acetate, and ethanol.

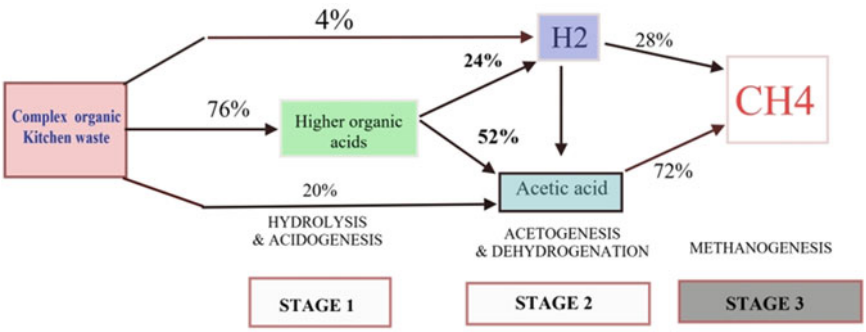

Fig. 6.6 Stages in kitchen waste conversion source http://www.fao.org/3/w7241e/w7241e0f.htm

In Fig. 6.6 decomposition of VFA has been mentioned which is degraded into acetate and hydrogen (VFA is a long-chain fatty acids, into acetate and H_2 by an *acetogen* and *Clostridium formicoaceticum*, respectively.

6.2.1.2 Pretreatment Methods for Food Waste

To increase the crystallinity of the food waste, they must be pretreated before actual hydrolysis. There are various types of pretreatment technology available, but their application is decided by the type of food waste. Mechanical, thermal, chemical, and biological types of pretreatment are existing and can be applied, thermal method (Ariunbaatar et al. 2014) at low temperatures (<120 °C) only. The result obtained was 647.5 ± 10.6 mL CH_4/gVS, thermal pretreatment at 80 °C for 1.5 h.

Chemical pretreatment include acidic pH, results in increased ammonia, and accumulation of volatile fatty acids.

The dairy waste, brewery waste, and livestock waste mostly are suitable for ammonia production (Meena et al. 2020).

Pretreatment of food waste using microwave (7.8 °C/min) resulted in biogas production with 24% higher COD solubilization (Paritosh et al. 2017).

Food waste valorization has been recommended in some case (Lytras et al. 2021); it has advantages that it yields almost pure methane and separates all other toxic components. Largest arising of food waste occurs from households; however, domestic food waste has been excluded from the scope of valorization to animal feed in REFRESH. This is due to the greater uncertainty regarding additional process controls required to mitigate risks and meet acceptable feed safety and quality standards.

Some researcher worked on optimization of H_2 production via methane route from waste oil (Rafieenia et al. 2019). Nonbiodegradable, recalcitrant organic food waste was pretreated with fungal mash with the prolonged hydrolysis, for the methane production (Ma et al. 2018). High-voltage pulse discharge (HVPD) pretreatment is the new technology to enhance the production of methane, and successfully it was able to enhance the production up to 160% (Zou et al. 2016).

6.2.2 Biogas Digester

Biogas digester is an airtight anaerobic digestion used for digestion of various kitchen wastes and other waste. Biogas digesters may be classified into (1) passive systems (low control of the anaerobic digestion process), (2) low-rate systems, and (3) high-rate systems (methane-forming bacteria is trapped in the digester to enhance the biogas production efficiency) (Alkhalidi et al. 2019).

Small biogas systems (portable bio-digester) often used with small volumetric capacity ranging from 1 to 10 m^3) biogas per day where feedstocks is kitchen waste producing biogas and bio-slurry (can be converted as organic fertilizers). As compared to small-scale biogas plants and industrial-scale plants, it has larger capacity of 1000–5000 m^3 biogas/day. Such large-capacity biogas is largely utilized in the municipal or industrial organic wastes to generate biogas.

Biogas used as cooking fuel known as LPG is produced mostly by PP mode so that biogas produced at large scale may be utilized properly.

Feedstock type generally varies in India, so digester type has to change every time (He et al. 2021; Song et al. 2014a, b).

6.2.3 Barriers in the Biogas Production (Mittal et al. 2018)

The following are barriers in commercial productions of biogas:

1. High cost of installations.
2. Lack of financial support.
3. There are variations in feedstock supply which may affect supply chains.

Plant profitability depends on various factors if really someone wants to do it in the long run in India.

6.3 Conclusion

In conclusion biogas has numerous advantages: (1) it can be elevated, (2) it can be bottled and easy to transport, and (3) biomethane is also used in CNG vehicles without engine modification (Vijay et al. 2015).

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Chapter 7
Food Processing By-Products and Waste Utilisation for Bioethanol Production

Naman Kaur, Aparna Agarwal, and Manisha Sabharwal

Abstract Globally, a rising trend in population and economy has caused an increase in the global demand for energy. Although, fossil fuels have been the most predominantly used fuel, the resources utilised for the production of fossil fuels have begun to deplete. This has resulted in the emission of various deleterious gases, which have consequently caused global warming including various alterations in climate, environment and biodiversity. In order to combat this challenge of depleting fossil fuel reservoirs and global warming, biofuels have been considered a suitable candidate for substituting fossil fuels. Biofuels are a sustainable and environment-friendly form of energy which are produced from biomass. Over the past few decades, researchers are persistently finding novel sources that can serve as the feedstock to generate biofuels. Of the variety of biofuels being generated, bioethanol has been recognised as a promising biofuel because of the various advantages associated to its chemical properties, cost of production, sustainability and impact on environment. Additionally, bioethanol is one such biofuel that requires carbohydrate source for its production. Therefore, the interests of various researchers have now inclined towards wastes generated from food industries. Food waste is generated from different sectors including agriculture, municipalities, industries, forestry and corporations. The food processing wastes are a rich source of various nutrients. Mostly, these wastes are either discarded in landfills or are used as an animal feed. However, their disposal has been reported to result in severe environmental hazards. Therefore, to avert this challenge of environmental pollution, researchers have considered valorisation of food waste into biofuel an efficacious strategy, especially bioethanol.

This chapter deals with the importance of utilisation of food waste for the production of biofuels, with primary focus on bioethanol production. The chapter also elucidates the types of feedstocks used for bioethanol production. It also delineates different types of food processing wastes and strategies to employ them for bioethanol production.

N. Kaur · A. Agarwal (✉) · M. Sabharwal
Department of Food and Nutrition, Lady Irwin College, University of Delhi, New Delhi, Delhi, India

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Keywords Biofuel · Bioethanol · Biomass · Food processing waste · Valorisation · Lignocellulosic feedstock · Second-generation bioethanol

7.1 Introduction

Currently, fossil fuel is a primary source of energy and of its 80% contribution; transport sector acquires 58% of it (Escobar et al. 2009). The persistent growth in global population and industrial economy has resulted in a decline in fossil fuels and, consequently, an increase in the demand for energy. The rapid depletion in the sources of fossil fuels and oil reserves has been found to be the leading cause for emission of harmful gases which are known to result in melting of glaciers, loss of biodiversity, change in climatic conditions and rise in sea level. The rising energy demand has also adversely affected the global economy by resulting in increased crude oil prices (Agarwal 2007). To meet the demand for energy while keeping into account the issues related to global warming, development of alternate energy has become a priority of utmost importance in the research and developments sector. A sustainable form of alternate energy, known as bioenergy which is produced from the biomass, has earned high endorsement in several sectors including government, public and private. Researchers across the world are persistently working to produce biofuel from sustainable biomass as it is an efficacious substitute for non-renewable fuels (Weldemichael and Assefa 2016). The biofuels can be derived easily from the biomass, and because of their biodegradable nature and combustion based on carbon dioxide cycle, they are not only sustainable but also environment-friendly. It has been estimated that demand for biofuels in automobile market may increase rapidly over coming decades as a result of their environmental benefits, which will subsequently lead to elevated growth in agriculture sector for higher yield and their by-products (Kim and Dale 2005; Demirbas 2008). There exist three types of biofuels, namely, solid, liquid and gaseous fuels. On the basis of chemical and complex nature of biomass used as the feedstock, biofuels are categorised into three generations which are first, second and third. The first-generation fuels, for example, biodiesel and vegetable oils, are those that are obtained from crop plants; the second-generation fuels which include bioethanol and biohydrogen are derived from agricultural wastes and/or by-products and energy crops which require fertile regions for their growth; and the third-generation fuels such as biogas and bioalcohols (ethanol and biobutanol) are those that are extracted from marine sources such as seaweeds and cyanobacteria. Although biofuels are principally contingent on terrestrial plants, which have the limitation of cultivable land exploitation, recently, marine sources have gained much interest for the production of biofuels because they do not require land for their growth and possess the ability to yield huge biomass in a required time duration (Demirbas 2008).

Another, such environment-friendly and sustainable feedstock for the production of biofuels is food processing waste, also known as food waste (FW) and food processing by-products. Mostly, FW is the final product generated from several food industries that has not been recycled and is disposed as waste. Several factors exist at

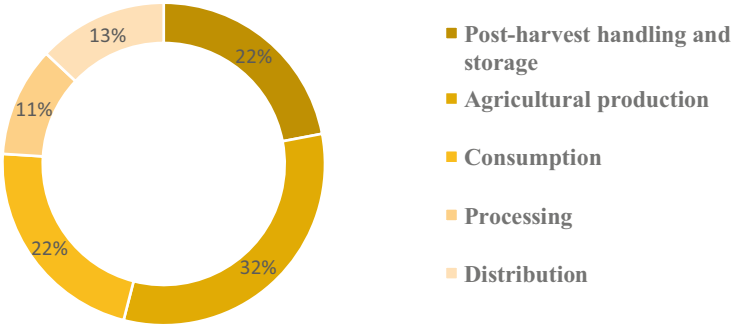

Fig. 7.1 Global relative food wastage in the food supply chain. (Adapted from Food and Agriculture Organization of the United Nations 2013b)

almost all the stages of food processing at an industry which lead to food wastage in considerable quantity (United Nations Industrial Development Organization 2012). It has been reported that every year nearly 1.3 billion tons of FW is discarded, which accounts to nearly one-third of global food production for human consumption (Gustavsson et al. 2011, 2013). It has also been reported that, in developed countries, most of FW is generated as a result of food disposal by consumers who buy more than the required amount and dispose the food not consumed by them, while in developing countries, the leading cause of food wastage is as a result of inappropriate harvesting strategies, lack of competent infrastructure, processing and packaging provisions and inefficacious marketing information (Zorya et al. 2011). Food wastage does not only pose challenges to a nation's economy but also leads to climate change, since production of food requires water, seeds, fertilisers, energy, pesticides as well as labour. In 2014, Food and Agriculture Organization of the United Nations stated that, besides averting the exploitation of natural resources, reduction in food wastage would also decline the demand to augment food production by 60% to meet the population needs by 2050 (Food and Agriculture Organization of the United Nations 2013a). The global relative food wastage in the food supply chain has been depicted in Fig. 7.1.

FW comprise of a wide range of organic constituents such as carbohydrates (35.5%–69%), proteins (3.9%–21.9%), organic acids, oil and fats (Kiran et al. 2014; Axelsson et al. 2012), of which the sugar and protein components are recovered and degraded to fermentable sugar and free amino nitrogen (FAN) (Pleissner et al. 2013). Application of biomass to produce biofuel enhances its value in comparison to its utilisation in the production of chemicals, electricity and animal feed (Lin et al. 2013; Tuck et al. 2012). Additionally, valorisation of FW to biofuels is capable of reducing the reliance on crude oil (Li and Yang 2016).

Of the many biofuels, bioethanol has been recognised as the most promising substitute of fossil fuels that can be generated from a variety of renewable sources which are rich in carbohydrates. Several countries including the USA, China, Brazil and Canada and various EU member states have announced agreement to programmes for the production of bioethanol in order to decrease the reliance on

fossil fuels (Zabed et al. 2016). Conventionally, bioethanol is produced from crops rich in starch content such as sugar cane, rice, corn and potato (Kiran et al. 2014). However, various food processing and agricultural wastes have also been identified as a suitable alternative for the production of bioethanol. These include vegetable and fruit peels, seeds, cheese and tofu whey, blood, bone, processed water and wastewater treatment sludge. These FW are composed of certain worthwhile nutrients which signify valuable biomass (Dar et al. 2019).

This chapter deals with the significance of bioethanol as a biofuel, feedstock and steps involved its production. It primarily focuses on describing certain food processing by-products and waste which instead of being disposed can be utilised in the production of bioethanol. This chapter emphasised on the need to envisage on FW which can be used a potential feedstock for the generation of biofuels such as bioethanol.

7.2 Applications of Bioethanol

Of all the commercially available biofuels, bioethanol is one of the most used liquid fuel, which can be combined with gasoline for regular use. This is attributed to the high oxygen content (35%) of ethanol which permits enhanced combustion of hydrocarbons while reducing the emission of carbon monoxide and other potentially hazardous hydrocarbons (Gebregergs et al. 2016). As a result, bioethanol has comparatively lower negative impact on environment than other fuels and, hence, is an environment-friendly biofuel with higher acceptability. Bioethanol can either be used directly or in combination with gasoline, thus, substituting gasoline with efficacious applications. The combination of bioethanol and gasoline has been found to be the most appropriate as a result of the high-octane number of ethanol (106–110) than gasoline (91–96) (Bhuvaneswari and Sivakumar 2019; Zabed et al. 2016). The property of bioethanol permits its combustion at a higher compression ratio with shorter combustion period, which results in a lower engine knock. Additionally, bioethanol also has a higher evaporation enthalpy (1177 kJ/kg at 60 °C) in comparison to gasoline (348 kJ/kg at 60 °C). Also, it has a relatively higher laminar flame speed (around 33 and 39 cm/s at 100 kPa and 325 K for gasoline and bioethanol, respectively) (Naik et al. 2010; Bayraktar 2005; Al-Hasan 2003). In addition, the higher heat of vaporisation of bioethanol (840 kJ/kg) in comparison to gasoline (305 kJ/kg) ascertains enhanced volumetric efficiency of bioethanol blend in comparison to that of pure gasoline, consequently augmenting power output (Lynd 1996). One such bioethanol-gasoline blend known as E85 (85% bioethanol and 15% gasoline) is utilised in various light-duty vehicles (Bhuvaneswari and Sivakumar 2019). In comparison to gasoline, bioethanol comprises insignificant proportion of sulphur; however, combining both results in the reduction of total sulphur content in the fuel and subsequent reduction in sulphur oxide emission, a carcinogen responsible to cause acid rain. Furthermore, bioethanol has been reported as a safer substitute to methyl tertiary butyl ether (MTBE), which is mostly used for

Table 7.1 Properties of gasoline and ethanol adapted from (Yüksel and Yüksel 2004)

Properties	Gasoline	Bioethanol
Chemical formula	C_nH_{2n} + 2 (n = 4–12)	C_2H_5OH
M/(g/Mol)	100–105	46.07
Octane number	88–100	108
r/(kg/dm^3)	0.69–0.79	0.79
Boiling point/°C	27–225	78
Freezing point/°C	−22.2	−96.1
Flash point/°C	−43	13
Auto-ignition temperature/°C	275	440
Lower heating value. 10^3/(kJ/dm^3)	30–33	21.1
Latent vaporisation heat/(kJ/kg)	289	854
Solubility in water	Insoluble	Soluble

gasoline as an octane enhancer (Zabed et al. 2016). The properties of gasoline and bioethanol are provided in Table 7.1.

7.3 Bioethanol Production

Bioethanol can be generated from a variety of food processing wastes and by-products. Since, bioethanol is mostly produced by fermenting the sugar constituents of plants and starchy crops, it is also known as grain alcohol (Shah and Sen 2011). Although any type of carbohydrate can be employed for the production of this fuel, the raw materials are typically classified into two categories, namely, sucrose-containing material and starchy crops. However, with recent advancement in technological development, lignocellulosic waste materials also known as cellulosic biomass like wood, bagasse and straw have also been recognised as suitable raw material for prudent bioethanol production (Demirbas 2008; Balat 2011; Shah and Sen 2011). However, studies have reported that since the process of producing bioethanol from lignocellulosic biomass is complex and longer in duration, it is expensive in comparison to starchy crops (Shah and Sen 2011). The raw material employed in the production of this fuel plays a vital role in determining the energy yield. Studies have reported that sugarcane and cellulosic bioethanol yield ninefold energy in comparison to the fossil energy. Over the past few years, of a variety of biomass used to produce bioethanol, sugarcane juice and molasses are most exploited yielding hydrated and anhydrous bioethanol (Kamani et al. 2019).

Additionally, several recent studies have revealed the potential use (by software simulation) of technologies that could contribute in the reduction of the environmental impact. Nevertheless, it is still crucial to investigate the processing cost, the purity of bioethanol derived from the different biomass and the practical implementation of the systems, as these are the chief challenges faced during the production of bioethanol (Dahiya et al. 2018). Besides, during bioethanol production, there is a

production of a large amount of waste which is required to be valorised. This is because they too can be reused to generate more bioethanol due to the presence of other valuable compounds (Kamani et al. 2019).

The renewable sources employed for the production of bioethanol are broadly classified into sugars, starch, lignocellulosic biomass and algae. Ethanol generated from sugars and starch-based raw material is known as the first-generation bioethanol, whereas ethanol produced from lignocellulosic biomass and algae are known as second and third-generation bioethanol, respectively. Although production of bioethanol from algae is still a new area which is currently confined to the laboratory research, other variety of biomass has shown greater potential as bioethanol feedstocks on commercial scale. The production of bioethanol from three major varieties of feedstocks varies significantly, especially with respect to obtaining sugar solutions. Sugar-based feedstock requires only an extraction process to obtain fermentable sugars; however, starch-based feedstock needs to go through hydrolysis in order to convert starch into glucose; and the lignocellulosic biomass requires to be pretreated prior to hydrolysis to modify cellulose structures to provide enzyme accessibility (Zabed et al. 2016).

7.3.1 Sugar-Based Feedstock

Bioethanol production from biomass broadly involves processes like enzymatic hydrolysis, fermentation and distillation/dehydration. In the first step, the homo- and heteropolysaccharide constituents of the biomass utilised for bioethanol production are hydrolysed either enzymatically or by dilute acids into sugars. This process is also known as saccharification, and it yields fermentable sugar-containing solution (Bhuvaneswari and Sivakumar 2019; Shah and Sen 2011; Gavahian et al. 2019)**.** The obtained solution can be further hydrolysed by yeast-derived invertase to release simple sugars, e.g. glucose and fructose. Subsequently, the simple sugars are fermented further using *Saccharomyces cerevisiae* yeast to yield bioethanol (Dudley 2004; Panda et al. 2018; Demirbas 2008; Gavahian et al. 2019). The last step involved in the production of this biofuel is distillation/dehydration, which is applied to the fermented broth to recover and concentrate the bioethanol. This step consumes extensive energy which accounts for a considerable portion of bioethanol production cost (Gavahian et al. 2019). Generally, the fermented broth comprises nearly 12% ethanol, which can be purified up to 96% by the process of distillation. The general steps involved in the production of bioethanol from sugar-based feedstock are represented schematically in Fig. 7.2.

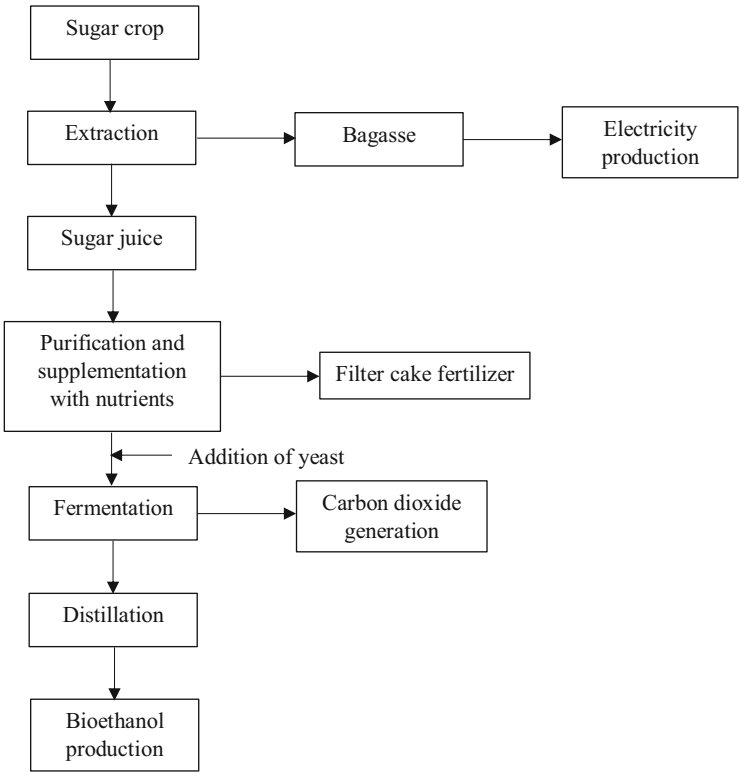

Fig. 7.2 Schematic representation of bioethanol production from sugar-based feedstock. (Adapted from Ramírez de la Piscina and Homs 2008)

7.3.2 Starch-Based Feedstock

Other than sugar-based feedstock, starch-based feedstock is also utilised for the production of bioethanol. Starch a combination of linear (amylose) and branched (amylopectin) polyglucans is hydrolysed using α-amylase enzyme which is active only on α-1,4 linkages of amylose, and not on α-1,6 linkages in amylopectin (Mousdale 2008). In order to produce bioethanol using this feedstock, hydrolysis of starch using α-amylase and glucoamylase is a vital step to obtain glucose syrup, which is subsequently converted to ethanol using *Saccharomyces cerevisiae* yeast. This step adds to the overall cost of bioethanol production from sugar-containing feedstocks (Ricardo Soccol et al. 2011). *Bacillus licheniformis* and genetically modified strains of *Escherichia coli* and *Bacillus subtilis* are employed for the production of α-amylase, while *Aspergillus niger* and *Rhizopus sp.* are used to produce glucoamylases (Shigechi et al. 2004). Bioethanol obtained from starch-based feedstock helps in enhancing enzyme application and yeast strains with high ethanol tolerance in comparison to bioethanol produced from sugar-based feedstock

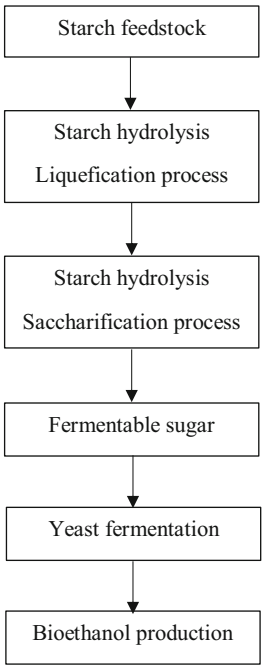

Fig. 7.3 Schematic representation of bioethanol production from starch-based feedstock. (Adapted from Woiciechowski et al. 2016)

(Schubert 2006). The general steps involved in the production of bioethanol from starch-based feedstock are represented schematically in Fig. 7.3.

7.3.3 Lignocellulosic Feedstock

The process of bioethanol production varies depending upon the type of feedstock employed. In some cases, lignocellulosic feedstock is utilised for bioethanol production. Lignocellulose is a complex of poly-carbohydrates comprising lignin, cellulose and hemicellulose. This type of feedstock is initially pretreated for delignification to extract cellulose and hemicellulose before hydrolysis. This step is essential to rupture the matrix, reduce the degree of cellulose crystallinity, expand the fraction of amorphous cellulose and essentially make lignocellulosic feedstock highly susceptible to further treatment like hydrolysis to enhance the yield of monomeric sugars. The pretreatment of the biomass can be done using different techniques, which may be physical, for example, reduction in the size of the biomass, microwave heating, pyrolysis and non-thermal irradiation; chemical, for example, wet oxidation and treatment with acids or alkalis; physico-chemical, for example, steam, ammonia fibre or CO_2 explosion; or biological, for example, microbial treatment using varieties of fungi (Sarkar et al. 2012). Following pretreatment, the pretreated biomass is subjected to enzymatic hydrolysis of

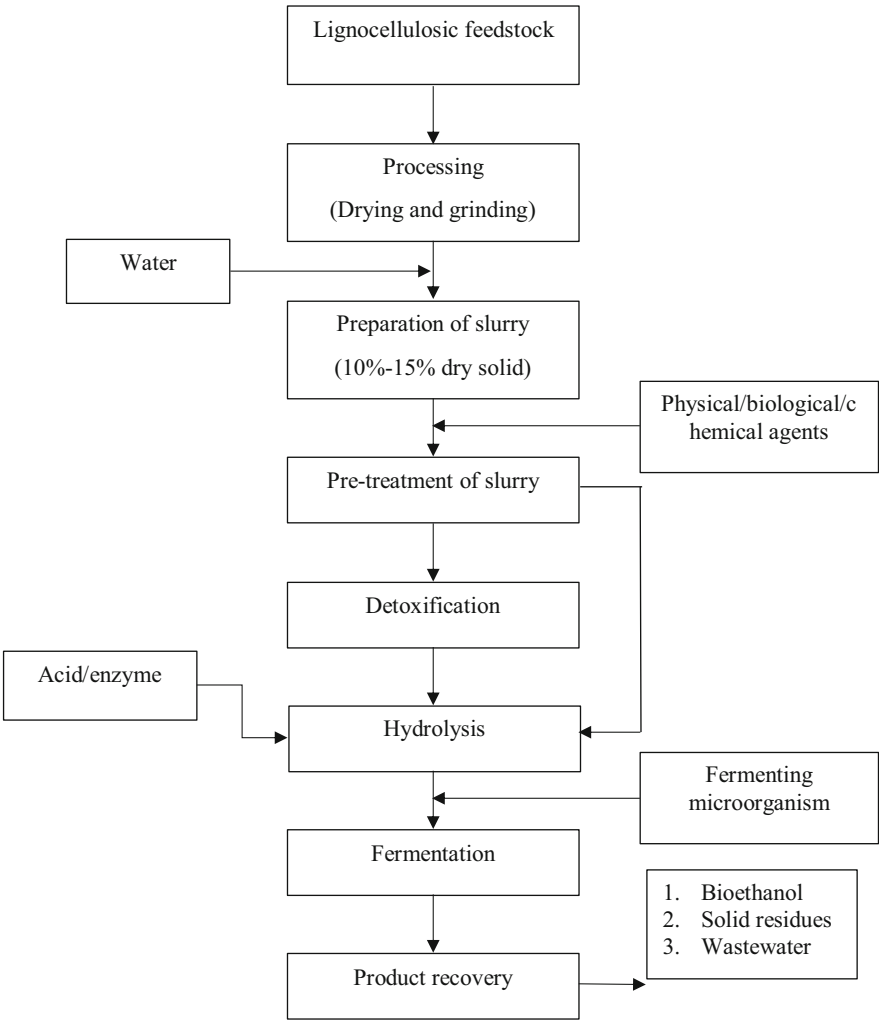

Fig. 7.4 Schematic representation of bioethanol production from lignocellulosic-based feedstock. (Adapted from Zabed et al. 2016)

cellulose and hemicellulose to produce fermentable form of sugars like glucose, galactose, arabinose, xylose and mannose. This step involves breaking down of glycosidic linkages to obtain hydrolysed sugars like pentoses and hexoses, which are then fermented to generate bioethanol (Sarkar et al. 2012; Demirbas 2008). The general steps involved in the production of bioethanol from lignocellulosic-based feedstock are represented schematically in Fig. 7.4.

7.4 Significance of Utilising Food Processing By-Products and Waste for the Bioethanol Production

Food Agriculture Organisation (FAO) has implemented to attain its specific target in the Sustainable Development Goals (SDGs), designed specifically to ensure food security for the rapidly rising global population. It has been estimated that about 44% to 47% of the total food waste is produced by households and a substantial part of this waste (23–28%) originates from the inedible portions of fresh fruits and vegetables including skin, peels, as well as trimming (Open Working Group on Sustainable Development 2015; De Laurentiis et al. 2018). Such wastes have been recognised as inevitable waste which is produced, regardless of the measures adopted for prevention, unless there is a change in the consumption patterns. Hence, considering the processing as well as treatment strategies such as valorisation of these resources to value-added products like biofuels in order to manage and leverage the potential of these resources is vitally important (Shehu et al. 2019). Cumulative (%) food wastage across India has been represented in Fig. 7.5.

Waste is generated from various sectors, including agriculture, municipalities, industries, forestry and corporations (Gosavi et al. 2017). Currently, waste has become a chief concern globally, especially in Europe and in developing countries such as India and China (Lin et al. 2013). FW is described as residue which persists following the processing of a primary product. A huge portion of solid and liquid fuel is generated from various food industries due to various reasons including the preparation, production and consumption of food, processing losses, inappropriate transport systems, contamination during storage and inappropriate packaging (Girotto et al. 2015). FW such as peels and seeds of fruits and vegetables, cheese and tofu whey, blood, wastewater and bone comprise of several worthwhile

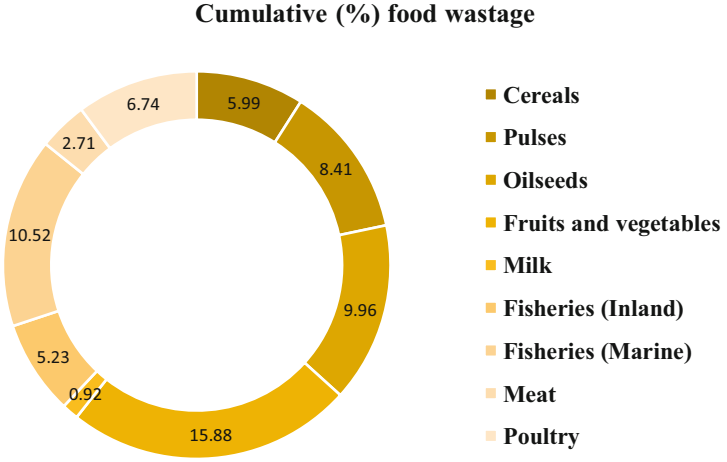

Fig. 7.5 Cumulative (%) food wastage. (Adapted from Ministry of Food Processing Industries 2015)

nutrients, hence, representing valuable biomass. However, if these are left untreated and unmanaged, their rampant decomposition will result in environmental pollution due to the release of toxic constituents and methane (Waldron 2007). Hence, utilising these wastes which would generally be disposed is an efficacious and cheaper strategy to generate bioethanol (Gosavi et al. 2017). Recently, significant consideration has been given to lignocellulosic biomass as a potential feedstock for the production of chemicals and fuels as well as bioethanol sustainably (Al Azkawi et al. 2018). Several countries describe bioenergy as energy which is derived from biodegradable wastes and residues from fruits and vegetables (Panda et al. 2018). Over the recent years, various forms of wastes have been utilised for the production of a variety of biofuels. Commonly, the waste generated from different sectors is discarded in dumping sites or landfills, which leads to land and water contamination (Hossain et al. 2011). This undesired environmental pollution can be avoided if the waste is employed for the production of biofuels (Tock et al. 2010). Initially, the production of first-generation bioethanol was identified as a potential solution for the challenges identified with fossil fuels; however, it was encountered with certain adverse effects due to the edible raw materials and the consumption of lands for the production crops to generate biofuels. Transforming the food crops into crops for the production of biofuels will not only increase the food cost but also lead to socio-economic problems. In order to overcome these challenges, researchers developed second-generation bioethanol from the waste biomass which has little or no cost, hence, reducing the overall cost of biofuel production. However, various developing countries have been encountering several challenges while disposing these wastes, which results in massive environmental annihilation such as greenhouse gas emission. Therefore, transformation of these wastes into a resource to generate energy aids in resolving two challenges, which includes reduction in the energy costs and providing an eco-friendly method for the disposal of what would otherwise cause pollution (Gebregergs et al. 2016).

7.5 Bioethanol from Food Processing By-Products and Waste

7.5.1 Bioethanol from Vegetable and Fruit

During the processing of fruits, about 30–50% of the by-products are produced on the basis of the variety of fruit processed. The by-products produced include preprocessing by-products such as fruit stems and stalks as well rotten fruits from sorting processes and processing by-products like seeds, pomace, peels and pulp. The chief component of the wastewater produced from fruit and vegetable processing plants comprises pectin, sugars, vitamins, starches and other constituents of the cell wall (Dar et al. 2019). The general steps involved in the production of bioethanol from vegetable and fruit waste are represented schematically in Fig. 7.6.

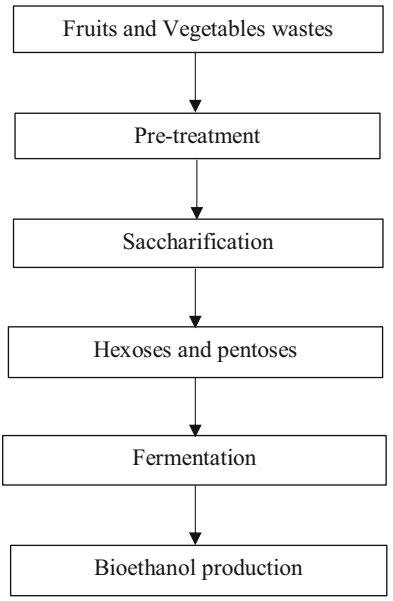

Fig. 7.6 General steps involved in the bioethanol production from fruits and vegetables wastes. (Adapted from Bhuvaneswari and Sivakumar 2019)

7.5.2 Bioethanol from Banana Wastes

Banana is one of the main fruit crops grown widely around the globe. It is a widely cultivated fruit crop in Asia and America (56% and 26%, respectively) as well as Australia. Every year, during the process of post-harvest transporting or grading, a huge portion of banana crop is rejected, wasted or discarded, which are then disposed in the lands which contributes in the environmental pollution. However, these low-cost wastes including rotten bananas and their peels can be utilised for the production of second-generation bioethanol as they comprise valuable sugars and minerals which are essential for the process of fermentation (Guerrero and Muñoz 2018). Besides glucose, the banana wastes also comprises various other carbohydrates, which can be converted into simple sugars by the process of enzymatic hydrolysis using enzymes like cellulases, hemicellulases and pectinases (Bhuvaneswari and Sivakumar 2019).

In order to produce bioethanol, banana waste is collected, washed, cut into small pieces and sun-dried. Following this, the sun-dried banana waste is crushed, ground well and blended with distilled water. At times, the banana waste is also utilised without drying. However, in such cases, it is essential to wash the waste and mash it well. In either case, the mashed or the powdered mixture of banana waste is required to be autoclaved, and, subsequently, the sterilised feedstock is subjected to further hydrolysis. To the feedstock, 0.5–2.5% (v/v) of diluted sulphuric acid is added, which is kept at 70 °C to 110 °C for 10–30 min. This mixture is then subjected to enzymatic hydrolysis at pH ranging from 4.5 to 5.5, using enzymes like cellulases, hemicellulases and pectinases (Wu et al. 2017) and is incubated at 50 °C for an hour.

Following this process, the sterile hydrolysate is mixed with 18–24 h grown yeast culture and is incubated anaerobically with constant shaking at 35 °C, pH 5–5.5 for 3–7 days. During this process, aliquots can be tested to examine the concentration of bioethanol generated. The bioethanol produced during this process is directly proportional to the water content of the feedstock. On achieving the desired concentration of bioethanol, it is then subjected to the process of distillation (Bello et al. 2014). Studies conducted to analyse the elements present in bioethanol have reported that the elements are either very low or negligible in concentration. The studies also revealed that the concentration of toxic minerals like lead, chromium and molybdenum was found to be zero ppm and that of minerals such as manganese, phosphorus, magnesium and calcium was higher (Hossain et al. 2011). However, despite the higher concentration, these minerals are not toxic to the environment, and, hence, bioethanol has been recognised as a sustainable fuel, and its production from banana waste exhibits a potential approach to generate second-generation bioethanol (Bhuvaneswari and Sivakumar 2019).

7.5.3 Bioethanol from Citrus Fruit Wastes

Another commonly cultivated crop across the world is citrus fruits. Production of oranges across the globe is nearly 50.2 million tons (Cypriano et al. 2018). A huge of amount of fruit is wasted if it is not stored, transported and retailed properly. During the production of orange juice, a variety of waste including citrus pulp floater, peels, fibres and internal tissues is generated, which contains high content of sugar, protein, pectin, celluloses and hemicelluloses. The citrus fruit waste when treated in the floatation tank leads to clogging and when disposed in inland sites results in environment pollution. However, the comparatively lower cost of these wastes and the presence of abundant sugars make them a suitable feedstock for bioethanol production (Girish et al. 2014). Rotten citrus fruits and peels are employed as a feedstock for the second-generation bioethanol production. This waste is fermented either by using a monoculture of yeasts or by co-culturing yeast with another yeast strain called *Candida parapsilosis* NRRLY-12969.The co-culturing of yeasts is an efficacious technique as the unavailable sugars like pentoses can also be fermented by the new yeast strain resulting in enhanced bioethanol production. Bioethanol production from citrus wastes involves collection, washing, surface sterilisation and drying in sun or oven. The dried waste is then ground, blended with distilled water and sterilised further. Subsequently, the cooled feedstock is blended with enzymes like cellulases, hemicellulases and pectinases for enzymatic hydrolysis. The enzyme hydrolysate obtained is then subjected to aerobic fermentation using a monoculture of yeast at 35 °C for 5 days and, subsequently, anaerobic fermentation from sixth to ninth day or by employing co-culturing technique using *S. cerevisiae* and *C. parapsilosis* (Girish et al. 2014). The bioethanol produced with this process is then purified by distillation and considered for further use (Bhuvaneswari and Sivakumar 2019).

7.5.4 Bioethanol from Date Fruit Waste

Dates are mostly cultivated in the Gulf countries, including Egypt, Libya, Algeria and Pakistan. Generally, dates are wasted in huge quantity during harvesting, storing and transporting as well as in retail outlets. Since, they are a rich source of carbohydrates, the waste obtained from dates has a high content of biodegradable sugars, and the bioethanol can be generated by bioconverting these sugars using yeasts under anaerobic conditions (Boulal et al. 2016). Bioethanol production from dates waste involves washing of the waste, immersing it in a water bath and rubbing and rinsing it with water. Date seeds are then removed and ground and placed in a water bath at 90 °C–95 °C to extract sugars. The temperature of the date juice obtained from a series of extraction is maintained at about 60 °C, in order to avoid contamination of the juice. Following acid hydrolysis, the hydrolysate obtained is fermented using a yeast culture at nearly 32 °C for 72 h, following which bioethanol produced is subjected to distillation. On repeating this process at least four times, bioethanol with 90% strength can be generated (Bhuvaneswari and Sivakumar 2019).

7.5.5 Bioethanol from Potato Processing Waste

Another crop that possesses high-value sugar for the production of bioethanol is potatoes. The potato crop is the third chief food crop that is cultivated worldwide, with 325 million tons yield every year (Birch et al. 2012). It has been estimated that nearly one-third of the world's total potato crop is "overstock", which is sufficient to yield 1200–7200 million litres of bioethanol per year (Tasić and Veljković 2011). Likewise, the gross proportion of potatoes wasted in potato processing has been estimated to be as high as 50%, with 5%–20% wastage during their growth and 18% wastage in the potato chip producing industry. Currently, majority of the waste produced from the potato industry is being employed as animal feed (Radunz et al. 2003). However, the starch present in processing wastewater produced from potato chip industry yields glucose which can be employed as a constituent for bacterial fermentation for bioethanol production (Abanoz et al. 2012). A study was conducted to optimise the hydrolysing conditions for solid potato waste generated from potato flake production, and the obtained medium was utilised for fermentation to bioethanol (about 31 g/L) using *Saccharomyces cerevisiae* (Izmirlioglu and Demirci 2010). Similarly, another study derived 20 g/L ethanol from medium comprising sugars released from fresh potato peel [12% (w/w)] using enzymes including amylase, pectinase or fungal β-glucanase. Additionally, it was also reported that the combination of potato mash and potato peel to the medium and employing the same three enzymes resulted in augmented bioethanol production (as high as 50 g/L) (Yamada et al. 2010).

7.5.6 *Bioethanol from Coffee Pulp and Husks*

Coffee processing also yields residues which include coffee husk, pulp and wastewater. Currently, coffee pulp is disposed into rivers and local streams, which results in acidification of water and subsequent destruction of aquatic life (Seboka et al. 2009). Coffee pulp a source with high nutrient content is mainly utilised to feed animals or employed as a fertiliser for coffee plants. Approximately 1 ton of coffee pulp is produced from every 2 tons of coffee cherries (Roussos et al. 1995). On the basis of dry weight, coffee pulp comprises 8.25% protein and 23–27% fermentable sugars. The hydrolysate, procured from diluted sulphuric acid, contains various fermentable sugars such as arabinose (up to 11.26 g/L), glucose (up to 6.31 g/L), sucrose (up to 3.96 g/L), maltose (up to 3.50 g/L), xylose (up to 3.23 g/L) and fructose (up to 3 g/L) (Urbaneja et al. 1996). A study conducted to investigate the feasibility of producing bioethanol from coffee pulp hydrolysed by dilute acid or distilled water and fermented with *S. cerevisiae* reported that to conduct hydrolysis, distilled water was the preferred choice over dilute acid (Kefale et al. 2012). 7.4 g/L bioethanol was produced from 4-h-long hydrolysis from boiling distilled water and subsequent fermentation for 24 h at 30 °C. Processing of 1 kg of coffee beans yields around 1 kg coffee husks, which is chiefly utilised as animal feed and fuel (Franca and Oliveira 2009). A study conducted to examine bioethanol production using whole and ground coffee husk as well as an aqueous extract of ground coffee husks as a substrate and *S. cerevisiae* as a fermentation microorganism reported that the production of bioethanol was as high as 13.6 g/L (Gouvea et al. 2009).

7.5.7 *Bioethanol from Grain Waste*

Cereal grains possess high starch content as a result of which they have been considered a good feedstock for biofuel production. The chief industrial use of grains is the isolation of the starch and subsequent processing. In most cereals, about 60% to 80% of dry matter is constituted by starch. Mostly, starch is extracted industrially from wheat as well as maize and to a lower extent from rice. Over the recent years, a rising trend has been observed in the portion of grain utilised for neither consumption nor for feed purposes. Most of the grain produced is utilised for the production of bioethanol other than the purpose of consumption and feed (Kamani et al. 2019).

7.5.7.1 Energy Crops

Energy crops are those crops whose partial or total production is employed as a raw material to yield renewable energy. These crops generate a huge quantity of biomass per unit of area and time. The chief characteristics to select energy crops are

accelerated growth, that is, short duration from planting to harvesting and the ability to grow under extreme weather and poor soil conditions where other crops may have lower and unstable yield (Dubois 2011). Generally, energy crops are categorised into two categories, namely, herbaceous and woody energy crops. The former crop type mainly belongs to perennial grasses like giant reed (*Arundo donax*), switchgrass (*Panicum virgatum*) and miscanthus (*Miscanthus spp.*), while the latter with a comparatively faster growth and shorter rotation includes poplar and eucalyptus. Both the crop types may result in the reduction of soil erosion and enhancement of soil carbon and soil fertility among poor soils. Additionally, herbaceous energy crops can also be cultivated lands with lower fertility and poor mineral composition without distressing the fundamental characteristics of bioethanol. Switchgrass and *Miscanthus spp.* are one of the most studied lignocellulosic energy crops. A study examined the conversion of switchgrass after using varying pretreatment strategies (sodium hydroxide, sulphuric acid and methanol). Of which methanol was found to be the efficacious pretreatment strategy and with final yield of 0.32 g of ethanol/g of glucose with 97% conversion yields (Smullen et al. 2017).

7.5.7.2 Rice Husks

Rice husks are agricultural food processing by-products that are available in abundance. The process of rice milling yields nearly 78% rice, broken rice and bran and 22% rice husk, of which rice husk comprises 50% cellulose, 25–30% lignin and 15–20% silica (Nagrale et al. 2012). In several rice-producing nations, a major portion of the rice husk is either incinerated or disposed, leading to environmental pollution (Eberemu et al. 2011; Krishnarao et al. 2001). Alternatively, the waste generated during rice harvesting and processing can also be employed as a low-cost feedstock for the production of bioethanol. A study investigated this theory, by pretreating the rice husk with lime followed by treatment with enzymes like cellulase, hemicellulose and β-glucosidase, which produced 9.8 g/L bioethanol with a yield of 0.49 g/g of available sugars after fermenting for 19 h at 35 °C using an *E. coli* strain FBR5. Additionally, on using saccharification coupled with fermentation for 53 h at 35 °C, a yield of an even higher bioethanol concentration (11.0 g/L) was acquired (Saha and Cotta 2008). Another study analysed bioethanol production by using *S. cerevisiae* from sugars released from rice husks by employing acid hydrolysis. The acid hydrolysis produced inhibitors which hindered the cell growth, however reduced the bioethanol yield by only 4% from the theoretical maximum (from 0.49 g/g glucose to 0.47 g/g glucose) (Moon et al. 2012).

7.5.8 Dairy

Although, dairy industry has not been correlated to massive environmental issues; however, its interaction with environment must still be taken into account, since

pollutants generated from this industry are predominantly organic in nature. Production of dairy products yields a variety of solid and liquid wastes along with certain by-products, which may include damaged or perished products, off-specification products, solids, curd, cheese, milk sludge and whey comprising proteins, fats and lactose, making their exploitation necessary (Prazeres et al. 2012).

7.5.8.1 Cheese Whey

Cheese whey, a by-product of cheese industry, is generated in huge quantity and can result in environmental pollution if disposed into rivers or farmland (Smithers 2008). Every year, more than 160 million tons of whey is produced in the world as a by-product of nearly about 18 million tons of cheese produced (Guimarães et al. 2010; Kosikowski 1979). Of the total cheese whey produced, 70% is utilised for the production of other products, and nearly 30% is used for feeding pigs or as fertiliser or dispensed in the environment. Whey powder is one of the products that are produced from whey and are utilised as a constituent of several food products for humans and animal feed (Božanić et al. 2014; Guimarães et al. 2010; González Siso 1996). Although, whey is often disposed, it is a rich source of vital nutrients such as lactose (5–6%), protein (1%), lactic acid (0.1–0.8%) and fat (0.06%) (Li and Yang 2016). Various researches have been conducted to investigate bioethanol production from cheese whey (Guimarães et al. 2010; Kargi and Ozmihci 2006; Koushki et al. 2012; Zafar and Owais 2006). Several studies conducted in the past reported lower yield of bioethanol from whey; however, efforts are being made for its enhancement. Another challenge faced during the production of bioethanol from cheese whey is the presence of lactose as a form of fermentable sugar, which comprises glucose and galactose. *S. cerevisiae*, a conventional bioethanol producer, is incompetent to deploy lactose due to the absence of β-galactosidase and also because lactose cannot be transported into the cell (Domingues et al. 2010). On the other hand, other yeasts, like *Kluyveromyces fragilis*, also known by other names like *K. marxianus*, *Candida kefyr* or *C. pseudotropicalis*, is capable of fermenting lactose. It has been reported that *K. fragilis* is proficient in fermenting lactose up to an economically feasible concentration of 20% albeit slowly. Furthermore, it has also been revealed that *K. fragilis* is impeded by moderate concentrations of sugar and salt found in whey and has a relatively lower bioethanol tolerance in comparison to *S. cerevisiae*. Another solution to the challenge is pretreating whey with β-galactosidase in order to hydrolyse lactose to galactose and glucose prior to using it as a constituent of fermentation medium. A study reported an Algerian strain of *S. cerevisiae* to be an efficient ethanol producer from pre-hydrolysed whey treated with β-galactosidase (Boudjema et al. 2015). However, a challenge faced by this approach can be attributed to catabolite repression, as galactose is metabolised only once glucose is completely consumed. Other strategies to overcome the challenge involved mutation to yield *S. cerevisiae* capable of utilising glucose and galactose concurrently from pretreated whey. A study ascertained that fermenting cheese whey low in lactose content [3–5% (w/v)] produced 2–3% (v/v) bioethanol post-fermentation. The same

study used unconcentrated (4.9% lactose) and concentrated (9.8% lactose) whey to compare *K. marxianus* and *C. kefyr* for the production of bioethanol and observed a production of nearly twice as much bioethanol yielded with the concentrated whey (4.0–4.6% compared to 2.0–2.2%) (Koushki et al. 2012). However, whey comprising 5–6% lactose and yielding only 2–3% (v/v) bioethanol is not considered to be economically feasible. This is attributed to the high distillation cost of culture broth with low bioethanol concentrations (Guimarães et al. 2010). A study was also conducted to investigate bioethanol production from 4.5% lactose containing whey and 5.5% lactose containing whey powder solution by employing *Escherichia coli* strain FBR5 without enzymatic hydrolysis (Akbas et al. 2014). Cheese whey powder whether in dry form or in the form of a solution has various benefits over whey with respect to bioethanol production, which include high lactose concentration and easy storage and transportation (Guo et al. 2010). Besides lactose, cheese whey powder also comprises proteins and vitamins, which are vital constituents of media required for the production of bioethanol production (Akbas and Stark 2016).

7.6 Conclusion

Food processing wastes (FPWs) are being produced in a huge amount. The management of this waste is essential to avert environmental damage which may result from disposing of these wastes in landfills. Although there exist several strategies to manage FPWs, the production of biofuel has been recognised as the most preferred strategy to combat the challenge of possible environmental pollution. Additionally, with a rising demand for energy, biofuels have been identified as a clean source of energy, which will play an integral role in addressing issues related to environmental, climatic, monetary and social security challenges which result from the utilisation of fossil fuel, the chief source of energy. Bioethanol has been recognised as promising biofuel given its eco-friendly nature and fewer negative environmental impacts compared to other fuels. Over the past few years, utilisation of FPWs has been an enticing field of research for several researchers worldwide, as the biofuels generated from these wastes serve as a suitable substitute to conventional fuels, thereby leading to significant reduction in the emission of harmful gases the greenhouse emissions. Therefore, employing FPWs for bioethanol production is a worthwhile in comparison compared to other valorisation processes. Therefore, this chapter emphasises on the need to envisage research to investigate other novel food wastes which can be deployed for the production of not only bioethanol but also other biofuels.

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Chapter 8
Utilization of Fruit-Vegetable Waste as Lignocellulosic Feedstocks for Bioethanol Fermentation

Manisha Verma and Vishal Mishra

Abstract Food waste is a challenge to the environment worldwide; hence recycling is required. Fruit and vegetable waste feedstock is a sustainable resource with a significant possibility for electricity, biogas production, and chemical solvents. Biomass-derived bioethanol is 10–15% of the global energy sources and resolves fuel scarcity, greenhouse gas emissions, and fossil fuel exhaustion. At present, bioethanol is a matter of global attention for reducing air pollution worldwide. Fruits and vegetable residues contain a high amount of simple and complex carbohydrates, and these sugars can be used as raw and fresh matter for the production of bioethanol using microbial culture. Currently, 80% of bioethanol is produced from foodgrain supplies such as sugar and starch. Recently, lignocellulosic biomass gathers more attention. This chapter purposes of explaining processes engaged in fruits and vegetable waste biomass pretreatment and fermentation process. The chapter also discussed fermentation conditions that affect fermentation, microbial culture, and ethanol yield.

Keywords Bioethanol · Solvent · Biofuel · Fruit waste · Vegetable waste · Fermentation

Abbreviations

$(NH_4)_2SO_4$	Ammonium sulfate
$Ca(OH)_2$	Calcium hydroxide
CO	Carbon monoxide
CO_2	Carbon dioxide
COD	Chemical oxygen demand
FVW	Fruit and vegetable waste

M. Verma · V. Mishra (✉)
School of Biochemical Engineering, IIT (BHU), Varanasi, Uttar Pradesh, India
e-mail: vishal.bce@itbhu.ac.in

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GHG	Greenhouse gas
KH_2PO_4	Potassium dihydrogen phosphate
KOH	Potassium hydroxide
NaOH	Sodium hydroxide
NH_3	Ammonia
SHF	Separate hydrolysis and fermentation
SSCF	Simultaneous saccharification and cofermentation
SSF	Simultaneous saccharification and fermentation

8.1 Introduction

Continuously rising demand and speedy exhaustion of conventional fuels raise the requirement of alternative sustainable energy are actually very urgent (Allen 2017). Speedy exhaustion of petroleum fuels causes depletion in oil reservoir levels while elevating the CO_2 and CO in the air (Uzair Ali et al. 2020). Air pollution, the greenhouse effect, and global warming are significant impacts of unlimited fuel consumption, alarming the earth's situation (Uzair Ali et al. 2020). Nowadays, the world's energy requirement relies on conventional fossil fuels, which will not withstand future energy demand. Continuous depletion in fossil fuel reservoir gives rise to its price hiking and the requirement of nonconventional future alternative of petroleum oil. So, environmental concerns bring many opportunities and build a substantial market for biofuel. Climate change concerns generate the need to reduce GHG emissions and encourage bioethanol demand to replace conventional fuels (Arto et al. 2016). One more issue is the continuous rise of waste dumping in an open area, harming the natural habitat and the dumpsite's nearby environment (Esparza et al. 2020). Producing energy from waste is reasonable, affordable, and effective. A vast range of renewable energy sources and technologies is available, including biogases, solid biomass, and liquid fuels. A biofuel is a biomass-generated fuel that involves several biochemical treatments and not originated from geological processes like fossil fuels. Biomass with complex or simple carbohydrates undergoes some pretreatment; later, it is converted into some soluble saccharides that are used to produce bioethanol (Demirbas 2007; Hafid et al. 2017). The biomass is classified into two main categories: starchy biomass (sugarcane and other sugar crops, by-products of sugar mills) and lignocellulosic (agricultural waste) feedstock. Previously, first-generation biofuels reported utilization of much starchy biomass like sugarcane, corn, and potatoes for bioethanol production. Yet, it has economic and socio-ethical barriers. Currently, second-generation biofuels are gaining more attention to utilize residual biomass for bioethanol production. Lignocellulosic feedstock involves agricultural residues such as bagasse, stalks, corn stover, straws, leaves, and switchgrass for the second-generation biofuels. Any agricultural waste biomass with a considerable amount of saccharide could be used as a raw material for bioethanol production. Pineapple, potato, and sugarcane are significant plant

sources that resulted in an excellent yield of bioethanol as by-products due to a high amount of saccharide in it. Bioethanol is an alternative biofuel for existing engines with a better octane rating with reliable production processes and easy adaptability (Bhuvaneswari and Sivakumar 2019).

8.1.1 *Fruit and Vegetable Wastes (FVW) as a Raw Feedstock for Bioethanol Production*

Food processing and nutrition sector founds a vital connection between agriculture and industry. Food industries generate abundant fruit peel waste by processing fruits and juices. Most of fruits contain 15–50% of peel. After consumption of mesocarp (fleshy part), peels are rejected as waste. For several production units, quantity of waste generated is greater than the product obtained (Alarcón García et al. 2015; Wadhwa and Bakshi 2013; Pathak et al. 2015). It is expected that, in upcoming years, majority of organic chemical compounds will be generated through bio-based processing using agricultural, forestry, municipal, and food waste feedstocks (Gnansounou and Pandey 2016). Fresh fruits or juice consumed is mainly in the ready form of consumption or concentrated form. Many studies stated that FVW biomass is rich in carbohydrates. Saccharides are one of the potential substrates for sustainable energy production. Research on FVW biomass utilization for biofuel manufacture caught much attention in various countries. Vegetable waste could be fresh or cooked parts generated during cultivation, harvesting, storage, marketing, and consumption. Rotten vegetables and vegetable peels are biodegradable waste generated in huge quantities every day, usually dumped on the open household area or landfills. This act emits a nasty odor and a big attraction for pigs, rodents, or scavengers and possibly transmitting various human diseases. The waste FVW biomass goes to landfills where the waste biomass spread nasty smell and generates methane (a GHG), and a massive quantity of hazardous leachate pollutes soil and groundwater. When we talk about waste management, there is some hierarchy to follow: (1) waste reduction or reuse, (2) recycling, (3) energy recovery or composting, and (4) treatment and disposal. Applying the identical waste management method for fruit and vegetable peels with a different biorefinery approach could be helpful for the valorization of FVW. The economic viability of the biorefinery approach for FVW could be attained by producing a various range of high-volume, low-value products such as animal feed and compost or less-volume high-value compounds such as pectin, essential oils, phenolic compounds, etc. (Joglekar et al. 2019). Figure 8.1 demonstrates the general representation of a biorefinery approach to process FVW. FVW is used either directly fresh (having moisture content) or dried, followed by size reduction. Based on the various varieties of FVW feedstock, different solvent extraction procedures are selected for the extraction of phenolic compounds (antioxidants), whereas further steam processing is performed for essential oil extraction. Remaining lignocellulosic biomass is kept in aqueous acidic

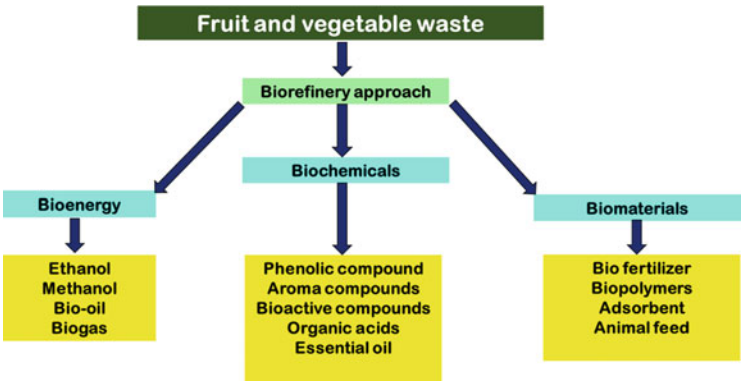

Fig. 8.1 General schematic of a biorefinery approach to process FVW

solution for its conversion into simple saccharides (reducing sugar). Hydrolysis step is significant to boost the fermentation and yield of products. Filtered solid hydrolysate residues could be used for either anaerobic digestion or gasification to obtain biogas and syngas, whereas fermentation is carried out with filtered liquid hydrolysate. Fermentation products are passed to distillation column to obtain pure ethanol (Joglekar et al. 2019).

After the fruit juice extraction, the leftover parts of the fruits are a rich source of lignocellulose, and they could be utilized as a fresh substrate for bioethanol fermentation (Bhuvaneswari and Sivakumar 2019). In a study, banana, pineapple, and plantain peels were used for simultaneous saccharification and fermentation at different temperatures (20–50 °C) by co-culture of *S. cerevisiae and A. niger* for a week (Itelima et al. 2013). It was detected that the highest pH and temperature for the banana peels fermentation were 6 and 30 °C. The optimal bioethanol yields were 3.98% v/v, 7.45% v/v, and 8.34% v/v for plantain, banana, and pineapple peels, respectively (Itelima et al. 2013). For citrus peel waste-based hydrolysates, the most effective conditions for bioethanol fermentation were observed (Patsalou et al. 2019). *Pichia kudriavzevii KVMP10* at 42 °C results in maximum bioethanol production of 30.7 g/L (Patsalou et al. 2019). Pineapple peels were utilized as a cheap and affordable raw material for bioethanol fermentation by *S. cerevisiae* (Casabar et al. 2019). NaOH was used in the pretreatment of pineapple peels for the high production of simple saccharides. *T. harzianum* inoculum was used for the hydrolysis process, which can efficiently raise the reducing sugar content of the pineapple peels after hydrolysis (Casabar et al. 2019). The maximum ethanol generation obtained was 5.98 g/L (at 48 h of incubation) which was followed by 5.31 g/L (at 24 h) and 4.5 g/L (at 72 h) of fermentation. Table 8.1 reports the use of FVW biomass for bioethanol production.

Promon et al. (2018) reported that vegetable waste biomass is rich in lignocellulose content and use nonedible vegetable waste to hydrolyze it to obtain D-xylose and glucose (Promon et al. 2018). Lignocellulose is mainly the composition of 10–15% lignin, 20–40% hemicellulose, and 30–50% cellulose (Wilson and Lee 2014).

Table 8.1 FVW biomass utilized for bioethanol production

Biomass feedstock	Pretreatment	Microorganism	Ethanol yield/ concentration	Reference
Green cabbage		*C. beijerinckii P260*	0.8 ± 0.0 g/ 100 g waste	Poe et al. (2020)
Red onion skins		*C. beijerinckii P260*	0.1 ± 0.0 g/ 100 g waste	Poe et al. (2020)
Red/green bell peppers		*C. beijerinckii P260*	0.9 ± 0.2 g/ 100 g waste	Poe et al. (2020)
Potatoes		*C. beijerinckii P260*	1.0 ± 0.0 g/ 100 g waste	Poe et al. (2020)
Rotten potatoes		*C. beijerinckii P260*	1.2 ± 0.0 g/ 100 g waste	Poe et al. (2020)
Tomatoes		*C. beijerinckii P260*	0.7 ± 0.0 g/ 100 g waste	Poe et al. (2020)
Simulated fruit and vegetable waste		Anaerobic mixed consortia from sludge	6.7 g/L	Wu et al. (2017)
Fruit waste		*Citrobacter* sp. *E4*	0.30 g/g	Sarkar et al. (2019)
Kitchen and garden waste (3:1)	0.05 M sulfuric acid at 120–180 °C	*Mucor indicus*	75.9%	Karimi and Karimi (2018)
Date residue		*S. cerevisiae*	38.0 ± 0.5%	Chniti et al. (2014)
Date residue		*C. pelliculosa*	34.0 ± 0.2%	Chniti et al. (2014)
Date residue		*Z. rouxii*	29.0 ± 0.1%	Chniti et al. (2014)
Orange peel waste	Acid-catalyzed steam explosion and enzymatic saccharification	*S. cerevisiae F15*	0.495 g/g	Santi et al. (2014)
Waste pineapple		*S. cerevisiae*	49.78% (v/v)	Aung et al. (2017)
Banana waste		*C. krusei* and *H. guilliermondii*	7.38% (v/v)	Utama et al. (2019)
Papaya waste		Indigenous yeast isolated	3.74% (v/v)	Utama et al. (2019)
Napa cabbage waste		Indigenous yeast isolated	1.18% (v/v)	Utama e tal. (2019)

(continued)

Table 8.1 (continued)

Biomass feedstock	Pretreatment	Microorganism	Ethanol yield/ concentration	Reference
Paddy straw and sapota peel waste	Cellulase enzymatic hydrolysis	*S. cerevisiae* and *Candida* sp.	3.9% (v/v)	Malik (2019)
Acerola bagasse	Acid pretreatment followed by enzymatic hydrolysis	*S. cerevisiae*	0.12 ± 0.13 g/ g of waste	de Oliveira et al. (2021)
Pupunha peel	Acid pretreatment followed by enzymatic hydrolysis	*S. cerevisiae*	0.12 ± 0.21 g/ g of waste	de Oliveira et al. (2021)
Mango peel	Acid pretreatment followed by enzymatic hydrolysis	*S. cerevisiae*	0.15 ± 0.07 g/ g of waste	de Oliveira et al. (2021)
Pupunha cluster	Acid pretreatment followed by enzymatic hydrolysis	*S. cerevisiae*	0.11 ± 0.07 g/ g of waste	de Oliveira et al. (2021)

Cellulose is a homologous glucose polymer linked by the β-1,4 glycosidic bond (Zhao et al. 2011).

8.1.2 Role of Microorganisms

Bioethanol production from FVW biomass includes several steps: pretreatment of waste biomass, saccharification of lignocellulose using enzymatic action, and ultimately fermentation. Each step somehow deals with the involvement of several microbial steps. Lignocellulosic biomass usually has a stiff texture, and it is made up of cellulose, lignin, and hemicellulose, making the raw feedstock unmanageable for complete enzymatic digestion (Zhao et al. 2011). However, commercial enzymatic conversion of lignocellulosic waste is limited by the cost of cellulase enzyme. Amid the various microbial sources of the cellulolytic enzyme, fungal strains are major cellulase producers compared to bacteria. A fungal species, *Trichoderma reesei*, is widely used for commercial cellulase production. There are three main types of cellulolytic enzymes produced by *T. reesei* are (1) b-glucosidases (EC 3.2.1.21), (2) cellobiohydrolases (EC 3.2.1.91), and (3) endoglucanases (EC 3.2.1.4), which are used to convert cellulose into glucose. Lesser b-glucosidase activity is the primary drawback of *T. reesei* (Zhang et al. 2010). Some novel fungal strains have been explored for cost-effective bioconversion of lignocellulosic waste. A fungus, *Chrysoporthe cubensis* (plant pathogen), has been examined as another source of

enzyme cellulases (Falkoski et al. 2013). Another study signifies the role of the *Phoma exigua ITCC 2049* (a fungus), which is usually a potato pathogen that could be utilized for cellulase production (Tiwari et al. 2013). A thermophile fungi *M. cinnamomea* and one more fungus *A. strictum* were discovered as a possible bioresource for cellulase production (Goldbeck et al. 2013; Mahajan et al. 2016). A unique b-glucosidase was obtained from *P. piceum*, which achieves maximum transglycosylation activity to yield cellulase inducers (Gao et al. 2013). Glucose xylose is another most abundant saccharide obtained after the hydrolysis and saccharification of lignocellulosic waste. Application of xylose conversion into fermentable saccharides has excessive significance for greater bioethanol yield. Then again, the native strain of *S. cerevisiae* is unable to ferment xylose into ethanol. Using synthetic biology, a novel strain design was developed for simultaneous utilization of acetic acid, xylose, and cellobiose (Wei et al. 2015). In this design to make possible the use of cellobiose, intracellular β-glucosidase encoding gene gh1–1 and cellodextrin transporter encoding gene cdt-1 from the fungi *Neurospora crassa* were expressed and amplified within *S. cerevisiae* (Wei et al. 2015). Additionally, the xylose-metabolizing genes XDH and XR from a yeast *Scheffersomyces stipitis* were expressed and amplified within the *S. cerevisiae*. To aid the reduction of acetate into ethanol, adhE gene from *E. coli* was expressed in the *S. cerevisiae*. The ultimate strain obtained exhibit all the three pathways for acetic acid, cellobiose, and xylose assimilation and its conversion into bioethanol, which significantly enhance ethanol yield compared to control strain (Wei et al. 2015). *S. cerevisiae* is generally admired for bioethanol production due to its extensive pH tolerance and less infection susceptibility.

8.1.3 Pretreatment and Detoxification of FVW

Household and pulp mill refuse fruit and vegetable biomass is an extensive range of lignocellulose-rich feedstock material. Pretreatment involves various procedures for converting lignocellulosic feedstock into its essential components such as lignin, cellulose, and hemicellulose. Pretreatment procedures mainly concern with lignin elimination, hemicellulose preservation, reducing the cellulose rigidity, and enhancing the porosity of the biomass (Chiaramonti et al. 2012). An economical way of pretreatment procedure should assist in increasing the availability of carbohydrates in the enzymatic hydrolysis step while reducing the loss of simple saccharides for hydrolysis and fermentation (Chiaramonti et al. 2012). Figure 8.2 lists different pretreatment methods for FVW biomass.

The primary purpose of an efficient pretreatment process is (Kumari and Singh 2018) (1) to obtain simple saccharides by hydrolysis, (2) to avoid loss of simple saccharides formed, (3) to limit the production of inhibitors, (4) to minimize energy requirements, and (5) to reduce bioethanol production cost. Biomass pretreatment procedures are categorized as (Kumari and Singh 2018) (1) physical/mechanical pretreatment, (2) chemical pretreatment, (3) physicochemical pretreatment, and

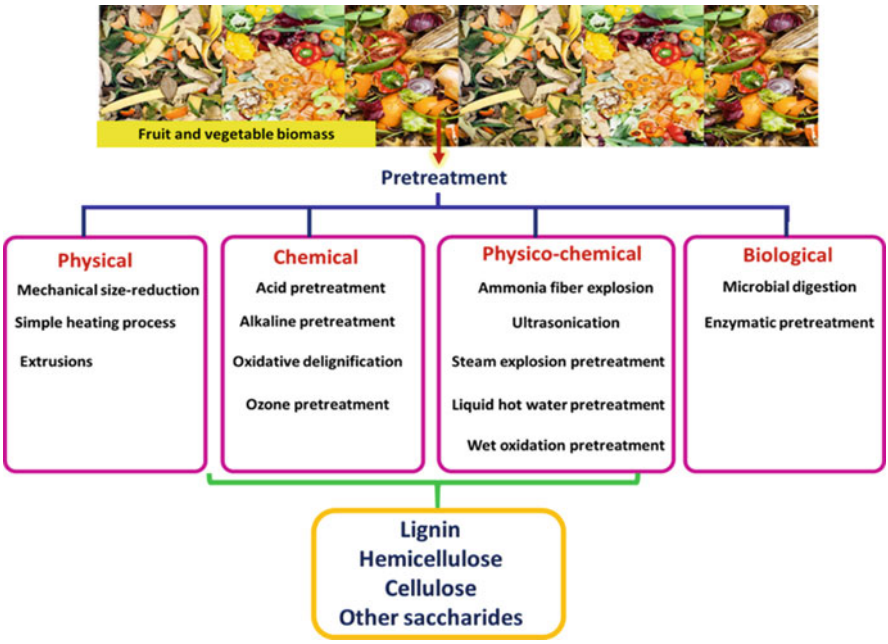

Fig. 8.2 Different pretreatment methods for fruit and vegetable biomass

(4) biological/enzymatic pretreatment. Table 8.2 represents various pretreatment methods utilized in waste biomass processing.

The initial stage of pretreatment involves the size reduction of lignocellulosic feedstock, but this size drop should not be too big or too little. Pretreatment approaches are chosen on the basis of the type and composition of feedstock used for bioethanol fermentation (Kumari and Singh 2018). Among different physical/mechanical pretreatment practices defined in Table 8.2, extrusion is supposed to be cost-effective and easy to process when combined with mixing, shearing, and heating. It assists in the release of a considerable quantity of simple saccharides. Microwave heating is a heating pretreatment approach that should be carried out at appropriate temperatures based on biomass composition (Li et al. 2012). Acid pretreatment is a well-recognized chemical approach generally used for lignocellulosic biomass and covert hemicellulose into its monomers and ultimately enhances bioethanol fermentation. Acids such as hydrochloric acid, acetic acid, formic acid, nitrous acid, nitric acid, maleic acid, phosphoric acid, and sulfuric acid have been utilized for acid pretreatment of lignocellulosic feedstock (Bagudo et al. 2014; Ajayi and Adefila 2012; Bai et al. 2016; Sen et al. 2016; Ranjan et al. 2013; Han et al. 2013). Acid pretreatment gets used in two ways: (1) high acid concentration at significantly lower temperatures and (2) low acid concentration at high temperatures (Sen et al. 2016). The typical downside of acid treatment is the simultaneous production of different inhibitors like furfural, acetic acid, and 5-hydroxymethyl furfural, which prevent the growth of microbial biomass (Taherzadeh and Karimi

Table 8.2 Pretreatment methods

Physical treatment		
Mechanical size reduction	Chipping, grinding, hammer milling, ball milling, disk milling	Nakagawa et al. (2007)
Simple heating process	Microwave irradiation	Gabhane et al. (2011)
Extrusions	Mixing, heating, and shearing of waste biomass	Simona et al. (2013)
Chemical pretreatment		
Acid pretreatment/ saccharification	High temperature and less acid conc. Low temperature and higher acid conc.	Sen et al. (2016)
Oxidative delignification	Hydrogen peroxide (H_2O_2) or peracetic acid	Gonçalves et al. (2014)
Alkaline pretreatment (solubilizing polysaccharides)	NaOH, $Ca(OH)_2$, KOH, and NH_3 are most frequently used alkalis for pretreatment	Wan et al. (2011)
Ozone pretreatment	Ozonolysis of lignocellulosic biomass to increase cellulose biodegradability	Appels et al. (2012)
Physicochemical pretreatments		
Ammonia fiber explosion pretreatment	Liquid ammonia and the steam explosion process	Bals et al. (2011)
Cell wall disruption	Ultrasonication	Sen et al. (2016)
Autohydrolysis or steam explosion pretreatment	Pressurized steam (20–50 bar, at 160–270 °C) for a few seconds	Baêta et al. (2016)
Liquid hot water pretreatment (hemicellulose solubilization)	High temperatures and pressure (160–220 °C) are used to maintain the liquid state of water and biomass kept in water upto15 min	Rogalinski et al. (2008)
Wet oxidation pretreatment	Biomass treated with water involving oxygen at a temperature >120 °C and 0.5–2 MPa pressures for <30 min	Zheng et al. (1995)
Biological pretreatment		
Cellulose and lignin digestion	Cellulase producing bacteria and fungi	Sindhu et al. (2016)
Enzymatic pretreatment	Cellulases and hemicellulases enzymes	Romano et al. (2009)

2008). However, concentrated acid pretreatment is exceptionally efficient for cellulose hydrolysis, owning to higher solubilization of cellulose and hemicellulose while the simultaneous exclusion of lignin from the feedstock (Kumari and Singh 2018). Acid and alkali pretreatment was more broadly applicable for the lignocellulosic feedstock. NH_3, $Ca(OH)_2$, KOH, and NaOH are primarily used alkalis for

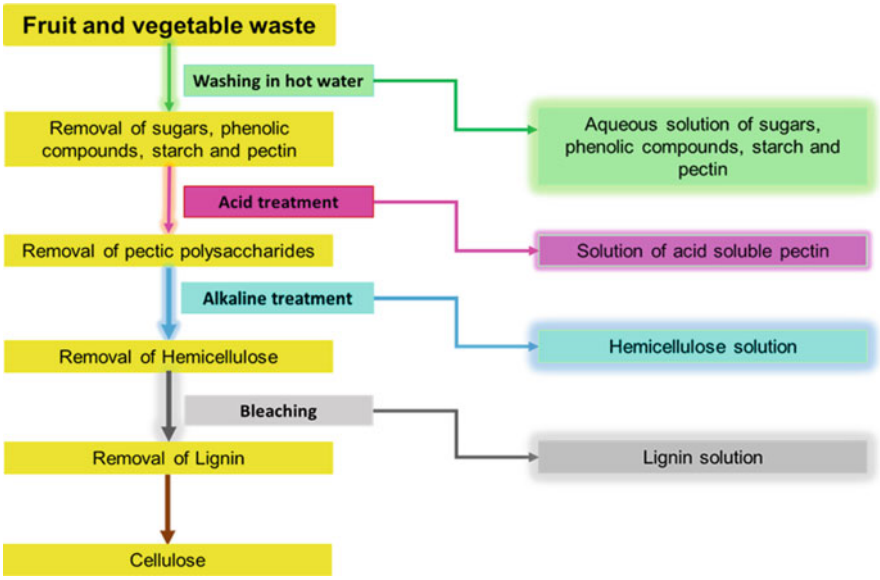

Fig. 8.3 Fractionation process for the pretreatment of FVW waste (Szymańska-Chargot et al. 2017)

pretreatment (Wan et al. 2011). The most promising microbes for biotic treatment are different white-rot fungi related to the Basidiomycetes class. *P. chrysosporium* is capable of lignin biodegradation and has extreme competence compared to the different acknowledged species of white-rot fungi as of its excessive growth rate (Sindhu et al. 2016). For valorization of FVW a combined way is much effective that include multiple pretreatment methods. In this fractionation method, FVW feedstocks are converted into its component at low cost, and simultaneously it will offer fractions of several valuable by-products. However, cellulose is the final product of FVW pretreatment which is further used for bioethanol fermentation. In this strategy FVW was washed in hot water and then kept in several solutions having acid, alkali, and oxidative agent sequentially. Such combined pretreatment methods are advantageous to obtain different fractions of sugar, lignin, pectin, cellulose, hemicellulose, and other bioactive components (Szymańska-Chargot et al. 2017). Figure 8.3 represents fractionation process for the pretreatment of FVW waste.

In a study, the consequences of different parameters were analyzed on the pretreatment efficiency using *C. subvermispora* for bioethanol fermentation and obtained up to 94% of cellulose degradation with 31.59% lignin digestion (Wan and Li 2010). Pretreatment is a significant part of bioethanol production from lignocellulosic feedstock. Pretreatment methods substantially impact the whole production process, and it directs the production of lignocellulose derivatives like acetic acid, 5-hydroxymethylfurfural, formic acid, furfural, and levulinic acid. If the accumulation of pretreatment derivatives is sufficiently high, it will act as an enzyme inhibitor for consequent stages of microbial fermentation (Cavka and Jönsson 2013).

The presence of inhibitors can be confirmed by (1) by adding some alkali like NH_4OH, $Ca(OH)_2$, and NaOH, (2) using enzymatic action of peroxidase and laccase, (3) thermal treatment or vaporization, (4) using liquid-liquid extraction or supercritical extraction, and (5) microbial treatment using *Trichoderma reesei* (Zabed et al. 2016).

8.1.4 Bioethanol Production

Bioethanol production from FVW could be achieved in three steps: (1) saccharification/hydrolysis, (2) fermentation, and (3) ethanol separation (distillation). Generally, bioethanol fermentation has been accomplished by using some bacteria such as *Zymomonas mobilis* or yeast (Ma et al. 2008). Yeast like *S. cerevisiae* can utilize carbohydrates and is effective for glucose to ethanol conversion (Chen 2011). Using yeast cell culture for anaerobic digestion of leftover biomass can reduce up to 30–50% of COD (Suwannarat and Ritchie 2015). Yeasts cell did not hold the whole range of amylolytic enzymes like glucoamylase, α-amylase, and β-amylase, which is essential for breaking down complex saccharides into glucose completely. YIR019C (FLO11, MUC1, STA4) and YIL099W (SGA1) are two genes in yeast cells that encode for α-glucoamylases only. Ethanol production can be performed two ways: either saccharification performed with fermentation simultaneously (SSF) or separated hydrolysis processing, followed by the fermentation step (SHF). In SSF, saccharification is carried out with fermentation, together in a single-chamber bioreactor; hence SSF is economically practical compared to SHF. In spite of that, the optimal parameters and conditions for saccharification and fermentation processes are diverse (Vohra et al. 2014). Ethanol production or fermentation appears to be a biochemical redox reaction inside the yeast cells that required an appropriate range of oxidation-reduction potential (Ma et al. 2016b). Since xylose makes a significant constituent in the hydrolyzed biomass, *S. cerevisiae* (widely utilized for ethanol production) performs glucose fermentation but could not metabolize xylose. An ascomycetous yeast, *P. stipitis*, was found able to ferment xylose, so the co-culture of *P. stipitis* and *S. cerevisiae* seems effective for elevating the fermentation of pretreated feedstock consisting of both glucose and xylose (Kordowska-Wiater and Targonski 2001). Studies disclosed the relevance of the co-culture method for bioethanol fermentation and generally include the following co-culture: (1) immobilization of *Z. mobilis* and unbound *P. stipites* co-culture, (2) *Z. mobilis* with *Candida tropicalis*, (3) *S. cerevisiae* with *P. tannophilis*, and (4) co-culture of *S. cerevisiae* with *E. coli* strain *KO11*. However, the best combination for xylose and glucose fermentation is the co-culture of *P. stipitis* with immobilized *Z. mobilis* (Chen 2011). The efficiency of the co-culture method is based on the growth rate of microorganisms on the diverse feedstock and the fermentation conditions such as pH and temperature (Cardona and Sánchez 2007). Figure 8.4 represents a generalized process involved in ethanol fermentation from FVW.

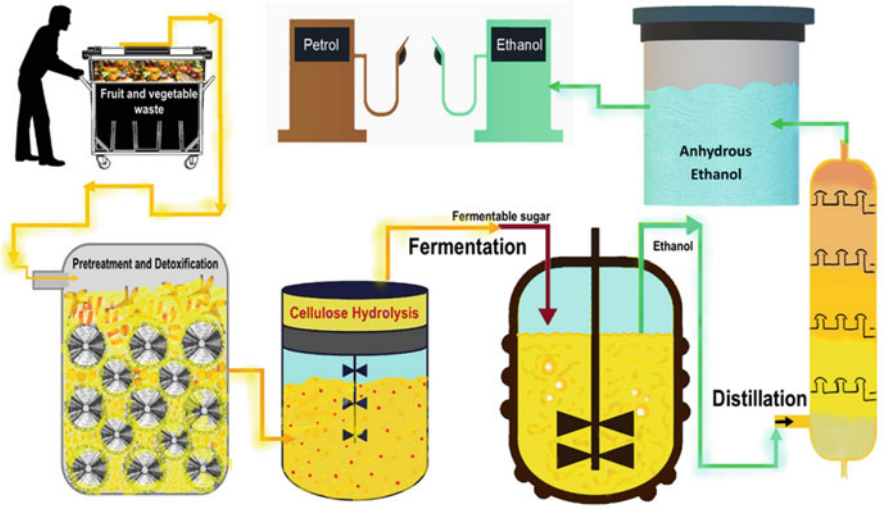

Fig. 8.4 The process involved in bioethanol fermentation from FVW

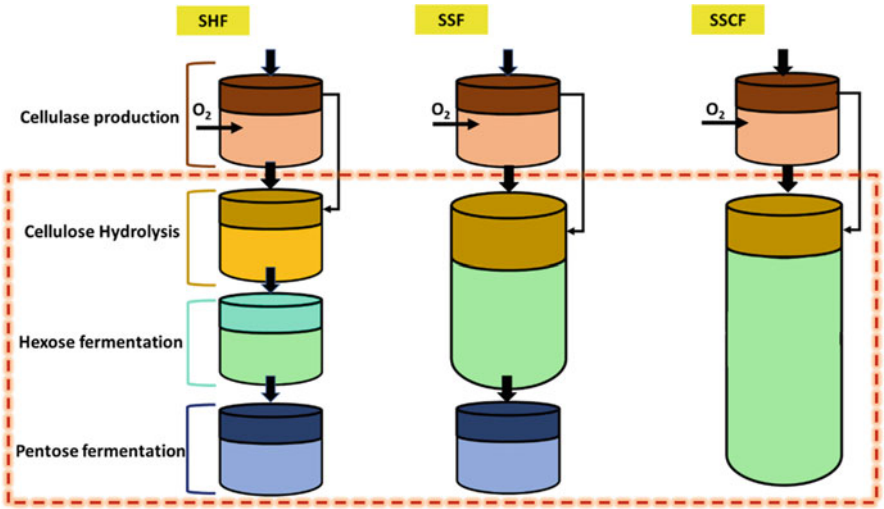

Fig. 8.5 Processing strategy in SHF, SCF, and SSCF

Bioethanol production mainly differs in the following three methods that are SSCF, SSF, and SHF. Fermentation is wholly detached from lignocellulose hydrolysis in SHF. Separate processing of enzymatic hydrolysis and fermentation enabled the enzyme operation at a high temperature and showed exceptional performance. Microbial culture needs a lesser temperature for sugar consumption and optimization during fermentation. Figure 8.5 depicts differences in processing of these three methods. In SSCF and SSF methods, hydrolysis and fermentation happen together

to maintain lower glucose concentration, so the entire process occurs quickly. In the SSF, glucose is isolated from pentose, whereas the SSCF procedure handles glucose and pentose in the same bioreactor (Canilha et al. 2012). SSF and SSCF processes are highly efficient and need a single bioreactor for production; hence, it is chosen over the SHF (Chandel et al. 2007). Ohgren et al. (2007) compared SSF and SHF process configurations for bioethanol production using pretreated corn stover with 8% water-insoluble solids. During SHF, pretreated corn stover slurry has significant concentration of inhibitors that affect enzyme hydrolysis negatively, whereas SSF minimized the negative impact of inhibitors. SSF minimize glucose inhibition (during hydrolysis). Hence SSF was determined as improved configuration in comparison to SHF (Ohgren et al. 2007).

Continuous or batch, repeated batch, and fed-batch mode are standard practices used for bioethanol production. In the batch mode of production, the substrate is supplied at the initial phase of the fermentation without adding or eliminating further substrate into the medium (Hadiyanto et al. 2013). Batch mode is recognized as minimal, easy, and flexible to run with control systems for fermentation. A closed-loop arrangement maintains an elevated sugar and inhibitor concentration during the initial and terminal phases of the fermentation, whereas the process deals with high product concentration (Thatoi et al. 2016). Batch mode of fermentation includes several processing benefits such as overall sterilization, no need for advanced labor skills, easy to control, easy handling of feedstocks, and flexibility for several product specifications (Jain and Chaurasia 2014), but the productivity is significantly less and requires high labor cost. The presence of a significant concentration of saccharides inside the bioreactor may cause inhibition of microbial biomass growth and ethanol production (Cheng et al. 2009). To overcome this inhibition for enhanced ethanol production, fed-batch mode of fermentation is preferred. Fed-batch fermentation involves feeding substrate to the reactor and avoiding the effluent removal; hence it is considered as a combination of batch and continuous mode. In a fed-batch, the substrate must be fed at a specific rate, whereas culture size varies. For higher ethanol yield in the fed-batch mode, it is essential to maintain lower substrate concentration because lower substrate concentration is suitable for converting fermentable sugar into ethanol (Jain and Chaurasia 2014). Fed-batch mode has several advantages: better availability of dissolved oxygen, lower toxicity, quick fermentation, and high ethanol yield (Cheng et al. 2009). Fed-batch has been effectively run in the SSF system by repetitively fed pretreated feedstock to attain high fermentation and ethanol yield (Azhar et al. 2017). The bioreactor is continuously loading with the substrate, culture medium, and nutrient media in a continuous processing method, whereas the culture quantity remains the same, and the final effluent (products) continuously drains off from the reactor. Some desired particular products such as microbial biomass, remaining sugar, and ethanol could be recovered from the bioreactor (Azhar et al. 2017). Small-size fermenters, greater ethanol yield, and economic effectiveness are the benefits of the continuous mode that make it chosen over batch and fed-batch modes. The ability of *S. cerevisiae* for fermentation and ethanol production is considerably falling with long cultivation time. The

risk of contamination is more in continuous mode than any other fermentation modes (Chandel et al. 2007).

8.1.5 *Ethanol Recovery by Distillation*

After fermentation, downstream processing began with several unit operations which are performed for bioethanol recovery from the fermentation broth. At first, liquid-solid separation is performed to separate solid fractions (containing residual saccharides) and bioethanol from fermentation broth. Definitely, filtration and centrifugation are the best choice for liquid-solid separation. To minimize the water content of hydrolysate, supernatant is driven to the rotary evaporator. Serial evaporation helps to attain pure condensate with the concentrated syrup. Evaporation is followed by distillation. Condensate consisting bioethanol will be circulated to the distillation unit. Separation of ethanol from condensate is based on the differences in the boiling points of water (100 °C) and bioethanol (~78 °C) mixture. If water and ethanol solution is very dilute, repeated distillation is preferred to attain >95% of ethanol concentration. Bioethanol recovery using distillation attains 99.6% efficiency to minimize the losses of evaporated portion of bioethanol (Avilés Martínez et al. 2012; Balat 2011).

8.2 Factors Affecting Fermentation

FVW has sufficient chemical nutrients for microbial growth and fermentation. Studies show no significant impact on ethanol production after adding external inorganic additives such as KH_2PO_4 and $(NH_4)_2SO_4$ to the production medium (Thongdumyu et al. 2014; Tang et al. 2008; Ma et al. 2008). However, when Ca^{2+} was added in ethanol fermentation by *S. cerevisiae KRM-1*, using kitchen waste, it has been found that Ca^{2+} can enhance the flocculation rate of yeast cells significantly (Ma et al. 2009). Besides nutrients, bioethanol fermentation depends on several fermentation conditions such as inoculation size, temperature for saccharification/fermentation, pH, moisture content, and fermentation time. Table 8.3 shows factors affecting bioethanol production and optimal condition for fermentation.

pH can alter the nature of proteins. Multiple enzymes are involved in the metabolic processes which occur during fermentation. A very low pH may alter the nature and structure of enzymes by causing the dislocation of chemical bonds. It is found that at higher pH, yeast tends to produce acids in place of bioethanol (Tahir et al. 2010). Microbial metabolic activities are basically enzyme-catalyzed reactions that further relied on several external factors such as temperature and pH. Temperature change deeply affects metabolic pathways by denaturing enzyme structure. Conventionally, saccharification is achieved at 95–105 °C (high temperature) using thermophilic α-amylase (Xu et al. 2016), whereas fermentation

Table 8.3 Factors affecting bioethanol fermentation

Factors	Effect	Optimum condition	Reference
pH	High pH cause enzyme inactivation	4–5	Ma et al. (2008, 2016b) and Tang et al. (2008)
Saccharification temperature	High pH cause enzyme degradation	50–60 °C	Hong and Yoon (2011) and Tang et al. (2008)
Fermentation temperature	Effect microbial activity	30–38 °C	Ma et al. (2008, 2016b), Le Man et al. (2010) and Tang et al. (2008)
Inoculum size	Small amount of inoculation affects slower cell growth	10% (v/v)	Ma et al. (2008, 2016b)
Moisture content/ solid to liquid ratio	Microbial growth and activity	1:0.5	Ma et al. (2008) and Uncu and Cekmecelioglu (2011)
Fermentation time	Ethanol productivity	40–48 h	Ma et al. (2008, 2016a) and Tang et al. (2008)

temperature is based on feedstock composition, and, generally, fermentation temperature varies from 25 to 30 °C (Vohra et al. 2014). The size of the initial inoculum significantly affects microbial cell density during fermentation. Small inoculum size leads to a more extended lag phase by slowing the cell growth and lowering ethanol production, whereas large inoculum yields overgrowth of cells, which further causes substrate competition for microbial population (Ma et al. 2008, 2016b). FVW has a high water content that significantly affects microbial growth and activity. A high solid to liquid ratio results in greater ethanol concentration, affecting microbial activity negatively (Ma et al. 2008). Whereas a low solid to liquid ratio does not affect microbial activity, however during the distillation process, the high moisture content in waste biomass demands enormous energy and ultimately enhances the production cost of ethanol. Fermentation efficiency is hugely affected by incubation time. Extended fermentation time requires additional energy consumption that will raise the production cost of ethanol. Adequate fermentation time avoids the accumulation of by-products (glycerol, organic acids) responsible for yeast activity inhibition (Ma et al. 2016a).

8.3 Ethanol as Biofuel

Bioethanol has high octane number, which measures performance. Higher octane number tends to the more significant compression that the fuel could endure prior to ignition. Lower octane numbers cause premature ignition and engine/cylinder knocking, so the gasoline engines require high compression ratios that can be achieved by a fuel having a higher octane number. Bioethanol is selected to be a fuel that can be utilized in high-performance engines due to its high octane number

Table 8.4 Effect of ethanol blending on different performance measuring parameters of engines

Fuel performance parameters	Effect on engine	Reference
Combustion efficiency	Increases	Bayraktar (2005, 2007)
Cylinder temperature	Increases	Thangavelu et al. (2016)
Cylinder pressure	Increases	Thangavelu et al. (2016)
Flame speed	Increases	Thangavelu et al. (2016)
Combustion duration	Decreases	Bayraktar (2005, 2007)
Combustion speed	Decreases	Bayraktar (2005, 2007)
Combustion temperature	Decreases	Bayraktar (2005, 2007)
Peak heat release rate (HRR)	Decreases	Thangavelu et al. (2016)
Engine torque	Significant improvement	Balki et al. (2014) and Topgül et al. (2006)
Engine knocking problem	Resolved	Thangavelu et al. (2016)
Cold start problem	Resolved	Thangavelu et al. (2016)
Brake power	Significant improvement	Yücesu et al. (2006)
Brake thermal efficiency	Significant improvement	Munsin et al. (2013) and Balki et al. (2014)
Volumetric efficiency	Improvement	Kiani et al. (2010) and Balki et al. (2014)
Brake means effective pressure	Improvement	Zhuang and Hong (2013)
Brake specific fuel consumption	Decreased for lower blends (E5–E20) and increased for higher blends (E60–E80)	Türköz et al. (2014)
GHG emissions	Reduced	Karavalakis et al. (2014)

(Dabelstein et al. 2000). High oxygen content increases combustion efficiency and reduced hydrocarbon emissions. Table 8.4 represents the effect of ethanol blending on various performance measuring parameters of spark ignition engine (Thangavelu et al. 2016).

While counting on GHG emission, significant reduction of unburnt hydrocarbon and CO was observed (Karavalakis et al. 2014). However, there is no remarkable decline in NO_X, CO_2 emissions, and other unregulated emissions like carbonyls, aromatics, particulate matter, etc. (Thangavelu et al. 2016). Bioethanol utilization does not require any alteration in the motor engine, and it does not emit greenhouse gases and eco-friendly and affordable production cost (Ritslaid et al. 2010; Sutjahjo 2018).

8.4 Future of Bioethanol in India

At current, the Indian population is highly dependent on conventional fuel resources. The Indian energy requirement is primarily relying on imported crude oil to satisfy its domestic consumption requirements. However, according to "National Policy on Biofuels 2018," bioethanol produced from sugar cane, sweet sorghum, sugar beet, corn, forestry/agricultural waste residues, rotten potatoes, cassava, bagasse, etc., are used for transportation or stationary fuel requirements. As per the policy, the government will also take some crucial steps for the adoption of biofuels. Indian government starts a 5–18% Ethanol Blended Petrol Program (EBPP), in which ethanol produced from various biomass feedstocks will be blended with petrol. Similarly, the commercialization and development of second-generation ethanol technologies have been promoted (Das 2020). Indian ethanol market is categorized as solvent, beverages, fuel and fuel additive, disinfectant, flavoring, and fragrance on the basis of its application. The government is emphasizing the biofuel production methods using waste biomass for ethanol production in the future. To reduce dependence on crude oil imports, the Indian government incentivizes sugar manufacturers to produce bioethanol for oil marketing companies (OMCs). Predictably, ethanol production will increase by three to fivefolds by 2030 to meet its 20% Ethanol Blended Petrol Program requirement. Bajaj Hindustan Sugar, Triveni Engineering & Industries Ltd., HPCL Biofuels Limited, India Glycols, Balrampur Chini Mills Ltd., Shree Renuka Sugars Ltd., Jeypore Sugar Company Ltd., Mawana Sugars Ltd., E.I.D Parry India Ltd., and Simbhaoli Sugars Ltd. are some of the key manufacturers in the Indian ethanol market. TATA Projects (an infrastructure company) got a project from BPCL (Bharat Petroleum Corporation Limited) for a bioethanol production plant with a capacity of 100,000 L/day in Bargarh, Odisha, India (Web Resource 1 2020). As the government of India is promoting cellulosic/agricultural feedstock for bioethanol production, it will be a boon for farmers to gain additional income from their agricultural waste. The burning of agricultural waste biomass causes air pollution, so the utilization of lignocellulosic feedstock sustains the country's Ethanol Blended Petrol Program as well as reduces environmental stress of greenhouse gas emissions.

8.5 Conclusion

The future of bioethanol is knotted with a greater extent on metabolic and genetic engineering of microorganisms and nonfood crops used during fermentation. The main aim of such bioengineering practices is to evolve such microbes that are able to perform efficient saccharification of lignocellulosic waste biomass or nonfood crops. Fruit and vegetable residues contain a high amount of simple and complex carbohydrates, and these sugars can be used as a raw and fresh material for the production of bioethanol using microbial culture. Currently, 80% of bioethanol is produced

from edible grains supplies such as sugar and starch. Recently, lignocellulosic biomass is considered for bioethanol production. More attention is needed toward cellulolytic enzyme production, as enzyme production charges more than 50% of the biomass saccharification cost. The enzyme used for cellulose hydrolysis can be improved by molecular engineering of enzymes themselves or genetic engineering of enzyme-producing microbes; so the production cost can be reduced. The second-generation bioethanol production uses two separate steps for saccharification and fermentation. SHF is favorable for these two steps to be carried out under their optimal conditions separately. For SSF, a microbe should be engineered or isolated to perform cellulose hydrolysis and ethanol fermentation simultaneously. Otherwise, co-cultures of two or more microbes could be utilized for combined saccharification and fermentation. Bioethanol produced from agricultural waste, fruit, and vegetable waste biomass is a sustainable fuel that obliging the engine to produce less greenhouse gas emissions. Fruit and vegetable waste has a lower cost and a wide range of availability, making it an excellent economical choice for bioethanol production.

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Chapter 9
Production of Bioethanol from Fruit Wastes: Recent Advances

Loveleen Kaur Sarao, Sandeep Kaur, Pardeep Kaur, Ankita, and Harmeet Singh Bakala

Abstract The enormous reduction of fossil fuel resources has resulted in the human race depending on energy sources which are renewable with bioethanol being one of them. Ethanol is a clear liquid alcohol. This is obtained via the fermentation of varied biological substances. This alcohol has several uses. One of its use in particular is gaining more importance. One of the most important renewable fuels is ethanol. It contributes to a reduction of the negative environmental impacts which occur as a result of the global use of fossil fuels. Production of fuel ethanol has gained attention worldwide. This is so, as several nations are looking for cutting down oil imports, boosting the economies at rural level and enhancing the quality of air. The huge usage of fuel ethanol globally requires a production technology which is cheap and sustains in the environment at the same time. The present research capacities for enhancing fuel ethanol production finds link to the nature of raw materials being used, the steps involved in processing and the process engineering issues which are related to this. The world ethanol production has reached about 29.03 billions of gallons. Presently during the energy crisis, ethanol production using cheaper sources of raw material employing efficient fermentative microorganisms is the way out for meeting increasing demand for ethanol. Producing value-added products by using wastes from agro-industries and the food processing units is gaining attention. In addition to production of energy, it curbs environmental pollution. Enormous quantities of wastes in the form of fruit peels, seeds, pomace, rags, kernels, etc. are generated by the food industry. These wastes are biodegradable in nature. To produce bioethanol, fruit waste serves as a promising lignocellulosic material. This is so, as it falls amongst the abundant renewable resources. Good-quality bioethanol

L. K. Sarao (✉) · Ankita · H. S. Bakala
Punjab Agricultural University, Ludhiana, Punjab, India

S. Kaur
Department of Agriculture, Maharishi Markandeshwar University, Ambala, Haryana, India

P. Kaur
Department of Biotechnology, Sri Guru Granth Sahib World University, Fatehgarh Sahib, Punjab, India

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is obtained from several fruit wastes. These wastes include banana peels, mango waste, apple pomace, kinnow peels, orange peels, grape pomace, papaya waste, etc. This fuel can be used in the engines for transportation purpose and curb the emissions. The pretreatment methods' choice serves an important role for improving output of the enzymatic saccharification. This makes the entire procedure economically reasonable. Employing recombinant cellulases to produce bioethanol is a way out for controlling the price of enzyme.

Keywords Fruit waste · Ethanol production · Biofuels · Bioethanol · Fermentation · pretreatment

9.1 Introduction

The excessive consuming of fossil fuels results in enormous pollution levels. This is much more evident in the large urban areas. The energy sources which are environmentally sustainable are required for finding a viable and long-lasting alternative for liquid petroleum. To tackle this issue, in the recent times, the addition of biofuels to gasoline is being done. This controls the carbon monoxide emission and unburnt hydrocarbons that lead to the formation of smog (Wyman 1994). Owing to the reduction of the resources which are based on fossil fuels, the mankind has been forced to be dependent on sources of energy which are renewable. One such energy source is bioethanol. Several different biological materials are fermented to obtain ethanol, which is a clear liquid alcohol. There are several uses of this alcohol. One use in particular is gaining a lot of attention. One of the most indispensable fuels which are renewable is ethanol. It helps in the lowering of the harmful effects on the environment which result owing to the global utilisation of fossil fuels (Lalitha and Rajeswari 2011). Producing this alcohol has been sped up because of its increased demand. This ethanol is in demand by several industries as it serves as an alternate energy source, solvent in industries, preservative and cleaning and disinfecting agent.

Ethanol is one of the most widely employed biofuel. It is made in a process which is similar to that of brewing beer. Usually, ethanol is produced via chemical synthesis of petrochemical substrates. It is also done by the microbiologically converting the carbohydrates which are present in the agricultural products (Dhabekar and Chandak 2010). In the present times, fuel ethanol generation has gained importance. This is so as several nations are on the lookout for curbing the import of oil, giving a boost to the rural economies and focussing on the improvement of the quality of air. The global ethanol production has reached about 29.03 billion of gallons (Fig. 9.1) with the USA being the first and Brazil being second largest producers amongst the top most producers of fuel ethanol (AFDC 2019). According to an estimate, we will be running out of the fossil fuels in the future. Therefore, converting of biomass to obtain fuel ethanol is trending. Three main types of raw material for producing ethanol are recognised. Producing ethanol using sugar- and starch-based materials is quite feasible when compared to the material which is

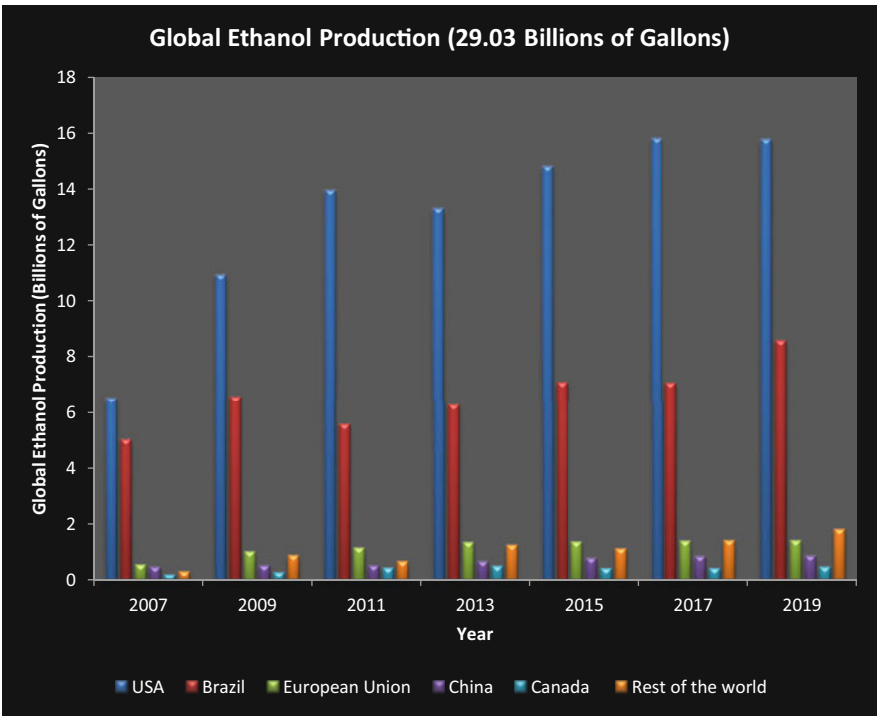

Fig. 9.1 Global bioethanol production output for the year 2019

lignocellulosic. This is so as there are technical challenges involved like pretreatment (Petersson et al. 2007). In addition to this, using high-end technology and methodologies involving complicated instrumentation having hefty costs of operation costs is a limiting factor for commercialisation and their application at industrial level in the nations which are still developing (Isarankura et al. 2007). Research is focused on designing and improving a process for producing a sustainable fuel for transportation by the use of feed stocks which are reasonable priced. All over the world, several different agriculture-based raw materials which are rich in fermentable carbohydrate components have been put to test. This has been done for bioconverting from sugar to obtain ethanol. Costing of the raw materials which are based on carbohydrate is limiting factor when industrial production is being considered at a large scale using the process of fermentation. The feedstock price is more than 55% of production cost. To produce bioethanol, cheaper feedstocks like lignocellulosic biomass and agri-food-based wastes are being thought of commercially (Campo et al. 2006). The worldwide production of different fruits and their largest producers have been depicted Table 9.1. The only possible way to produce ethanol using cheap raw materials is making use of the fermentative microorganisms which are efficient. By doing so, the huge demand of ethanol in the current scenario of energy crisis can be met effectively (Pramanik and Rao 2010). One of the

Table 9.1 Worldwide production of different fruits in 2020

Fruit	Production	Largest producer
Apple	86 million tonnes	China
Mango	56 million tonnes	India
Pineapple	28.2 million tonnes	Costa Rica
Grape	23.38 million metric tons	Spain
Banana	153 million tonnes	India
Papaya	13.3 million tonnes	India
Citrus fruits	124 million tons	Brazil
Orange	79 million tonnes	Brazil

Table 9.2 India's rank in comparison to other countries in context of export of Fresh Fruits in 2020

Fruit	Rank of India
Apple	39
Citrus fruits	3
Orange	3
Grape	9
Mango	1
Papaya	7
Pineapple	5
Banana	1

potential solution which can lead to reduction of cost involved in the energy and input for ethanol production is making use of the fruit biomass which is ripe as the raw material for fermentation and enzymatically hydrolysing by employing microbial enzymes (Hammond et al. 1996). Amongst the fruit crops, India occupies the first rank in comparison to other countries in context of export of mango and banana (Table 9.2 and Figs. 9.2 and 9.3).

The fruits which are pulpy are quite prone to rotting or spoilage owing to their nature. The spoilage happens during harvesting, during the storage period, during the phase of marketing and also during its processing. This leads to a lot of wastage and losses. As per the India Agricultural Research Data Book of 2004, production of fruits and vegetables in India was estimated to be around 150 million tonnes. The generation of waste was estimated to be 50 million tonnes. In such commodities, the estimated loss is nearly 20 to 30% of the entire produce. This amounts to a total loss of Rs. 30,000 crore every year. As per report of FAO (FAO 2003), the amount of total waste which was generated from the fruits was calculated to be around 3.36 million tonnes (MT). This figure was calculated based on entire production of 16.8 MT. This was 6.4 MT for banana. The unsuccessfulness and the non-ability to salvage and reutilisation of this material keeping in view the economics lead to the unwanted wastes and reduction of the natural resources (Essien et al. 2005). The wastes which are generated from the food processing units which are solid wastes in nature could be utilised as useful raw materials for producing secondary metabolites which find significance industrially by microorganisms. The main by-products which are obtained after the processing of several fruits are the peels. These peels

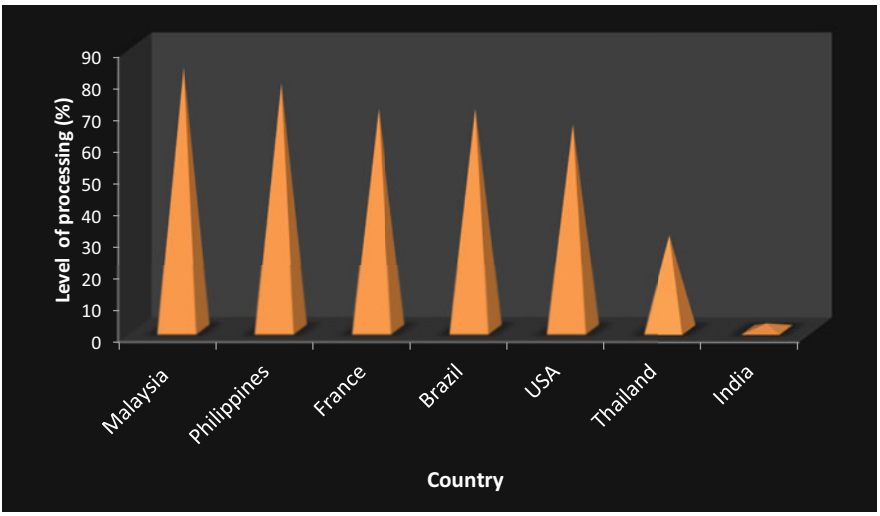

Fig. 9.2 Level of processing of different fruits and vegetables worldwide

serve as an efficient source of several bioactive components which have several useful effects.

A major portion of fruit peels (nearly 20–30% in case of banana and nearly 30–50% in case of mango) are disposed of as wastes by processing units. This disposal leads to various environmental issues (Zhang et al. 2005). Such wastes obtained from the processing of fruits could be used as a potential feedstock for production of bioethanol. This can also serve as a useful alternative for disposing off the residues which cause pollution (Wyman 2001). Some of the research reports show varied practical applications of such wastes obtained from fruit (banana and mango). Some of these are producing the microbial enzymes which can be utilised industrially (Essien et al. 2005), alcohol production (Hammond et al. 1996), wine production, vinegar production, biogas production (Guneseelan 2004) and food to be used for livestock (Onwuka et al. 1997). The number of reviews on production of ethanol from other feedstocks such as those based on sucrose- or starch-based material is quite few. Production of ethanol via pretreated enzyme saccharified fruit wastes by using simple fermentation methods has not been studied much.

9.2 Advantages of Bioethanol

Based on the numerous benefits of ethanol, it is being used as a fuel. The benefits are low thermal energy content (nearly 45% less per gallon as compared to diesel), cheaper cost and relatively lower emission than gasoline or diesel. As compared to petrol, ethanol possesses a high octane number (99) while that of petrol being 80–100. Owing to this, the pre-ignition does not take place upon employing ethanol.

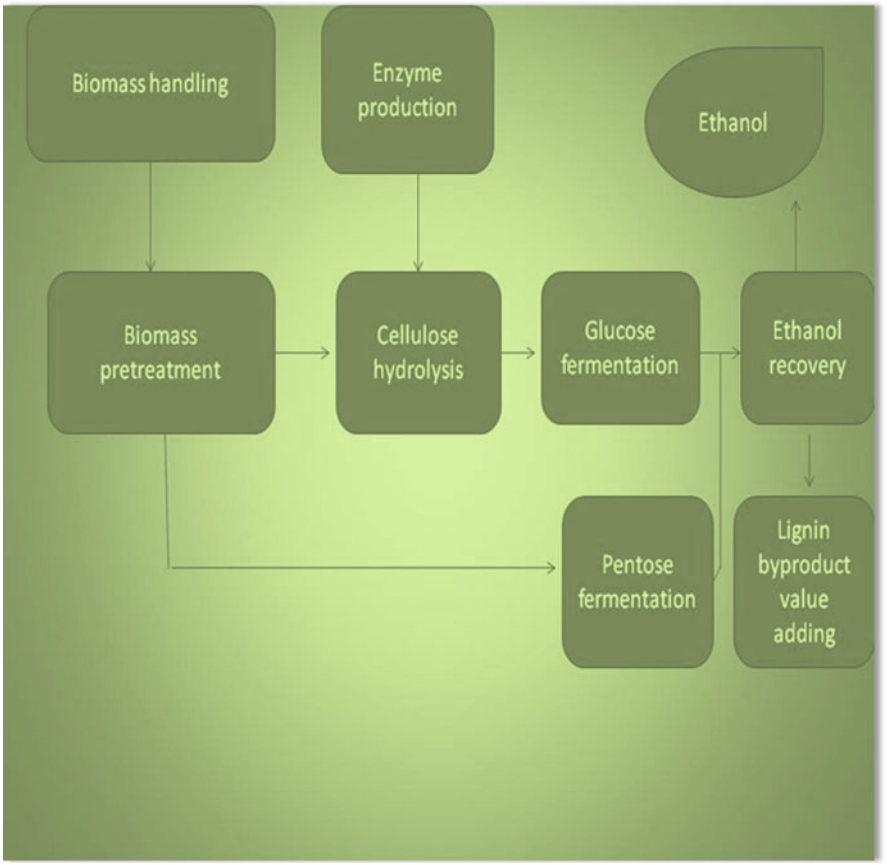

Fig. 9.3 Flow diagram for the production of bioethanol

Therefore, ethanol is being largely used as a fuel additive which is competitive along with gasoline. In rare instances the pure form is used (Oliveira et al. 2005). Nearly 90% lowering of vehicle CO_2 emission is achievable by putting to use bioethanol to produce gasoline (War and Singhs 2010). The Government of India uses a mix of ethanol (10%) to the petrol. This is done for achieving cost cutting and the consumption quantity of petrol. Producing ethanol by utilisation of different agro residues is of primary importance. This is because of the easy availability of cheap raw material (Mishra et al. 2012).

The fuels which could be put to use as an alternative to gasoline and diesel are the biofuels. These biofuels are gaining attention all over the world. The biofuels are eco-friendly and are renewable fuels. As a result of this factor, they are thought of as the best alternatives to be used for SI and CI engines. These biofuels could be put to use in pure form or could be used by blending along with gasoline and diesel to be used in the IC engines. The commonly available feedstocks and agricultural waste can be used to produce biofuels. Biodiesel is another source for alternative fuel.

This is generally produced using animal/vegetable oils and alcohol-based fuel such as ethanol, which in turn is obtained via fermenting sugarcane or corn. This method is quite common in the USA. In nations like Brazil, ethanol has become a common fuel being used and is available at fuel stations. A modern form of biomass energy is the ethanol which is obtained from biomass. This ethanol has a potential for being a sustainable fuel for transportation to be used in gasoline engines (Wang 2000). With the ever-increasing price of oil, producing biofuels is a blooming and a profitable business.

With a view of developing novel technologies for biofuels production and improvising the ones available, it's mandatory to address challenges and opportunities of biofuels with respect to food security and the needs for a development which is sustainable (FAO 2008). As per Osanaiye Akin et al. (2005), the production of ethanol via fermentation has to face a lot of competition with production of ethanol from sources which are petroleum-based. However, with increase in the value of petrochemical, attention was diverted to fermentation of ethanol (Ahmeh et al. 1988). As the renewable material (waste) is cheap or even free at times, therefore it is readily available and quite economical. There are certain bottlenecks in ethanol production which have been depicted in Fig. 9.4.

9.3 Present Scenario

Currently, biofuels like bioethanol, biodiesel, biohydrogen and methane obtained using lignocellulosic biomass are being generated by the utilisation of agro waste instead of the energy crops because they pose a competition for the food crops. The agricultural wastes are in abundance which pose a disposal issue. A way out is to use lignocellulosic biomass. By doing so there can be reduction in the competition that occurs between the food and fuel (Mahro and Tim 2007). The lignocellulosic biomass material of plant material like wood, grass and the residues of crop offers possibility of a renewable and a source of sugars which is relatively greenhouse gas favouring and could be utilised for generating ethanol. The potential involved in utilisation of the lignocellulosic material for bioethanol production is very well recognised. The main source for ethanol production is carbohydrate. This can easily be found in several parts of plants. In India, the ethanol production is commonly done using grain sorghum or corn. For producing ethanol, various different plants or their parts can be used. To name a few, sugarcane, wheat, sawdust and yard clippings can be used.

It was reported that the naturally available resources along with *S. cerevisiae* constitute the highest bidders for commercially producing ethanol. A continuous energy supply can be assured by the conversion of renewable non fossil carbon, like organic waste and biomass having all growing organic matter (plants, grasses, fruit wastes and algae) into fuels (Wyman 1996).

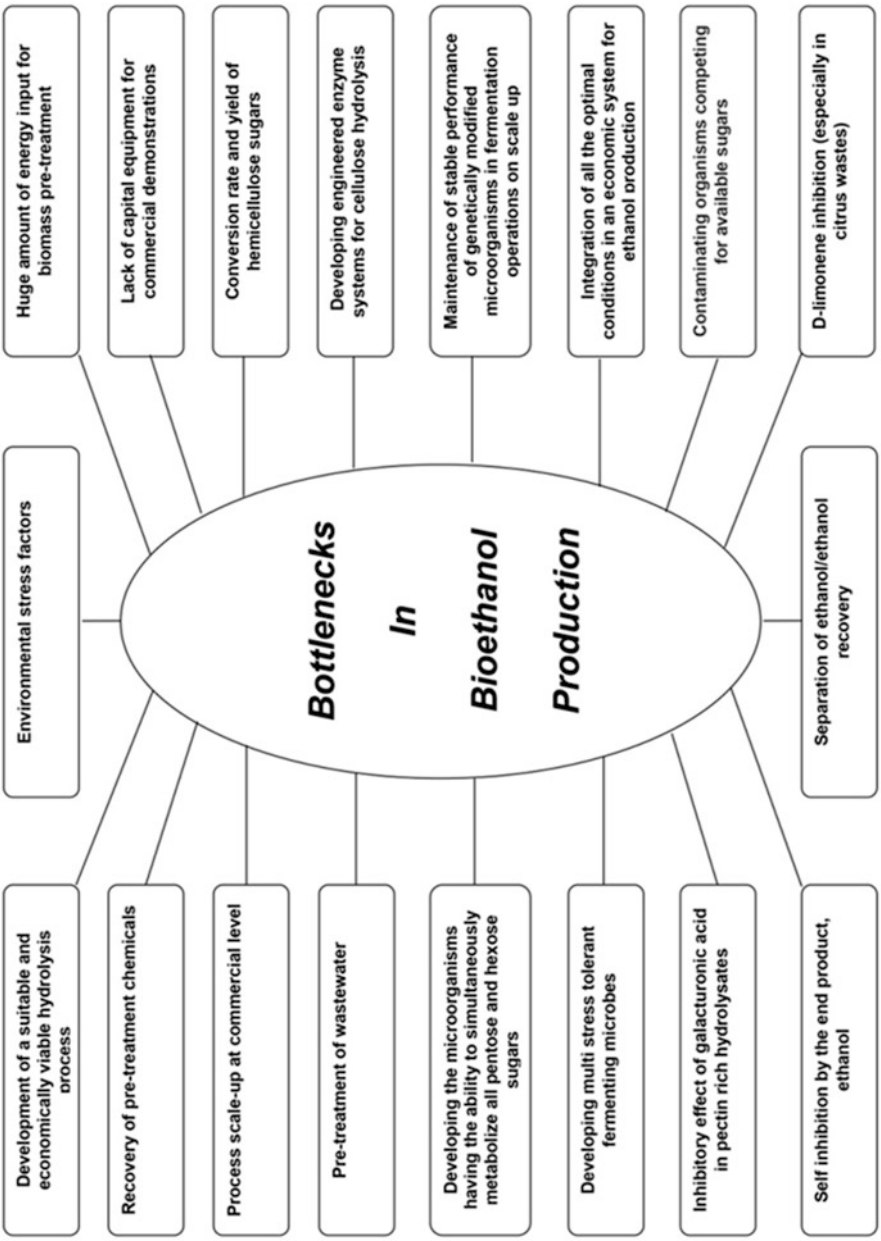

Fig. 9.4 Bottlenecks in bioethanol production

9.4 Ethanol as a Biofuel for Renewable Energy

A source of energy which is obtained using organic matter or biomass and could be employed for the production of heat and electricity or can be use a transportation fuel is referred to as bioenergy (United Nations 2007). Particularly, the liquid biofuels like ethanol are commonly called as bioethanol and biodiesel. These are the major bioenergy producers. This is often seen in transport sector (United Nations 2007). In the present times, the ethanol being used is generally made via fermentation and subsequent distillation of starchy crops like the corn and wheat (EPA 2010).

Any crop which produces fermentable sugar can be used to produce bioethanol. These include sugarcane, sugar beets and the parts of crops which are unused like the fruit waste. Using these crops to produce ethanol poses a threat to land to be used for food (United Nations 2007). In future, this can be sorted out as cellulosic biomass like trees and grass can also be used to produce ethanol (EPA 2010). The lignin in the structure of these biomass restricts the access to the usable material to produce ethanol (United Nations 2007). A commonly used blend of ethanol being supplied in the market which is utilised for fuelling majority of the vehicles is E10. It is called as gasohol as well. It's a mix (10%) of ethanol in gasoline (EPA 2010). E85 is a blend of high concentrations of ethanol (85% mixture of ethanol in gasoline). This blend is commonly used. Only the flex fuel vehicles can use this mixture (EPA 2010). Besides the E85, the flex fuel vehicles have the ability to operate by putting to use a mix of ethanol and gasoline (EPA 2010). The ethanol concentrations (anhydrous) have the capability to reach close to 100% as a fuel when it's not mixed up with gasoline.

The use of a high concentration of ethanol in gasoline is beneficial, and one of the main advantage is that it is cheap. In 2009, an estimate was made that E85 costs $2.13/gallon (on average). The cost of usual gasoline is around $2.67/gallon (EPA 2010). There is a backdrop in such type of comparison as the ethanol possesses lesser energy as compared to gasoline. An estimate was made that the E85 vehicles got worse mileage (20–30%) as compared to the vehicles powered by gasoline (EPA 2010). "It can be concluded that a 30 MPG gasoline vehicle, a comparable flex-fuel vehicle which runs on E85 will be getting around 21-24 MPG". If we look at the cost per mile, the vehicle with 30 MPG will be costing around $0.089/mile. The flex fuel vehicle which is comparable will be costing around $0.089/mile–$0.101/mile.

Even though the price on an average is low, the issue here is the profitability involved in using the ethanol as fuel. This could be traced back to the energy which is being used for the production and distribution of ethanol from its basic source. Such sources currently are the.

starch crops (EPA 2010). Generally, there are five basic steps which are involved in the ethanol production. These are (1) pretreating the crops, (2) recovering the sugar, (3) fermenting the sugar for producing ethanol, (4) distilling the ethanol to obtain higher concentrations (5) ethanol drying.

The crop has to be treated physically when it is grown and is harvested. It is cleaned, chopped into thin pieces. At times it is even ground to obtain the fine

material. The recovery of the sugars is done by different methods from various crops. Either enzymes could be used, or the simple methods for extraction are employed. Then, the fermentation of these sugars is done via yeasts for producing ethanol. Distillation of ethanol is done using columns (in series) for obtaining ethanol in higher concentrations. With the rise of ethanol concentrations, separating ethanol from water gets tedious. This is so because of the azeotropic conditions of vapour-liquid equilibrium. This limits the capacity of distillation. Next, the ethanol is further dried. This is done in order to enhance overall concentration of ethanol without the vapour-liquid equilibrium hindering it.

9.5 Bioethanol Economy

One of the very important economic considerations is the price of biofuels. There needs to be competitive scenario of biofuels with each other as well as with the mineral-based fuels like diesel and petrol. This ensures the availability of market for the biofuel. This will provide an incentive to the people for converting to a source of energy which is renewable. Hence, during the analysis of crop rotations, the optimisation of the cost should also be given consideration (Murphy and Power 2009).

If we consider till now, the bioethanol cost was higher considerably as compared to the cost involved in the supply of fossil gasoline. Special policies had to be enacted by the national governments to encourage the generation and usage of the bioethanol in the transport segment.

Commonly, the three below outlined approaches could be distinguished for the policies and regulation supporting implementation of biofuels:

1. Policies based on taxation.
2. Policies/subsidies based on agriculture.
3. The fuel mandates (Smith 2008).

Currently, instead of the green sector, the agricultural sector and green lobbies are the ones promoting the development and promotion of biofuels. As a matter of fact, the majority of the biofuel programmes are dependent on the government programmes and subsidies. This creates a possibility of leading to a market distortion and is high in cost for the governments. In several nations in the future, with a high price of oil which is sustained and the progression of more efficient and cheaper technology which is steady, the biofuels can turn as a cost-effective alternative (De Fraiture et al. 2008).

There is high volatility in cost of raw material. This affects the production cost of bioethanol to a great level (Yoosin and Sorapipatana 2007). About 60–75% of entire production cost of bioethanol is represented by the feedstocks.

The technology of production using the crops which contain sugar or starch is mature relatively. It is quite likely that this will not be improved for lowering the production cost. In Brazil, bioethanol obtained from sugar cane is priced around US $0.23–0.29/L (Kojima and Johnson 2005). In EU and the USA, the cost of

bioethanol derived from sugar and corn is at US$0.29/L (Mitchell 2008) and US $0.53/L (Christensen and Smith 2008), respectively.

If we compare the energy content, the cost of producing the biodiesel is low as compared to that of producing the bioethanol. There is a significant effect of the raw materials' price on the economy of producing ethanol via fermentation. This is so because cost of raw material accounts for over 50% of cost involved in production (Classen et al. 1999).

Therefore, it's important to supply cheaper raw materials in order to have a low cost of production. The majority of the wastes (fruits and vegetables) which are obtained from the processing industries are seasonal. Hence, their decomposition does not happen rapidly. Peels of mango, citrus, tomato, pineapple, etc. constitute these wastes. When mechanically dried, these wastes can be stored all round the year. *Saccharomyces cerevisiae* (yeast) and Zymomonas *mobilis* (facultative bacterium) are promising candidates for producing alcohol industrially. With respect to the productivity of ethanol and tolerance, *Z. mobilis* has more advantages as compared to *S. cerevisiae.*

Commercially, ethanol production is done using yeast. This is so as the yeast causes the fermentation of glucose for producing ethanol as the only product virtually. It's also recognised because of its high ethanol tolerance power and quick rate of fermentation. It is also insensitive to the concentration of substrate and temperature (Linden and Hahn-Hägerdal 1989).

Zhou et al. (2007) analysed and discussed the economics involved in the making of citrus ethanol. As a benchmark, the economic model used in process of cellulose to ethanol was employed. The cost of the project and the operating cost (fixed) were estimated for the process involving peel to ethanol. It was estimated that the cost of production of citrus ethanol was nearly $1.23/gal. This was higher than the cost involved in corn ethanol which was $1/gal but lesser as compared to the cost of cellulose ethanol which was nearly $1.35–1.62/gal.

The economic effect was examined, involved in converting xylose to obtain ethanol for wood to the ethanol plant. An estimate was made that the maximum potential reduction in cost of ethanol by using xylose was estimated at $0.42/ gallon from a price of $1.65 (Hinman Norman et al. 1989). The sensitivity involved in the cost of ethanol to yield, concentration of ethanol and the rate of xylose fermentation were studied. It was concluded that the cost of ethanol gets influenced mainly by the fluctuations in yield and the concentration of ethanol, while the rate had least importance.

Analysis was done of several biocatalysts involved in xylose conversion. The best found yeasts for this were *C. shehatae* and *P. Stipitis.* As per Renewable Fuels Association, the industry of ethanol created around 147,000 jobs in several departments of the US economy in the year 2004. Over $2 billion was provided as tax revenue to government at all the levels. The US Department of Energy (DOE) has made an estimate that for each one billion gallon of ethanol being produced, the creation of 10,000–20,000 jobs will occur.

9.6 Types of Fruit Wastes

There is a generation of huge quantities of waste from the industries dealing with food. These wastes are in the form of peels, seeds, pomace, kernels, rags, etc. (Fig. 9.5) . These wastes are biodegradable in nature. Such waste has ample content of carbohydrate. There is an upsurge in the manufacturing of the processed fruit products. Therefore, the quantity of waste being obtained from the related industries is also increasing proportionately. Huge quantities of such waste create disposal issues as the environmental pollution being caused by their disposal has to be ruled out. The manufacturing of beneficial by-products from such wastes is the only means to dispose these wastes effectively.

Huge quantities of effluents as well as the solid wastes are generated by the processing industries related to fruits and vegetables. There is high organic load in the effluents. Besides this, there are cleaning and blanching agents, suspended solids like soil particles and certain fibres. There may also be residues of pesticides which get washed off from raw material.

The primary solid waste is the organic material which includes the fruits and vegetables which are discarded. The issues related to odour are observed when there is poor management of solid waste and the effluents. This is also observed when the processing of onions is done or there is preparation of ready to serve meals. Most of the fruits' and vegetables' waste obtained via the respective industries involved in processing are seasonal. Hence, their rapid decomposition does not occur. When these wastes are dried mechanically, these substrates like peel of citrus, peel of mango, peel of pineapple and wastes of tomato processing can be stored all over the year (Reddy et al. 2011).

Two types of wastes are generated after using fruits. One type is solid consisting of peel or skin, seeds, stones, etc., while the other type is a liquid waste comprising of juice and wash water (Hemalatha 2012). In certain fruits, the portion discarded could be quite high. It is 30–50% in mango, 20% in banana, 40–50% in pineapple and 30–50% in orange. Hence, there is a common but serious waste disposal issue. This could lead to the problems of rats and flies in and around the processing room.

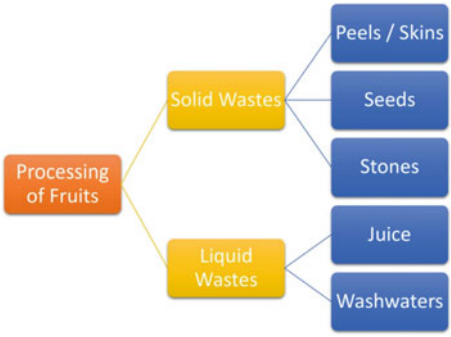

Fig. 9.5 Different types of wastes produced upon processing of fruits

In case there is no plan of utilising the waste, it must be either buried or fed to animals. This should be done away from the site of processing.

A main problem in utilising the fruit waste is ensuring the waste possesses a reasonable quality microbiologically. Hence, the waste produced same day should therefore be only used. It is not recommended to stock up wastes to be used at the end of week's production. A major area under focus is producing the value-added products using the wastes. These wastes refer to those obtained from food processing and the agro-industrial sector. This is so because it leads to reduction in the environmental pollution besides producing energy. Annually, 1.05 billion tons of such waste is available (Anonymous 2004). Generally, a majority of this is disposed of. This accounts for increasing pollution in the environment. As heavy transportation costs are involved in this, the disposal process also becomes an expensive step.

The yearly production of mango peels in India is approximately around 0.4 to 0.6 million tons (Anonymous 2004). This waste is either employed as feed for cattle or dumped in open lands. This dumping leads to environment pollution. Processing of the mangoes is done to the maximum extent. This leads to the generation of solid and liquid wastes of high quality.

While preparing the raw materials, we get solid wastes (stones, stalks and trimmings) and fibrous material. This constitutes around 40–50% of the entire fruit wastes. From this 5–10% is constituted by the pulp waste, and 15–20% is the kernel (Anonymous 2004; Madhukara et al. 1993; Pandey et al. 2000).

The liquid obtained subsequent to washing of the fruit, packing, blanching, cooling and after cleaning the plant and machinery is referred to as the liquid wastes. It is both necessary and challenging to use up these mango wastes. An industry which processes 5 tons of mangoes (totapuri) in an hour generates 6 tons of peels per day as waste after 8 h of work.

While producing orange juice, around half weight of the fruits' is disposed of as waste. The wastes are in the form of peels, seeds, juice vesicles and membranes (Braddock 1999). Presently, such wastes (solids) are spread out on the soil areas near the locations of the production. Such is done as a last utilisation as raw materials to cattle feed or their burning (Garcia-Castello et al. 2006). This method of handling the wastes leads to leaching on the soil and groundwater which is uncontrolled. This leads to enhanced amounts of organic components which severely threatens the environment.

There is extensive cultivation of oil palm trees, *Elaeis guineensis*, in the tropical and humid regions to produce edible oil (Yong et al. 2007). When the red coloured fruit of palm oil trees grow in huge bunches, the empty fruit bunches (EFB), accounting for nearly 20% of the entire oil palm biomass, are removed during the processing oil (Yong et al. 2007). Each year nearly 14.9 and 37.7 million tons of EFB are generated in Malaysia and worldwide, respectively (Akhtar et al. 2010). These bunches have abundance of cellulose and hemicelluloses. These fractions are not digestible with ease. Such bunches constitute the basic materials which should be subject to the waste treatments in the palm industry.

9.7 Fruit Wastes (Substrates) Suitable for Production of Ethanol

Bioethanol production could be done using various raw materials. These raw materials are generally classified into three different categories: (1) sucrose-containing feedstocks (sugar cane, sweet sorghum, sugar beet), (2) starch-rich materials (corn, wheat, potatoes) and (3) lignocelluloses- containing materials (grasses, wood). The main issue with bioethanol is the supply of raw material for production. In addition to this, the cost of the raw material is quite unstable and therefore has a huge effect on the cost involved in production of bioethanol. These days, the research work has focus on biomass which is lignocellulosic. This is one of the most potential feedstocks. This is attributed to its supply and low price (Prados et al. 2010). Fruit waste serves as good lignocellulosic material for producing bioethanol. This is attributed to the fact that it's an abundant renewable resources.

To produce ethanol, the most suitable feedstocks are the crops containing high sugar content. These are sugarcane, fruits, sugar beets, molasses and fruits. This is so as sugar is their main component which could be easily converted for obtaining (Ensinas et al. 2009). Owing to less lignin and abundant sugar contents, such fruit-based residues could turn out to be promising substrate for the production of ethanol as compared to the recalcitrant lignocellulosic-based feedstock such as rice straw, corn stover and wheat straw. Insoluble polysaccharide fractions are also present in fruit residues. These are cellulose, hemicelluloses and pectin. These can be hydrolysed enzymatically to obtain sugars by employing mix of hydrolytic enzymes like cellulase and pectinase (Wilkins et al. 2007c). Even substrate flexibility is offered by the fruit residues in the process of conversion of biomass to ethanol.

During the grading step, banana waste is discarded owing to the imperfections. Bioethanol can be produced from banana biomass used as a raw material (Hossain et al. 2011). Nearly 30% of the bananas which are harvested in Australia are rendered useless at the packaging stage itself (Clarke et al. 2007).

The wastes of banana which is rejected because of the imperfections are generally thrown away as enormous dumps of wastes. This contaminates the water sources. This dumping can also lead to environmental issues and affect the well-being of the living organisms (Tock et al. 2009). Hence, for checking the environmental issues occurring as a result of waste decomposition, it's beneficial to generate energy using banana wastes as the generation source for biofuel.

The banana fruits and the leftover biomass associated with it are amylaceous and lignocellulosic materials. Hence, there is a requirement for them to be hydrolysed for changing them to glucose. This glucose is further fermented to get ethanol (Carrasco et al. 1992; Kumakura and Xin 1993). There is a high content of starch (53.2% w/w) in banana pulp. This makes it one of the appropriate materials to carry out acid hydrolysis. The flower stalks exhibit high content of cellulose (40.9% w/ w). Hence, it's the best raw material for carrying out enzymatic hydrolysis. The banana skin has the higher LHV. Therefore, we can think about it as an appropriate raw material to be used as fuel in utility plant. Banana and cooking banana (*Musa* spp.) production

systems lead to accumulation of an appreciable amount of discard because of high market demands in terms of quality. The ripened fruits possess a good amount of sugar content, which we can process with ease to obtain ethanol (Sophie et al. 2011) .

An agro waste rich in pectin is the lemon CPW. It's a good feedstock to produce bioethanol. This is attributed to its high content of carbohydrate (Marín et al. 2007; Mielenz et al. 2009; Boluda-Aguilar et al. 2010). Ethanol production using orange peel was documented by Grohmann et al. (1994). The production of ethanol using fruits of banana (Manikandan et al. 2008) and peels of pineapple (Ban-Koffi and Han 1990) has been carried out. Decomposing mango peel is difficult. Owing to the complex composition of mango peel, decomposing it takes a lot of time. There are reports related to ethanol fermentation using fruits and vegetable wastes such as mango peels giving good returns. There are good amounts of reducing sugars present in dried as well as fresh mango peels. This leads to its usage as raw material for producing ethanol and developing cheap medium. The mango (*Mangifera indica* L. var. Criollo) fruit has a cumulative carbohydrate amount ranging from 14 to 16% at maturity. It is rich in vitamins A and C, minerals, fibres and antioxidants. For fermentation, mango pulp is a suitable substrate. It possesses good amount of carbohydrate and is easily found in Mexico. In mango pulp, sugars are available in degradable form. The yeast cells can therefore metabolise the sugar content as such. Substrates like these are quite economical (Lin and Tanaka 2006).

Out of the various substrates, cashew is thought of as a cheap substrate for the production of ethanol (Rocha et al. 2007). Various authors have reported using oranges, mandarins, grapefruits and CPWs for producing bioethanol (Grohmann et al. 1994, 1995a, b; Wilkins et al. 2007a, b, c; Talebnia et al. 2008; Wilkins 2009; Boluda-Aguilar et al. 2010).

The pineapple cannery wastes are promising substrates to obtain ethanol. It has sugars, vitamins, proteins and certain other growth factors. This may also lead to lowering of the disposal cost pertaining to waste (Chye and Meng 1975; Prior et al. 1980; Alain et al. 1987) as the cannery is supposed to pretreat the wastes prior to disposing of with a view to bring down load of organics.

If we look at large-scale industry dealing with apple juice, around 75% of apples are used for juice, while the rest 25% are the by-products (such as apple pomace). Annually, in India, over 500 industrial units dealing with the processing of apples are reported to produce 1.3 MT of apple pomace. This incurs a cost of ten million dollars for disposal every year. Commonly, apple pomace is just put in open lands. This pollutes the environment. Nearly 10,000 tonnes of apple pomace is the only part which is used. The pomace is one of the fruit parts. Therefore, it exhibits a capacity for being changing to obtain several consumable and industrial products. Pomace is a rich (amount per 100 g) in carbohydrates (11.8 g), pectins (16.95 g), crude fibres (2.3 g) and mineral (0.3 g). Hence, it's a storehouse of various nutritional components (Mahawara et al. 2012).

Producing bioethanol using apple pomace serves as a good option. This is so as it is supplied at a cheap rate, and there is minimum requirement of land. The manufacturing in laboratory is not dependent on the climatic condition outside while the fermentation progresses. Many studies deal with bioethanol generation

via SSF of apple pomace either alone (Hang et al. 1982; Ngadi and Correia 1992) or combined with molasses (Kaur 1989) or utilisation of SSF for enriching the nutrients (Hang 1988).

Several studies have been published where citrus peel wastes (CPWs) have been converted to ethanol (Grohmann et al. 1994, 1996; Oberoi et al. 2010; Wilkins et al. 2007a, b). CPWs are put to use for production of certain products which commercially important. These are ethanol, enzymes, microbial biomass, organic acids, volatile flavouring compounds and antioxidants (Dhillon et al. 2004). Amongst the cheaper substrates which are readily available to produce ABE, the spoilage date fruit is a good choice (Mohamed and Abdel-Wahab 2012). Majority of the pineapples are consumed either fresh or as processed fruit (generally canned). Only best-quality fruits are picked up for processing and shipping (Tanaka et al. 1999). There is no suitable market for poor-quality food. So, it's left to rot at the farms. Major chunk of the pineapples are subject to processing to obtain juice. This leaves behind huge quantity of waste pulp. Such wastes are unusable. This waste which is pulpy in nature still has substantial quantity of sucrose besides the starch and hemicellulose fractions. Hence, it is anticipated that the juice from the rejected fruits and the other wastes could be utilised for a fermentation process to produce ethanol.

The peels of banana and beet wastes are common agri-based wastes. These wastes have a rich carbohydrate content. They also have other basic nutrients which support microbial growth (Dhabekar and Chandak 2010). In Nigeria, *Carica papaya* (pawpaw) is very common fruit consumed as an edible item as well as medicinal product. It's consumed either as a fresh fruit or as desert after processing (Desmond 1995). The unripe and mature pawpaw fruit which is unripe and mature is utilised to produce papain. This is done by making incisions on rear side of the fruit to obtain the latex for production of papain. Huge amounts of pawpaw wastes are obtained through plantations being cultivated to obtain papain. Disposing them is an issue of concern. Therefore, trial was done for processing these wastes to obtain ethanol, having industrial applications (Osanaiye Akin et al. 2005).

The grape pomace is taken as a waste product having very less economic value. The chemical analysis of the grape pomace exhibited appreciable quantities of sugars which could be fermented. The retaining of these sugars is done after the pressing of grapefruits. To obtain ethanol, the hydrolysis of such complex polysaccharides could be done. Constant testing of new substrates is being done via fermentation to get ethanol (Pimentel and Patzek 2005; Teles et al. 2007; Ye et al. 2007; Hossain and Fazliny 2010; Oyeleke and Jibrin 2009).

9.8 Pretreatments of Fruit Wastes for Ethanol Production

The fruit waste serves as a good lignocellulosic source to produce bioethanol. This is so as these are available as abundant renewable resource. The choice of the method for pretreatment serves a major role for enhancing efficiency of enzymatic saccharification, therefore, rendering entire method cost-effective (Senthilguru et al. 2011).

Pretreatment conditions optimal for a higher efficiency to produce ethanol using residual fruit biomass peel were investigated by Lalitha (2011). The residues were given hydrogen peroxide (alkaline) pretreatment and sulphuric acid pretreatment. Three weeks of fermentation was done after this using *Fusarium solani*. Pretreatment method led to removal of lignin effectively. The generation of ethanol in the culture samples was observed via high-performance liquid chromatography (HPLC). Giving the alkali-based pretreatment with the use of H_2O_2 (2%) at a pH of 13 and soaking for 8 h removed the 45% lignin. The ethanol obtained was found to be 115 mg/L. Upon acidic pretreatment, 0.2 mol/L H_2SO_4 fermenting for 15 days, the bioethanol obtained was 12 g/L in 1 day. An appreciable removal of lignin from the residue having fruit biomass peel led to high amount of ethanol production.

A turbid juice was obtained when the pineapple cannery was chopped mechanically and pressed. This led to a production of nearly 450–500 L of juice (Nigam 1999). Liquefied effluents obtained at different steps of processing were mixed with the above and subject to a short high-temperature treatment (at 80 °C for 15 min). This was followed by cooling and centrifugation (15 min), and a clear liquid was obtained. High temperature assisted in the lowering of total solids to a great extent. This also lowered number of microbes. Lemon (*C. limon L.)* CPWs were chopped into less than 7 mm particles. These were put in a pressure reactor (HRS Spiratube, model T-Sensation 12 L capacity) to carry out the steam explosion pretreatment (Boluda-Aguilar and López-Gómez 2013).

Such reactor and almost same steam explosion pretreatments were done with mandarin CPW and were documented by Boluda-Aguilar et al. (2010). Subsequent to thermo-hydrolysis, the entire steam (nearly 6 bar abs) was let out. This was done to rapidly lower pressure in reactor. Hence, this led to rapid decompression of the water vapour in the biomass. This causes the cell walls' disruption. In accordance with findings of Boluda-Aguilar et al. (2010), the test related to steam explosions were done in moist condition (having a water/biomass ratio of 1:2 w/w, this is equal to dry matter concentration of nearly 14%). The reaction time was 5 min with steam at a temperature of 160 °C. The let out was from 6 bar (abs) to an atmosphere vessel which had a connection with a condenser.

Analysis of four various ways of production was done: acid hydrolysis of amylaceous materials (the banana fruit and banana pulp) and enzyme-based hydrolysis of lignocellulosic materials (banana skin flower stalk). The banana plant cultivation, the feedstock transportation, the hydrolysis, the fermentation, the distillation, the dehydration, the residue treatment and the utility plant are considered (Arredondo et al. 2010). Kinnow mandarin (*Citrus reticulata*) waste which was dried, ground and hydrothermally pretreated was used to produce ethanol via simultaneous saccharification and fermentation (SSF) by Oberoi et al. (2011b). The oil palm empty fruit bunches (EFB) were pretreated using the aqueous ammonia soaking to produce bioethanol (Young Hoon et al. 2011). The pretreatment of EFB was done at the optimum temperature 60 °C, 12 h and 21% (w/w) aqueous ammonia.

Tanaka et al. (1999) did the enzymatic hydrolysis of pineapple waste to produce ethanol. This was done at 50 °C for 1 day. Usage of enzyme is done at a protein concentration of 0.3 mg/mL. The specific activity was of 1.82 (units/mg) in a filter

paper assay method. Termination of the reaction is achieved by raising the temperature of the waste suspension in boiling water for about 10 min. A chemical pretreatment process by the use of alkaline peroxide or acid hydrolysis was carried out on fruit biomass peel residue. This was done for removal of lignin. Lignin is a physical barrier for cellulolytic enzyme (Lalitha 2011). To the mango and banana fruit wastes, liquid hot water treatment and dilute acid pretreatment by the use of dilute H_2SO_4 were given to produce ethanol (Arumugam and Manikandan 2011).

The starch-rich fruits of papaya which were spoiled were chopped into the pieces and subjected to different processing methods such as boiling, mashing and autoclaving (Balasubramanian et al. 2011).

The peels of apple, papaya, turnip and banana were normally cut to the size of 1–2 cm. They were washed using the tap water till they were free of dust and clean. In the sunlight, these peels were air dried for some days. These were completely made dry in oven at 60 °C for 48 h. Then, these dry peels were diluted using distilled water in a ratio of 1:6. Then, these were boiled for 30 min prior to extraction (Kandari and Gupta 2012).

9.9 Ethanol Production Using Different Fruit Wastes

The ethanol production using various fruit wastes has been discussed below and depicted in Table 9.3.

9.9.1 Kinnow

The production of ethanol by SSF of the dry, grinded and hydrothermally pretreated kinnow mandarin (*Citrus reticulata*) wastes was investigated by Oberoi et al. (2011b). The ethanol concentrations and productivity of 42 g/L and 3.50 g/L/h, respectively, were obtained by the validation experiment using 6 FPU/gds cellulose and 60 IU/gds pectinase at temperature of 37 °C for 12 h in a lab scale batch fermenter.

Sandhu et al. 2012, studied the potential of utilising the crude filtrate extract (CFE) which was obtained using new isolated strain of *Aspergillus oryzae*. The fermentation was done using novel thermotolerant strain of *Pichia kudriavzevii* for producing ethanol using kinnow peel waste (KP) via SSF. HPLC analysis revealed that prehydrolysis of KP with CFE at 3 cellulase filter paper units/g dry substrate (FPU/gds) at a temperature of 50 °C produced 24.87 ± 0.75 g/L glucose, 21.98 ± 0.53 g/L fructose, 10.86 ± 0.34 g/L sucrose and 6.56 ± 0.29 g/L galacturonic acid (GA). Besides these, non-significant amounts of arabinose, galactose and xylose were also produced. The saccharification and fermentation of hydrothermally pretreated KP was done simultaneously at substrate concentration of 15% (w/v) in a 2.5 l lab scale fermentor using *P. kudriavzevii* at a temperature of

Table 9.3 Ethanol production from different fruit wastes

Fruit waste	Fermenting microorganism	Pretreatment/ hydrolysis	Ethanol production	Reference
Kinnow mandarin (*Citrus reticulata*) waste	*Saccharomyces cerevisiae*	Hydrothermal	3.50 g/L/h	Oberoi et al. (2011b)
Kinnow peel waste	*Pichia kudriavzevii*	Hydrolysis (CFE)	2.82 g/L/h	Sandhu et al. (2012)
Kinnow wastes and banana peels	*Saccharomyces cerevisiae* G and *Pachysolen tannophilus* MTCC 1077	Steam	26.84 g/L	Sharma et al. (2007)
Mandarin (*Citrus unshiu*) peel	Yeast	Popping Enzymatic	90.6%	Seong Choi et al. (2012)
Banana and mango (pulp and peels)	*Saccharomyces cerevisiae*	Liquid hot water treatment (LHW) Dilute acid pretreatment (DAP)	35.86%	Arumugam and Manikandan (2011)
Banana fruit peels	*Saccharomyces cerevisiae*	Liquid hot water treatment (LHW) Dilute acid pretreatment (DAP)	13.84%	Arumugam and Manikandan (2011)
Banana waste	*Saccharomyces cerevisiae*, type II	Cellulase Pectinase	4.1%–07.1%	Hossain et al. (2011)
Musa spp. discard	–	–	118–266 L ethanol	Sophie et al. (2011)
Banana fruit and its residual biomass	Yeast or bacteria	Acid hydrolysis of amylaceous material enzymatic hydrolysis of lignocellulosic material	7.4–79.4 kg/t wet biomass	Arredondo et al. (2010)
Banana peels	*Saccharomyces cerevisiae var. ellipsoideus*	Acid	44.5–66.1%	Tewari et al. (2003)
Ripened red banana	*Saccharomyces cerevisiae*	–	1.3%	Shyam Kumar et al. (2011)
Hydrolysed peels of red banana	*Saccharomyces cerevisiae*	–	0.27%	Shyam Kumar et al. (2011)
Green unhydrolysed banana peels	*Saccharomyces cerevisiae*	–	0.02%	Shyam Kumar et al. (2011)

(continued)

Table 9.3 (continued)

Fruit waste	Fermenting microorganism	Pretreatment/ hydrolysis	Ethanol production	Reference
Banana peel wastes	Five different mutant strains of *Saccharomyces cerevisiae*	Sulphuric acid and steam	9 g/L (fourth mutant strain)	Manikandan et al. (2008)
Dry and grinded banana peel biomass (BP)		Hydrothermal	2.3 g/L/h	Oberoi et al. (2011a)
Banana peels	*S. cerevisiae*	–	1.90%	Dhabekar and Chandak (2010)
Mango peel extract (direct fermentation)	*S. cerevisiae* CFTRI 101	Enzymatic pectinase, TriZyme 50	5.13%	Reddy et al. (2011)
Mango peel extract (with nutrient supplementation)	*S. cerevisiae* CFTRI 101	Enzymatic pectinase, TriZyme 50	7.14%	Reddy et al. (2011)
Citrus wastes	Baker's yeast	Dilute acid	39.64 L/ton	Mohammad et al. (2010)
Citrus peel wastes	*Saccharomyces cerevisiae*	Steam explosion	26.97–39.60 g/L	
Citrus processing wastes	*Saccharomyces cerevisiae*	High-pressure steam	4%	Zhou et al. (2008)
Mandarin citrus peel waste (MCPW)	*Saccharomyces cerevisiae* yeast CECT 1329	Steam explosion	50–60 L/ 1000 kg raw MCPW	Boluda-Aguilar et al. (2010)
Lemon (*Citrus Limon L.*) peel wastes	*Saccharomyces cerevisiae*	Steam explosion	60 L/kg fresh lemon peel biomass	Boluda-Aguilar and López-Gómez (2013)
Citrus processing waste	*Saccharomyces cerevisiae*	Steam Acid Base	76% to 94%	Widmer et al. (2010)
Beet waste	*S. cerevisiae*	–	2.15%	Dhabekar and Chandak (2010)
Apple pomace	*Saccharomyces cerevisiae* Montrachet strain 522	Enzymatic	5.1% (without saccharification) 6% (with saccharification)	Miller et al. (1982)
Apple pomace	*S. cerevisiae*	–	18.1–19.3%	Ngadi and Correia (1992)
Apple pomace	*S. cerevisiae*	Cellulase and pectinase	20–30 g/kg	Khosravy and Shojaosadati (2003)
Apple pomace (natural fermentation)	Natural fermentation	–	3.956%	Jain and Singh (2006)

(continued)

Table 9.3 (continued)

Fruit waste	Fermenting microorganism	Pretreatment/ hydrolysis	Ethanol production	Reference
Apple pomace (inoculated fermentation)	Yeast strains (Y2, Y5 and Y12)	–	4.074% (Y5)	Jain and Singh (2006)
Apple pomace (75%) + molasses (25%)	Yeast strain Y5	–	5.02%	Kumar and Sahgal (2008)
Apple pomace	*S. cerevisiae* MTCC 173, *A. foetidus* MTCC and *Fusarium oxysporum* MTCC 1755	–	16.09%	Chantanta et al. (2008)
Rotten pineapples waste	*Saccharomyces cerevisiae*	–	8.7%	Hossain and Fazliny (2010)
Pineapple cannery waste	*Saccharomyces cerevisiae* ATCC 24553	Heat treatment	3.75 g/L/h	Nigam (1999)
Juice of rotten/discard pineapples and waste materials of production of pineapple juice (with no nutritional supplementation)	*Zymomonas mobilis*	Enzymatic cellulase	59.0 g/L	Tanaka et al. (1999)
Juice of rotten or discarded pineapples and the waste materials of the production of pineapple juice (with nutritional supplementation)	*Zymomonas mobilis*	Enzymatic cellulase	42.5 g/L	Tanaka et al. (1999)
Pineapple waste	*Saccharomyces cerevisiae* and *Zymomonas mobilis*	Cellulase and hemicellulase	8%	Ban-Koffi and Han (1990)
Industrial pineapple waste	*Saccharomyces bayanus* 1926, *Saccharomyces cerevisiae* 1102, *Saccharomyces cerevisiae* 1319	Cellulase and hemicellulase	5%	Prados et al. (2010)
Grape pomace	*Pichia rhodanensis* isolate 1	Acid Enzymatic	18.5 and 16.1 g/L	Korkie et al. (2002)

(continued)

Table 9.3 (continued)

Fruit waste	Fermenting microorganism	Pretreatment/ hydrolysis	Ethanol production	Reference
	Saccharomyces cerevisiae Y294	yeast Irradiation		
Oil palm empty fruit bunches (EFB)	–	Aqueous ammonia	18.6 g/L	Young Hoon et al. (2011)
Different fruit peels (papaya, banana and apple)	*Saccharomyces cerevisiae*	–	5.90–4.94%	Kandari and Gupta (2012)
Fruit biomass peel residue	*Fusarium solani*	Alkali	115 mg/L	Lalitha (2011)
Fruit biomass peel residue	*Fusarium solani*	Acid	12 g/L	Lalitha (2011)
Fruit waste	*S. cerevisiae*	Fungi (*Phoma* sp.)	2.4%	Senthilguru et al. (2011)
Pineapple fruit	*Saccharomyces cerevisiae* and *Candida albi*cans	–	2.16%	Mishra et al. (2012)
Carica papaya (pawpaw) agricultural waste	*Saccharomyces cerevisiae*	–	3.83 to 5.19%	Osanaiye Akin et al. (2005)
Spoiled papaya	*Saccharomyces cerevisiae*	Boiling Autoclaving	7.4 mg/mL	Balasubramanian et al. (2011)
Spoilage date palm (*Phoenix dactylifera* L.) fruits	*Clostridium acetobutylicum* ATCC 824 and *Bacillus subtilis* DSM 4451	–	21.56 g/L (acetone, butanol and ethanol)	Mohamed and Abdel-Wahab (2012)
Rotten rambutan	*Saccharomyces cerevisiae*	Enzymatic	5.9–9.8%	Hadeel et al. (2011)
Orange peels	–	Acid	3.37 g/L/h	Oberoi et al. (2010)
Orange peels	*Mucor indicus*	Enzymatic hydrolysis	0.33 g/g	Ylitervo (2008)
Orange peels	Recombinant *Escherichia coli KO11*	Cellulase, pectinase and β-glucosidase	2.8–4.8%	
Orange peels	*Saccharomyces cerevisiae*	Pectinase Cellulase Novozyme	4–5%	Grohmann et al. (1994)
Cashew apple juice	*Saccharomyces cerevisiae*	Gelatin, sodium or potassium metabisulphite	7.62%	Neelakandan and Usharani (2009)
Syzygium cumini (jamun)	*Saccharomyces cerevisiae*	Acid	1.21 g/L	Mutreja et al. (2011)

(continued)

Table 9.3 (continued)

Fruit waste	Fermenting microorganism	Pretreatment/ hydrolysis	Ethanol production	Reference
Mangifera indica (mango)	*Saccharomyces cerevisiae*	Alkali	0.658 g/L	Mutreja et al. (2011)
Fruit wastes	*Citrobacter* sp. strain E4	–	2.96 g/L	Debapriya et al. (2019)
Fruit wastes	*Saccharomyces cerevisiae*	–	–	Mohammad et al. (2018)
Fruit pulp	*Saccharomyces cerevisiae RK1*	Dilute acid	0.67%–1.32%	Kamlesh et al. (2019)

40 °C after a 3-h prehydrolysis. No oligosaccharides were obtained in SSF procedure. The generation of ethanol levelled off with the passage of 12 h. This resulted in ethanol concentration and productivity of 33.87 g/L and 2.82 g/L/h, respectively. Potential of SSF by using the crude enzymes and *P. kudriavzevii* to scale up the ethanol generation by employing the kinnow peel was demonstrated by this.

9.9.2 Kinnow and Banana Peels

The analysis of the role of certain fermentation parameters such as inoculums' size, incubation period, temperature and agitation time on the production of ethanol using kinnow waste and banana peels was done by Sharma et al. 2007. The SSF was done by the use of cellulase and co-culture of *Saccharomyces cerevisiae* G and *Pachysolen tannophilus* MTCC 1077. The kinnow wastes and peels of banana (steam pretreated) were the substrate put to use for to ethanol generation in a ratio 4:6 (kinnow wastes/banana peel). A temperature of 30 °C, an inoculum concentration of *S. Cerevisiae G* 6% (v/v) and *Pachysolen tannophilus* MTCC 1077 4% (v/v), an incubation time of 2 days and an agitation for initial 1 day were reported as best for producing ethanol utilising the two wastes together. Biomass (pretreated and subject to steam explosion) subsequent to enzymatic saccharification which contained 63 g/L reducing sugars used for fermentation involving both hexose and pentose fermenting strains of yeast under the optimised condition. This resulted obtaining ethanol, yield and fermentation efficiencies of 26.84 g/L, 0.426 g/g and 83.52%, respectively. In this investigation, efficient use of kinnow wastes and the banana peel for obtaining bioethanol with the use of optimised fermentation parameters was reported.

9.9.3 Mango/Banana Waste

The analysis of composition (chemical) of fruit waste (both pulp and peel) of banana and mango was carried out via laboratory experiments for exploring the possible applications of these for the production of bioethanol (Arumugam and Manikandan 2011). Fermentation of DAP hydrolysate of the mixed fruit pulp exhibited a highest ethanol production of 35.86%. This corresponds to a fermentation efficiency of about 70.31% at 48 h of incubation. The experiment also revealed that the hydrolysates which were obtained via the H_2SO_4 (dilute) pretreated banana fruit peels gave a maximum yield of 13.84% ethanol having fermentation efficiency of 27.13% at 42 h of incubation. This investigation hinted that fermentation of hydrolysates which we get from dilute acid pretreatments and then subjected to enzymatic saccharification of the mixed fruits pulp (banana and mango) and banana fruit peel was appreciable for a high output of ethanol at the optimised condition.

9.9.4 Banana Waste

The fermentation of the banana waste was done by the use of *Saccharomyces cerevisiae*, Type II under the anaerobic conditions (Hossain et al. 2011). This was done for determining the bioethanol production. Nearly 4.1% to 07.1% bioethanol was obtained using the fermented fruit waste of banana. The obtained bioethanol had a viscosity and acid value as per the American Standard for Testing Materials (ASTM) and European Norms (EN) standards. This investigation reported the use of combination (skin and pulps) of the rotten fruits was quite apt to produce bioethanol as renewable energy. This led to checking of the economics involved in initial process.

An investigation done by Sophie et al. (2011) assessed quantitative production potential of the ethanol using the discard of *Musa* spp. It was reported by them that annually, the production of 118–266 L ethanol could be done using the banana and the discard of cooking banana being collected at a rate of nearly 1.4 to 3.4 t/ha.

An investigation was done by Arredondo et al. (2010) in which an energy analysis was done for obtaining anhydrous ethanol which was achieved via hydrolysis of starch and cellulosic and hemicellulosic materials found in banana fruit and its residual biomass. The analysis of four production channels was carried out: the acid hydrolysis of the amylaceous material (banana fruit and banana pulp) and enzymatic hydrolysis of lignocellulosic material (banana skin and flower stalk). Amylaceous material gave the best indices. For this the mass performance ranged from 346.5 L/t to 388.7 L/t. The net energy value (NEV) varied from 9.86 MJ/L to 9.94 MJ/L, and the energy ratio was noted to be 1.9 MJ/MJ. In case of the lignocellulosic material, these values were less favourable. The mass performance ranged from 86.1 to 123.5 L/t, NEV from 5.24 to 8.79 MJ/L. The energy ratio was in the range of 1.3–1.6 MJ/MJ.

The dried and ground biomass peels, the ripe and waste banana and the hydrolysed peel of the green and red bananas were utilised to produce ethanol by using the *Saccharomyces cerevisiae* in shake flask cultures. Different concentrations of the substrate (1%, 2.5%, 5%, 7.5% and 10% (w/v)) were given with inoculums (1%). The maximum yield of ethanol was reported in *Saccharomyces cerevisiae* in the ripened banana (red) and their peels (hydrolysed) nearly 1.3% and 0.27% (v/v) in 10% substrate concentration. In green unhydrolysed banana peels (with 1% substrate concentration), the least yield of about 0.02% of alcohol was obtained.

The kinetic studies for obtaining ethanol using the banana peel wastes by utilisation of the five different mutant strains of *Saccharomyces cerevisiae* were done by Manikandan et al. (2008). The fourth mutant strain gave the maximum production of ethanol at 9 g/L. Tewari et al. 2003, investigated saccharification of banana peel using the acids, enzymes and steam. This was done in order to investigate potential of banana wastes related to the ethanol fermentation using the *Saccharomyces cerevisiae var. ellipsoideus*. The content of reducing sugar increased over tenfold by the hydrolysis of substrate by employing sulphuric acid (2.5%) at 15 psi for about 15 min. The maximum saccharification was 26.7% and 28.3% (wt basis) and 56.4% and 59.9% (CH_2O basis) with 2.5% acid at 10 and 15 psi for 15 min. More increase in the concentration of the sulphuric acid and the treatment time left unfavourable impact on hydrolysis. There was a sixfold increase in saccharification by steaming without pressure. The steam under pressure of 10 psi for about 30 min gave good saccharification.

The maximum saccharification was attained on hydrolysing the cellulose of the banana wastes using the cellulase enzyme from *Trichoderma reesei* QM 9414. Yield of 1.38 and 0.78% (v/v) and 44.5 and 61.1% ethanol (mg/g reducing sugars) was noted from cellulose and the acid hydrolysed (2.5% at 15 psi for 15 min) banana peel, respectively.

The dried and ground banana peel biomass (BP) was pretreated via the hydrothermal sterilisation. After this, it was used to produce ethanol via the SSF (Oberoi et al. 2011a). The concentration of cellulase and pectinase, the temperature and the time producing of ethanol using banana peel via the SSF was done using central composite design (CCD). A high coefficient of determination (R^2) value of 0.92 for producing ethanol was revealed by ANOVA. The validation was done in a lab scale batch fermenter based on model graphs and the numerical optimisation. The concentration of cellulases,and pectinases and the temperature and time obtained were 9 cellulase filter paper unit/gram cellulose (FPU/g cellulose), 72 international units/gram pectin (IU/g-pectin) at a temperature of 37 °C and time duration of 15 h, respectively. The experiment performed in batch fermenter by use of optimised parameters led to a higher concentration of ethanol. This was more than the prediction done made by the model equation. Fermentation time was saved here. It was reported that both the hydrothermal pretreatment and SSF can be carried done successfully in a single vessel. Utilising the optimised process parameters assisted for achieving a significant productivity of ethanol. This indicated the commercial feasibility of the process. Ethanol concentrations and the ethanol productivities of 28.2 g/L and 2.3 g/L/h, respectively, from banana peel were reported. Dhabekar and

Chandak (2010) documented that producing ethanol by the banana peels is nearly 1.90% equivalent to dextrose.

9.9.5 Mango Waste

There are two types of wastes, i.e. solid wastes (stones and peels) and liquid wastes (wash water and juice), which are produced by the processing industries dealing with mango fruit. Reddy et al. 2011 did a study to find the suitability of the dried mango peel to produce ethanol. Ethanol (5.13%, w/v)) was generated by direct fermentation of the extract of the mango peel. The nutrients like the yeast extract, wheat bran extract and peptone were used as supplements in the mango peel medium. They documented that addition of the nutrients enhanced ethanol production significantly to about 7.14% (w/v).

9.9.6 Citrus Wastes

Bioethanol production by applying the steam explosion and enzymatic hydrolysis pretreatment on the lemon (*Citrus limon* L.) citrus peel waste was carried out (Boluda-Aguilar and López-Gómez 2013). The processing was carried out of the steam exploded lemon peel waste via the sequential and simultaneous hydrolysis and fermentation. They reported that ethanol production in excess of the 60 L/1000 kg fresh lemon peel biomass could be generated. Mohammad et al. (2010) employed an integrated process to produce ethanol using the citrus wastes (CWs). A dilute acid process was carried out for the hydrolysis of CWs. This was done in pilot plant reactor having an explosive drainage system. In the hydrolysates, sugars were present which were converted to ethanol by the use of baker's yeast. The yield of ethanol nearly 0.43 g/g of the fermentable sugars was reported. About 39.64 l ethanol was produced from 1 ton of CWs having 20% dry weight. Zhou et al. (2008) carried out a study and reported that the wastes obtained after citrus processing could be fermented and nearly 4% w/v ethanol could be produced.

For bioethanol production, study was done on mandarin (*Citrus reticulata L.*) citrus peel waste (MCPW) by Boluda-Aguilar et al. 2010. The coproducts obtained were D-limonene, galacturonic acid and citrus pulp pellets (CPP). Contents of D-limonene and the influence they have on the production of ethanol were investigated as well. Concentration of different sugars, galacturonic acid and ethanol were analysed for measuring the saccharification and fermentation (HF and SSF) efficiency of the processes which was reported by the MCPW pretreatment involving the steam explosion. The ethanol amounting to nearly 50–60 L/1000 kg of raw MCPW was obtained. The CPP yield could be optimised via control of the dosage of the enzymes and pretreatment involving the steam explosion. This could reduce the enzyme requirements significantly.

Widmer et al. 2010, investigated the pretreatment of citrus processing wastes (CPW) for different times, pH and temperatures. Limonene is a fermentation inhibitor. For removal of limonene below 0.1%, the pretreatments at temperature of 160 °C for more over 4 min along with steam purging were required. The hemicelluloses were well solubilised after the pretreatment at 160 °C. The solubilisation of only 70% of pectin was done in the natural CPW. When acid-modified CPW (pH 2.8) was used, more than 80% of the pectin was solubilised. The pectin was quickly destroyed by the pretreatment at a temperature of 160 °C on the base modified CPW (having initial pH 6.8). The dissolved solids were lowered significantly, and they were viscous as well (excessively). After the pretreatments at a temperature of 160 °C for nearly 8 mins in CPW within a pH range of 2.2 to 8.2, the amount of total sugars fermentable remained unchanged. The ethanol yields on the basis of sugar content following the enzymatic hydrolysis after the 48 h of simultaneous saccharification and fermentation varied from 76% to 94%. The yields of ethanol were lower slightly but were similar statistically upon using the base modified pretreatments.

Effects of the D-limonene concentrations, the enzymatic loadings and the pH on the ethanol production via the SSF of the citrus peel wastes using the *Saccharomyces cerevisiae* were investigated at a temperature of 37 °C by Wilkins et al.. Before SSF, the citrus peel went through a steam explosion procedure. This was done in order to remove over 90% of initial D-limonene which was there in the peel wastes. The yeast growth is inhibited by the D-limonene. The experiments were carried out in which the addition of the D-limonene was done back to the peel for determining the threshold inhibition amount. The ethanol concentration after a time interval of 24 h was lowered in fermentations with the initial concentration of D-limonene being higher or being equal to 0.33% (v/v) and the final (1 day) D-limonene concentration higher or being equal to 0.14% (v/v). The ethanol production was lowered when the enzyme loadings were (IU or FPU/g peel dry solids) pectinase (25), cellulose (0.02) and beta-glucosidase (13). The ethanol production was found to be highest with initial pH of peel waste being adjusted to around 6.0.

Seong Choi et al. 2012, designed a biomass popping pretreatment system. They used fire burner along with horizontal cylinder which was rotating on an axis. This was done for ethanol production using the mandarin (*Citrus unshiu*) peel (MP). The popping pretreatment was done at temperature of 150 °C for about 10 min in the absence of a chemical treatment. Popping pretreatment decreased the particle side (<1 mm) and lowered the concentration of D-limonene (yeast fermentation inhibitor) from 0.21% to about 0.01%. The enzymatic hydrolysis of the pretreated MP was carried out in a 50 mM sodium acetate buffer (with a pH 4.8) at a temperature of 45 °C for about 6 h. The total saccharification rate was approximately 95.6%. Concentration of the fermentable sugars increased to 10% (glucose 7.1% and fructose 2.9%) by the vacuum evaporation process. The consequent fermentation at a temperature of 30 °C and pH 5.0 for about 12 h in lab bioreactor augmented yields of ethanol to 90.6% in comparison to 78% at 36 h using the raw MP.

9.9.7 Beet Waste

Dhabekar and Chandak (2010) documented that the yeast *S. cerevisiae* exhibits appreciable attributes for producing ethanol. This was nearly 2.15% in case of the beet wastes in comparison to dextrose with 2.05% (v/v) production of ethanol on the fourth day. They also reported that the production of ethanol with banana peels is nearly 1.90% same as dextrose.

9.9.8 Apple Pomace

The supply of apple pomace occurs at a very cheap price. There is very little land requirement. In the laboratory, the manufacturing during the fermentation process is not dependent on the outer weather conditions. Hence, the ethanol production using the apple pomace is an attractive option. Many studies pertaining to the ethanol production via the SSF of the apple pomace as the only substrate (Hang et al. 1982; Ngadi and Correia 1992) or combined with molasses (Kaur 1989) or by utilising SSF for enriching of the nutrients have been done (Hang 1988).

The saccharification and ethanol fermentation using the apple pomace was done by Miller et al. (1982). Best yield of ethanol was reported by using *Saccharomyces cerevisiae* Montrachet strain 522 at 7.73% or 6.48% saccharification. They got an ethanol yield of 5.1% (w/w) by utilising 100 g aliquot of the apple pomace. Ngadi and Correia (1992) reported the SSF of the apple pomace. The moisture content was 77% and 85% (wb), and the mixing speeds were 2, 20 and 40 rpm. Culture used was *S. cerevisiae*. Average maximum concentrations of ethanol at 18.1% and 19.3% (db) were obtained at 85% and 77% (wb) pomace moisture levels, respectively. Average ethanol concentrations of 10.8%, 10.3% and 9.3% (db) were reported at the bioreactor mixing speeds of 2, 20 and 40 rpm, respectively. Besides this, the highest concentrations of ethanol were achieved sooner at 2 and 20 rpm as compared to 40 rpm.

An ethanol yield of 20–30 g/kg of apple pomace was reported under condition of fermentation of the apple pomace. The yeast used was *S. cerevisiae*. The moisture content was 75% (w/w), an incubation temperature of 30 °C and a nitrogen source of 15% (w/w) and phosphorus source at 0.08% (w/w). The inoculum concentration was 500,000,000 cells/kg The ethanol yield was 20–30 g/kg of the apple pomace. This yield was dependent on the conditions of the fermentation and the pretreatments of the substrate saccharification using the cellulase and pectinase (Khosravy and Shojaosadati 2003). The fermentation of apple pomace was done utilising different strains of yeasts (Y2, Y5, and Y12) *S. cerevisiae.* This was done to analyse the fermentation of the apple pomace. In the natural fermentations, production of ethanol was 3.956% after the time period of 72 h of fermentation. In inoculated fermentation, the Y5 strain treated sample led to maximum yield of ethanol of 4.074% at a time duration of 72 h of incubation as documented by Jain and Singh

(2006). Investigation was done by Kumar and Sahgal (2008) on the yeast strain Y5. This strain when inoculated into the substrate combination of 75% apple pomace and 25% molasses led to a highest ethanol (5.02%) production at 72 h of fermentation. Chantanta et al. (2008) investigated that when all the cultures *S. cerevisiae* MTCC 173, *A. foetidus* MTCC and *Fusarium oxysporum* MTCC 1755 were utilised in combined form for fermentation of the apple pomace, the ethanol production was 16.09% (v/w).

9.9.9 Pineapple Wastes

Hossain and Fazliny (2010) obtained bioethanol using the rotten pineapples wastes via the fermentation using the commercial yeast, *Saccharomyces cerevisiae*. They documented that the optimal yields of bioethanol was 8.7%. On the analysis of the anhydrous ethanol, they did not find any dangerous elements in its acceptance as a fuel for transportation as per the ASTM standard. Nigam (1999) investigated continuous ethanol production of ethanol using the waste from the pineapple cannery by utilisation of respiration deficient strain *Saccharomyces cerevisiae* ATCC 24553 at 30 °C and pH 4.5.The maximum yield of ethanol (92.5%, theoretical) was noted at a dilution rate of 0.05/h. The maximum values noted for the volumetric ethanol and biomass productivities were 3.75 gp/L/h and 0.63 g/L/h, respectively. These values were at dilution rate of 0.15/h. Maximum specific productivity of ethanol was found to be 0.98 gpg/L/h.

Tanaka et al. (1999) studied the ethanol production using juice of rotten/discarded pineapples. Wastes obtained after production of pineapple juice by *Zymomonas mobilis* were also studied. Nearly 59.0 g/L of ethanol was obtained in the undiluted pineapple juice. There were no nutritional supplementation and no optimisation of pH. About 42.5 g/L ethanol was reported by utilising 125 g/L sucrose medium which was enriched using 10 g/L yeast extract and minerals.

Saccharomyces cerevisiae and *Zymomonas mobilis* were allowed to grow on wastes of pineapple. Characteristics of their alcohol production were compared (Ban-Koffi and Han 1990). Wastes of pineapples consisted of cellulose (19%), hemicellulose (22%), lignin (5%) and cell soluble matters (53%). The concentration of the soluble sugars, which consisted of sucrose (5.2%), glucose (3.1%) and fructose (3.4%), was comparatively less, and pretreatment of substrates was required. The pretreatment of the pineapple wastes using cellulase and hemicellulase and followed by fermentation using *S. cerevisiae* or *Z. mobilis* reported nearly 8% ethanol using the pineapple wastes within a time span of 48 h.

Prados et al. (2010) utilised the industrial pineapple wastes for the production of ethanol. To obtain bioethanol, three different processes were analysed from pineapple waste. These methods were direct fermentations (DF) of extracted liquor, the consecutive saccharifications and the fermentations (CSF) of blended wastes and the simultaneous saccharification and fermentation (SSF) of blended wastes. Testing of three various industrial yeasts (CECT: *Saccharomyces bayanus* 1926,

Saccharomyces cerevisiae 11,020, *Saccharomyces cerevisiae* 1319) was done. Cellulase and hemicellulase (Sigma Aldrich, Spain) were utilised to carry out the hydrolysis of cellulosic material (1 g/kg × 1.2 U/g hemicellulase and 6 g/kg × 0.87 U/g cellulose). In context of the fermentation experiments, for the non hydrolysed materials, the best output was observed upon sterilisation of the waste materials. The pH was regulated to 5, and following a time span of 72 h of fermentation, the mean yield of 5% ethanol was noted.

9.9.10 Grape Pomace

The isolation and evaluation of yeast strains were done by Korkie et al. (2002). These yeast strains were associated with the grape pomace and their ability to carry out hydrolysis of the complex polysaccharides found in grape pomace was done. The fermentable sugars were used for the production of ethanol. The pomace polysaccharides were hydrolysed partly by two *Pichia rhodanensis* isolates. Slight enhancement in the quantity of ethanol generated was observed as a result of the fermentation of the pomace. It was revealed by this study that appreciable amount of ethanol was obtained using residual sugar associated with grape pomace.

9.9.11 Oil Palm

The ethanol production by using the oil palm empty fruit bunches (EFB) which were pretreated using aqueous ammonia soaking was analysed by Young Hoon et al. (2011). An ethanol production nearing 18.6 g/L, 65.6% of theoretical highest yield and 0.11 g/L/h of production was reported by utilising the pretreated EFB. The simultaneous saccharification and fermentation were done for 168 h with glucan loading (at 5% w/v), cellulose (60 FPU) and β-glucosidase (30 CBU) per gram glucan.

9.9.12 Fruit Peel

The ethanol production by using the different fruit peels was investigated by Kandari and Gupta (2012). A maximum ethanol production was reported within 36 h of fermentation in papaya peel extracts. This was followed by banana and apple peel extracts (5.90 to 4.94%). The optimisation of pretreatment condition for high efficiency of production of ethanol by using the fruit biomass peel residues was done by Lalitha (2011). The fermentation of the residue was done with *Fusarium solani*. With the alkaline treatments involving H_2O_2 (2%) at a pH 13 sand soaked for 8 h, the production of the ethanol produced was 115 mg/L. Upon acidic treatments of

0.2 mol/L H_2SO_4 and fermentation for about 15 days, the ethanol production was 12 g/L in 24 h.

The concentration of ethanol was obtained using the fungi- treated fruit waste. This was inoculated using 3 mL of the second day *S. cerevisiae* culture (Senthilguru et al. 2011). The ethanol yield was 2.4% (v/w) of the fruit waste (100 g). Mishra et al. (2012) used the yeasts (*Saccharomyces cerevisiae* and *Candida albi*cans) to produce ethanol by using the fruits of orange, sweet lime and pineapple. They reported prominent rise in quantity of ethanol produced via the submerged fermentation. This was more in comparison to the value reported by solid state fermentation. The maximum ethanol content (2.16% v/v) was obtained from the pineapple under the solid state fermentation conditions.

9.9.13 Pawpaw

The dry active baker's yeast and brewer's yeast strains (*Saccharomyces cerevisiae*) were utilised for carrying out the fermentation of *Carica papaya* (pawpaw). It is an agricultural waste (Osanaiye Akin et al. 2005). The ethanol contents of about 3.83–5.19% (v/v) were obtained by the fermented pawpaw. Higher ethanol yield was reported by the brewer's yeast as compared to the baker's yeast. The saccharification for 48 h along with the with nutrients supplementation enhanced the ethanol yield significantly.

9.9.14 Papaya

The collection of the spoiled starch-rich fruits of papaya was done. They were analysed for ethanol production by Balasubramanian et al. (2011). Different processing methodologies were subjected to the substrate. The methods such as boiling, mashing and autoclaving were used. Following these the bacterial (*Lactobacillus*)-mediated saccharification was done. The process of the alcoholic fermentation was done on the bacteria saccharified substrates by using the *Saccharomyces cerevisiae*. Following a fermentation period of 42 h, 7.4 mg/mL concentration of ethanol was found in the broth.

9.9.15 Date Palm

For producing acetone, butanol and ethanol (ABE), fruits of spoilage date palm (*Phoenix dactylifera* L.) were utilised as the substrates. The consortium of *Clostridium acetobutylicum* ATCC 824 and *Bacillus subtilis* DSM 4451 (Mohamed and Abdel-Wahab 2012) was used. A total production of ABE of 21.56 g/L was attained

at 75 g/L spoilage date fruit homogenate. Maximum productivity of ABE at 0.30 g/L/h and the yield of ABE at 0.42 were noted at 75 g/L spoilage date fruit homogenate. The microbial consortium was used with no addition of a reducing agents and N_2 flushings. The production of the ABE was enhanced significantly by adding the 5 g/L yeast extract and 1.6 g/L or ammonium nitrate to the spoilage date fruit homogenate. Combining the yeast extract and ammonium nitrate significantly enhanced the production of ABE. It was suggested by these results that the use of spoilage date fruits could be done effectively to commercially produce ABE.

9.9.16 Mixed Fruit Wastes

Debapriya et al. (2019) directly converted the fruit wastes to ethanol utilising marine bacterial strain *Citrobacter* sp. E4. The ethanol tolerant strains were isolated from marine water of Digha and Shankarpur, West Bengal, India. These were analysed for the ethanol production utilising the various domestic wastes. These wastes included, paper, kitchen, garden and fruit wastes. The efficiency of the strain E4 was highest in ethanol production via the fermentation of the kitchen and fruit wastes. A production of 2.96 g/L of ethanol was reported by using the fruit waste via the (HPLC). The yield of ethanol production was obtained as 0.13 g of ethanol/g of reducing sugar present in fruit waste.

Mohammad et al. (2018), carried out the bioethanol production from fruits and vegetable wastes by using *Saccharomyces cerevisiae.* The aim of present study was determining bioethanol percentages using fruits and vegetables' waste produced via fermentation procedure utilising the yeast, *Saccharomyces cerevisiae*, and analysing chemical content and glucose amount in producing bioethanol. They concluded that maximum bioethanol yields were obtained utilising pineapple waste. High concentrations of elements were recorded in oranges' bioethanol; glucose contents were also reported higher in orange wastes.

Kamlesh et al. (2019) used mixture of three fruits, namely, banana, grapes and mango as possible substrates to produce cellulosic ethanol by modifying parameters such as aeration. Pretreatment, hydrolysis and fermentation were carried out during this study. The well-known yeast *Saccharomyces cerevisiae RK1* was used. Fermentation of mixed fruit pulp without sucrose and fruit pulp with sucrose produced 0.67% ethanol and 1.32% ethanol, respectively.

9.9.17 Rambutan

Bioethanol production was attempted by Hadeel et al. (2011) using the rotten rambutan. Yeast *Saccharomyces cerevisiae* was employed to ferment fruit wastes of rambutan. The chemical contents, the viscosity and the acid value of bioethanol obtained were in accordance with the American Society for Testing and Materials

(ASTM) standard specifications. There were not present many harmful chemicals in the bioethanol.

9.9.18 Orange Peels

Analysis of orange the peels as a fermentation feedstock was done by Oberoi et al. (2010). Process conditions for increased ethanol production were investigated. The primary hydrolysis of the orange peel powder (OPP) was done at acidic concentration ranging from 0 to 1.0% (w/v) at temperature of 121 °C and a pressure of 5 psi for a time duration of 15 min. HPLC of the sugars and the inhibitory compounds revealed an increased production of hydroxymethyfurfural and acetic acid and decline in the concentrations of sugar when the level of acid was beyond 0.5% (w/v). The secondary hydrolysis of the pretreated biomass got from the primary hydrolysis was performed at acid concentration of 0.5% (w/v). The response surface methodology (RSM) by utilising the three factors and two-level central composite design (CCD) was used for optimising effects of temperature, pH and fermentation time on the production of ethanol from the OPP hydrolysate. This was carried out at the shake flask levels. Based on the result obtained through the optimisation experiments and the software for numerical optimisation, a validation investigation was done in a 2 L batch fermenter. The pH was 5.4 and the temperature was 34 °C for time span of 15 h. Separate fermentation was done of the hydrolysates obtained via the primary and secondary hydrolysis processes. The employed parameters were optimised using the RSM. They obtained an ethanol yield of 0.25 g/g on biomass basis (YP/X). The ethanol yield of was obtained on 0.46 g/g on a substrate consumed basis (YP/S). An appreciable volumetric productivity of ethanol (3.37 g/L/h) was obtained by using this method at fermenter level. This indicated towards promising further scale-up studies in the future.

Ethanol was produced by the use of orange peels by employing the fungus *Mucor indicus* (Ylitervo 2008). Upon preliminary aerobic cultivation on the enzymatically hydrolysed orange peels, the yield of ethanol, 0.33 g/g after a time span of 26 h, was obtained. Grohmann et al. (Grohmann et al. 1994, 1996) documented producing ethanol using orange peels. Converting the monosaccharides in the orange peels' hydrolysates for obtaining ethanol using the recombinant *Escherichia coli* KO11 was studied in a pH-controlled batch fermentations at temperatures 32 and 37 °C. pH values and concentrations of the peels' hydrolysates were varied for determining the approximate optimised conditions and the limitations involved in such fermentations. Quite appreciable yield of ethanol was observed using this microbe at a moderate ethanol concentrations (28–48 g/L). pH ranges of 5.8– 6.2 seemed to be to appropriate. All the major monosaccharides in the orange peels' hydrolysate were converted by the microorganism to obtain ethanol. Lesser quantities of acetic acid and lactic acid were also produced.

To such previously carried out investigations related to the enzyme-based hydrolysis of polysaccharides in orange peels, an extension was done. The commercially

available cellulase and pectinase enzymes were used to the more high and more practical concentrations of the orange peel solids by Grohmann et al. 1994. The maintenance of high yields of saccharification was possible. This was true even at substrate concentrations as high as 22–23%. Though rate of solubilisation and saccharification lowered by two to threefold. The yeast *Saccharomyces cerevisiae* was used to investigate the fermentability of such hydrolysates. This study indicated presence of certain inhibitory components. The removal of such components could be done by filtering hydrolysed peel. After adjusting the pH with the calcium carbonate, the fermentation of filtered hydrolysates was done successfully.

9.9.19 Cashew Apple Juice

Utilising the immobilised yeast cells of the *Saccharomyces cerevisiae*, the production of ethanol using the cashew apple juice was investigated by Neelakandan and Usharani 2009.

Under optimum conditions, maximum yield of ethanol (7.62%) was achieved. The optimum conditions comprised of substrate concentration −10%, pH-6, temperature—32.5 °C and an inoculum concentration of 8% (v/v) in 24 h. This study revealed the possibility of an effective usage of the cashew apple juice for the production of bioethanol. This could be achieved by employing the optimised parameters of fermentation by the use of technology involving the immobilised yeast cells.

9.9.20 Jamun and Mango

Mutreja et al. (2011) carries out the ethanol production from jamun (*Syzygium cumin*) and mango (*Mangifera indica)*. The simultaneous saccharification and fermentation (SSF) were done by employing the recombinant cellulase and the yeast *Saccharomyces cerevisiae*. Three pretreatments were given, namely, alkali, acid and steam explosion. The acid pretreatment of jamun at a temperature of 30 °C yielded maximum ethanol (1.21 g/L). The alkali pretreatment mango yielded the maximum ethanol (0.658 g/L).

9.10 Conclusions

The enormous use of the fuel ethanol globally demands technology for its production should be economical as well as sustainable environmentally. The ongoing research tendencies to improve the fuel ethanol productions finds link to nature of the raw material employed, the stages involved in the processing and the related issues

pertaining to the process engineering. The fruits of banana and its residues (organic) are the feedstocks having potential to be used for production of ethanol. This can be achieved via hydrolysis, fermentation and distillation. By following such procedures, the agricultural waste could be used for producing ethanol and reduction of the issues related to the environment.

The bioethanol which is produced using the banana biomass is good quality wise. It can be employed to run engines for transportation. They are reported to produce less amount of emissions. Besides this, this could be utilised in the environment recycling procedures for the management of waste management. Appreciable quantities of ethanol can be generated from the market-oriented production systems using the bunches of banana which do not comply with the quality standards. Ethanol can also be produced from low-input agroforestry systems. In such systems cultivation of the *Musa* spp. is being done as a secondary crop. These are partially left for rotting in fields. Lemon CPW is another potential feedstock which could be utilised for bioethanol and galacturonic acid production. The processing via SSF of the steam exploded lemon CPW, with a low enzymatic concentration as well, gave appreciable amounts of ethanol.

The simultaneous saccharification and fermentation can be done of both kinnow wastes and banana peels. These haven't been commercially exploited for such industrial applications. They are disposed of poorly but can be used effectively for ethanol production. The apple pomace, kinnow peels and mango waste can also be used for producing ethanol. The pineapple wastes have a relatively lesser amount of sugars for the fermentation of alcohol. Hence, the pretreatment for enhancing the sugar level is required. The ethanol yield was enhanced by the use of high substrate concentration for fermenting.

For the bioethanol production, the waste of pineapple could be utilised as an economical material. The partial valorisation of the pineapple industries' residues is represented by these. The spoilage date palm fruits could be utilised as inexpensive renewable substrate for the producing ABE. With respect to this, further focus needs to be drawn to determine utilisation of ethanol produced for optimising the economic returns. This employs producers by replacing on their own the gasoline consumption on the farms or selling it in a regional market for ethanol.

An appreciable removal of lignin from peel residue fruit biomass peel residue led to increased ethanol production. Using recombinant cellulases for the production of bioethanol is a strategy for lowering the cost of enzyme. Certainly, there is a scope for enhancing ethanol yield via process optimisation. Carrying out the process using the optimised conditions of fermentation can be employed to scale up to the pilot scale and subsequently to a commercial fermenter level. Hence, this will make the whole process economical. The fruit wastes are an attractive lignocellulosic material to produce bioethanol. This is so as fruit wastes are most abundant renewable resources. Choosing correct pretreatment methods helps in increasing the efficiency of the enzymatic saccharification hence, rendering the entire procedure cost-effective. Using the recombinant cellulases for the production of bioethanol is a smart strategy for lowering the cost of enzyme.

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Chapter 10
Trends in Biodiesel Production from Algae and Animal Fat Wastes: Challenges and Prospects

Javaria Bakhtawar, Muhammad Irfan, Hafiz Abdullah Shakir, Muhammad Khan, Shaukat Ali, Shagufta Saeed, Tahir Mehmood, and Marcelo Franco

Abstract The maximum and uncontrolled use of fossil fuels is being faced from last few decades due to extreme energy requirements, leading the entire globe in search of any renewable and biodegradable energy source having no harm to environment. For this purpose petroleum fuels are being replaced with biofuels which are considered to be eco-friendly and cost-effective. Biodiesel is proved to be most fascinating fuel in last few years and can be obtained from algae, animal fat waste, and waste cooking oil. It is safe to use fuel with low or no emission report. Algae are broad aquatic group and are biodiesel feedstock, can be easily grown, and have specific features leading to significant biodiesel production. The other big source of biodiesel is animal fat waste which can easily attained due to maximum usage of poultry and meat in daily life. Regardless of the easy availability of these resources, there are yet some issues like pretreatment conditions, algae growing, etc. which need to be resolved for significant biodiesel production. Sustainable biofuel economy can be pursued if the environmental conditions affecting biofuel are completely understood. As an end result, sustainable energy supply with low emitting gases will replace the petroleum fuel industry.

J. Bakhtawar · M. Irfan (✉)
Department of Biotechnology, University of Sargodha, Sargodha, Pakistan
e-mail: irfan.ashraf@uos.edu.pk

H. A. Shakir · M. Khan
Insititute of Zoology, University of the Punjab New Campus, Lahore, Pakistan

S. Ali
Department of Zoology, Government College University, Lahore, Pakistan

S. Saeed · T. Mehmood
Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Science, Lahore, Pakistan

M. Franco
Department of Exact Sciences and Technology, State University of Santa Cruz (UESC), Ilhéus, Brazil

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10.1 Introduction

During last few decades, usage of fossil fuels has been increased due to extreme energy requirements causing severe environmental drawbacks leading to discover some fuel alternatives in industries. Presently European Union transport sector accounts on nearly 30% consumption of total energy mainly depending on use of petroleum fuel that is known to be a source of emitting pollutants and is considered to be indefensible due to their diminishing reserves. As a result, the requirement for developing uncontaminated and renewable diesel energy alternative is there and has drawn general interest and consideration within the scientific society. Liquid biofuels are supposed to be a renewable and reasonably affordable choice to balance petroleum fuel (Lourinho and Brito 2015). So due to the diminishing petroleum assets and exhausting of toxic gases from petroleum diesel, biodiesel has fascinated in the last few years like a renewable along with eco-friendly fuel. Moreover it is also biodegradable because it is utterly made by using vegetable oil or animal fats. Biodiesel holds little sulfur along with metals and polycyclic aromatic hydrocarbons, while petrochemical diesel may have up to 20% polycyclic aromatic hydrocarbons (Vasudevan and Briggs 2008).

Biodiesel can be distinct as a combination of monoalkyl-esters of extended chained fatty acid (FAME) derivatives of renewable natural resources like fats of animal, vegetable oils, algae oil, and remaining of cooking oil (Banković-Ilić et al. 2012; Yan et al. 2014; Lourinho and Brito 2015; Priya and Thirumarimurugan 2020). Because of a fuel source, biodiesel has a lot of profits, more than petroleum diesel. It is nontoxic and capable to four times quicker biodegradable in contrast to petroleum fuel (Tariq et al. 2012; Lourinho and Brito 2015). It holds almost no sulfur and has generally low emission report, advanced combustion effectiveness, and a better flash point. Moreover, it is renewable and safe and can be used without going under any modification of existing engines (Helwani et al. 2009a, b; Abbaszaadeh et al. 2012; Aransiola et al. 2014; Lourinho and Brito 2015).

Biodiesel can be obtained by converting animal fats, vegetable oils, and cooking oil through a process called transesterification (Kour et al. 2019). There are three means for biodiesel yield from oil plus fats (Fig. 10.1). Biodiesel may be produced by using soybean, sun flower, jatropha, and palm fruits. Biodiesel obtained from vegetable oil is usually 50% more costly as compared to that of obtained from waste cooking oil (Phan and Phan 2008). Oleaginous microorganisms like bacteria, algae, cyanobacteria, and yeast are also being used in biodiesel production. Some yeast species like *Lipomyces lipofer*, *Rhodosporidium toruloides*, *Trichosporon pullulan*, and *Cryptococcus albidus* are competent to produce lipids (Meng et al. 2009; Fu et al. 2018; Kour et al. 2019).

Presently the main barrier faced in biodiesel widespread commercialization and production is its production expenditure (Abbaszaadeh et al. 2012). As a result a

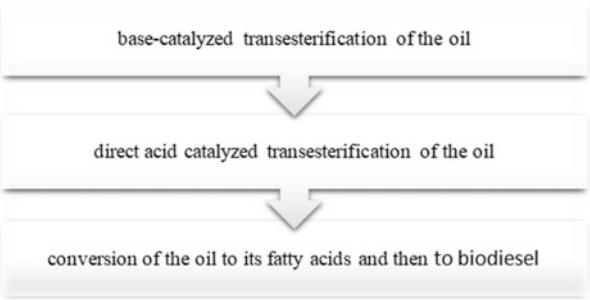

Fig. 10.1 Basic routes for biodiesel production by using oils and fats (Kour et al. 2019)

broad research is being conducted on biodiesel production from different technologies. There are four principal methods for biodiesel production (Abbaszaadeh et al. 2012; Lourinho and Brito 2015). These four methods such as heterogeneous transesterification have a huge potential to prevail over main issues caused by biodiesel traditional production by homogenous catalysts. However these technologies cannot be further improved due to partial mass transfer faced by diffusion problems between phases, since the conversion of oil in to esters is slightly slow process (Leung et al. 2010; Lam and Lee 2010; Lourinho and Brito 2015).

Primary methods for biodiesel production:

1. Direct utilization of vegetable oil.
2. Microemulsion.
3. Thermal-cracking.
4. Enzymatic catalytic transesterification.

10.2 Biodiesel Production by Using Algae

Algae are broad group of aquatic organisms having the capability of carrying photosynthesis as well as efficient conversion of solar energy. They can be divided into two classes based on their size (1) macroalgae and (2) microalgae (Koutra et al. 2018; Kour et al. 2019). The earliest shape of life appeared on earth is microalgae, and presently it has become a main object to produce biodiesel due to its unique features (Falkowski et al. 2004). Biofuel is eventually a mean of collecting solar energy and accumulating it in energy-intense chemicals. Feedstocks having high capability of using solar energy by mean of photosynthesis are desired (Fan and Burton 2009).

10.3 Algae Production Processes and Conversion Processes

Algae production process can be classified in three broad classes based upon energy cause and operating method: heterotrophic, photoautotrophic, and mixotrophic. These growth modes can take place in closed bioreactor system plus open raceway ponds (Chen et al. 2011; Juneja et al. 2013). For maximum biofuel yield, various schemes involving combination of diverse growth system in bioreactors are proposed. Algae feedstock can be produced from range of substrates like lignocellulosic material, sugar from molasses or starch depending upon growth system and bioreactor configuration (Brennan and Owende 2010; Juneja et al. 2013). Starch and lipid parts of algae can be preceded into liquid fuel by undergoing diverse processing techniques such as thermochemical liquefaction (Zou et al. 2009); pyrolysis (Du et al. 2011), transesterification (Sharma and Singh 2009), and fermentation (Ueno et al. 1998). Pros and cons of these conversion processes are listed in Table 10.1. Majority thermochemical conversion techniques like pyrolysis, combustion and gasification involve little-moisture content biomass. This creates a dare due to increased energy supplies for algal feedstock drying. Still, hydrothermal liquefaction method can employ wet slurry for algae oil yield by reducing cost linked with drying (Goyal et al. 2008). Anaerobic digestion (biochemical conversion process) produces methane and carbon dioxide (Cantrell et al. 2008). The fermentation of sugars obtained from the starch portion can be employed for ethanol yield by using algae biomass (Ueno et al. 1998).

Table 10.1 Advantages and disadvantages of conversion processes, involved in biofuel production through microalgae (Juneja et al. 2013)

Process	Advantages	Drawbacks
Pyrolysis	Maximum bio-oil production potential (almost 57.5% w/w for fast pyrolysis (Miao and Wu 2004)	Less moisture contented biomass is required essentially. High-energy portion required for feedstock drying
Thermochemical liquefaction	Algal wet slurry is able to use. Reduced power and cost supply. High production potential (almost 60% w/w (Duan et al. 2013)	Reactors are composite and costly
Fermentation	Coproducts can be used. Sugar conversion of into bioethanol is achievable	Lengthy processing durations necessary. Biomass has to be preprocessed to get converted into sugars
Transesterification	Obtained renewable fuel has improved physical properties. Biodiesel has an existing market which helps in simplifying of commercialization	It is limited in lipids conversion and do not exploits protein and carbohydrate portion of feedstock

10.4 Algal Pretreatment for Biodiesel Production

Generally some algae species can be used for bioethanol production after their alkaline pretreatment by using NaOH (Harun et al. 2011). Algae biomass pretreated with H_2SO_4 (sulfuric acid) facilitates the hydrolysis of starch and oligosaccharides and can produce ethanol (Nguyen et al. 2009). A microalgae specie *Chlamydomonas reinhardtii* gathered high starch content through photosynthesis. Hydrolytic commercial enzymes altered the starch into glucose, and almost 23.5 mg ethanol was obtained from 1gram of algae biomass (Choi et al. 2010). In an additional report, *Chlorella vulgaris* produced 0.4 g ethanol/gram biomass after pretreating with dilute acid (Lee et al. 2011). *Schizochytrium* sp. produced almost 11.8 g/L bioethanol from 25.7 g/L of glucose after hydrothermal fractionation (Kim et al. 2012). *Dictyochloropsis* that is a green microalgae has been reported for biodiesel production; gas-liquid chromatography was carried out to determine its fatty acids (Afify et al. 2010). Spirulina sp. was reported for biodiesel yield after in situ transesterification (Xu and Mi 2011) and *Schizochytrium limacinum* produced biodiesel after undergoing through transesterification methodology (Johnson and Wen 2009). The strain of *Stichococcus bacillaris* has been reported as highly biodiesel producing strain (Olivieri et al. 2011).

10.5 Utilizing Microalgae to Produce Biodiesel

If all the transport fuel of the USA is replaced by biodiesel, then it will cost almost 0.53 billion m^3 yearly at current expenditure rate (Chisti 2007; Fan and Burton 2009), so oil crop, soap stock, jatropha oil, and waste cooking oil are unable to fulfill the demand. Situation can be dramatically altered by using microalgae for production of biodiesel. Microalgae are grown up in a well-planned system having significant access to CO_2, nutrients, and water which contribute more than average photosynthetic capability as compared to those of land crops (Vasudevan and Briggs 2008).

Microalgae can produce a lot of chemical intermediates along with hydrocarbons that can be transformed into various fuel types such as diesel, alcohol, hydrogen, and methane. The *Chlorococum* specie has been reported as a significant substrate to produce bioethanol (Harun et al. 2010). These are marine organisms distinguished by having high biomass and photosynthetic competence along with significant oil portion that proves them a valuable biodiesel producing source without causing any struggle with food crops. Algae attain high oil portion such as in *Botryococcus braunii* it is 75% of total dry basis. The main component of typically algal oil is unsaturated fatty acids like palmitic acid all along with noteworthy content of extremely unsaturated species (Balat 2011; Chaker Ncibi and Sillanpaa 2013).

Additionally, microalgae grow up exceptionally fast and usually twofold their biomass in 34 h. During the exponential growing, this period can be reduced to 3.5 h.

It has been reported that microalgae biomass productivity is 50 times greater than switchgrass which is ever best growing terrestrial plant (Demirbaş 2006; Fan and Burton 2009). Department of Energy (United States) sponsored a broad study regarding microalgae biomass production for to obtain biodiesel yield (Sheehan et al. 1998). Microalgae has a great oil content, usually 20–50% (Chisti 2007), and some microalgae are said to have 80% of oil portion by weight of their total dry mass (Metting 1996; Spolaore et al. 2006).

Presently, large-scale microalgae production is possible in open ponds typically in raceway ponds sometimes (Terry and Raymond 1985) as well as tubular photo-bioreactors (Mirón et al. 1999; Grima et al. 1999). Even though raceways are inexpensive, the biomass productivity was lesser than photo-bioreactors. Major drawback of open systems is the loss of water through evaporation at a speed similar to ground yield and is also vulnerable to contamination by means of surplus species, being open to the environment (Schenk et al. 2008). Photo-bioreactors save energy, water, and chemicals unlike open raceways. It can give a restricted atmosphere that can be customized to the precise demands of vastly productive microalgae in order to achieve a significant annual oil production (Chisti 2007). For that reason, the selection of crop growing systems is the main choice that can considerably influence the cost-effectiveness and competence of production method of microalgal biofuel (Lee 2001; Janssen et al. 2003; Li et al. 2008; Fan and Burton 2009).

Microalgae are supportive for biodiesel production because of holding the subsequent attractive characteristics (Miyamoto 1997; Fan and Burton 2009):

- Expenditures related to microalgae harvesting and shipping off are comparatively low, as compared to the other biomass supplies like conventional harvest.
- Microalgae are capable of any chemical treatments.
- Commonly algae can be cultured under those environmental conditions which are inappropriate for conventional harvest.
- Microalgae are competent for carbon dioxide (CO_2) fixation in atmosphere. Thus they support to drop the atmospheric CO_2 level which is a worldwide continuous rising issue.

Many universities like University of Utah have conducted the projects related to microalgae biodiesel production, and many projects are ongoing in the world for biodiesel production by using algae. For example Victor Smorgon Group and International Power Hazelwood are operating a 6-month pilot trial of a method from Green Fuel Technologies Corp, which utilizes microalgae in a photo-bioreactor to seize CO_2 from the furnace gases. Victor Smorgon Group will convert algal biomass oil into biodiesel for biodiesel production by using of canola oil. In a different plan, Solazyme Inc. is working on genetically engineering of a green eukaryotic microalga (*Dunaliella*) for improving its performance. *Dunaliella* is previously being used to obtain beta-carotene and be able to accumulate considerable extent of lipids which are appropriate for biodiesel production (Seefeldt 2007; Fan and Burton 2009).

In an earlier study, two dissimilar methods supercritical CO_2 extraction and thermochemical liquefaction for biodiesel production by oil extraction from

microalgae were compared. Results suggested that thermochemical liquefaction was more effective as compared to supercritical carbon dioxide extraction by quantitative viewpoint, but fatty acid decomposition might take place in the operative situation. Moreover, almost required temperature was 350 °C and 395 °C to attain significant extracted oil amount. Regardless of the apparent brilliant prospect of utilizing microalgae for biodiesel production, this is yet far beyond from being set for definite execution at commercial level (Aresta et al. 2005; Fan and Burton 2009). In an earlier report, it has been stated that the major challenge for its commercial level execution is the principle cost of photo-bioreactors (Vasudevan and Briggs 2008). However Chisti has stated that microalgae biodiesel production can be attained affordably by some improvement level in algae through metabolic and genetic engineering (Chisti 2007). Moreover, algal biodiesel production cost can be further decreased by integrating biorefinery idea and using advanced photo-bioreactor engineering (Fan and Burton 2009).

10.6 Process Used to Obtain Biodiesel from Algae

Recently many researchers have performed studies for biodiesel production by using algae from different methods one of which is dewatering of intact algal cells for algal biomass production. This technique is being applied to extract natural lipids present in algal biomass and their esterification by using a catalyst in the alcohol presence. This technique also carries the separation of a water-soluble portion having glycerin from the water-insoluble portion having fuel esters and later on distillation of fuel esters under vacuum. The whole method lead in the obtaining of a jet oil blend supply from short fuel esters and a diesel blend supply from long fuel esters (Kale et al. 2012; Chaker Ncibi and Sillanpaa 2013).

In another study biodiesel production from algae was reported by cultivating oil-producing algae for algal oil extraction which was latterly converted into biodiesel. Algal oil was extracted from oil-producing algae by biologically rupturing the vesicles and cell wall of algae by using glycol-proteinase (a structured enzyme system) like enzymes or a virus or blend of all these biomaterials (Oyler 2012; Chaker Ncibi and Sillanpaa 2013).

Microorganisms can also be directly transformed into biodiesel. For example, a single-step process was developed in a research for direct alteration of algae specie *Nannochloropsis Salina* into biodiesel by undergoing supercritical ethanol production conditions. This ethanol was further used under supercritical conditions for instantaneous extraction and transesterification of algae lipids for yielding of fatty acid ethyl esters. This study resulted that optimum 67% of fatty acid ethyl esters yield was attained at a temperature of 265 °C following 20 min of reaction time at a ratio of 1:9 weight/vol of dried algae and ethanol (Chaker Ncibi and Sillanpaa 2013; Reddy et al. 2014).

10.7 Biodiesel Production by Using Animal Fat Waste

Biodiesel production by using animal fat waste is now in trend. Because of the huge utilization of poultry and meat, a large amount of animal byproducts are produced. About 17 million tons/year byproducts are produced in only European Union (Zalouk et al. 2009) by slaughtering of beef, sheep, pig, chicken, and dairy cattle. After interpretation results depict that up to 12 million tons can be classified as edible in food feed-related zones after being processed (Toldrá et al. 2016), while others are used to produce in energy generation for biodiesel and other biofuel productions (Rosson et al. 2020; Toldrá-Reig et al. 2020).

In terms of energy, specifically biodiesel production is the most attractive one (Baladincz and Hancsók 2015). In this way the use of inedible animal byproducts assures the most attractive utilization in biodiesel production. Biodiesels can both be obtained from animal fat wastes and by vegetable oils. Vegetable oil is leads to costly biodiesel production so animal fat waste is being preferred for alternative to produce biodiesel. Moreover, biodiesel produced from animal fat waste, is having attractive lubricating features and has high octane number as compared to that of fossils diesel (Nigam and Singh 2011) as well as reduces the CO_2 emission (Mansir et al. 2018). CO_2 is the most common gas and contributes up to 72% in greenhouse gases (Toldrá-Reig et al. 2020).

In previous few decades, production of biodiesel by using animal fat waste has become a hot issue. For this purpose many researches have been conducting studies for its production by using low-grade feedstocks, improving efficiency of bioreactors to carry transesterification with the option of reusing of catalysts (Lawan et al. 2020). In actual, the 60 to 80% of the entire expenditure of biodiesel production relies upon the raw material in the shape of fats or oil which is used in biodiesel production (Bušić et al. 2018), so it is significant to choose the best material because they may be affected by climate, geographic location, or agriculture (Mahlia et al. 2020).

Many countries like Indonesia, the USA, Malaysia, and Brazil and some European countries are using biodiesel as a renewable and biodegradable energy source (Balat and Balat 2010). In 2019 total world biodiesel production was almost 35–45 million tons, and it is continuously increasing per year (Flach et al. 2019). World's principal producer for biodiesel is the European Union having more than 202 plants and above 14 million tons production in 2019 (Ramos et al. 2019; Bockey 2019), while US biodiesel production capacity till 2019 was 8.3 million tons by using 91 plants (Toldrá-Reig et al. 2020).

Biodiesel can easily be employed in present diesel engines without undergoing any specific modification. As compared to conventional diesel, biodiesel has a poor carbon to hydrogen proportion along with high oxygen content that leads to less emission of hydrocarbons, sulfur, and monoxides (Bhatti et al. 2008; Xue et al. 2011). The key challenge of producing cost-effective and viable biodiesel is possible overcome by using animal fat waste (Gumahin et al. 2019).

It has been researched that a large number of animals are slaughtered every year producing significant amount of waste along with fats which need to be properly

Table 10.2 Composition of fatty acids in beef tallow, poultry fats, mutton tallow, and pork lard (Toldrá-Reig et al. 2020)

Fatty acids	Pork lard (Toldrá et al. 2004)	Beef tallow (Realini et al. 2004)	Mutton tallow (Castro et al. 2005)	Poultry fat (Zduńczyk et al. 2011)
Myristic	1.6	1.6	2.2	0.4
Palmitic	25.1	21.6	21.1	21.6
Docosapentaenoic	0.2	–	–	0.3
Stearic	12.6	17.7	11.6	6.3
Arachidonic	0.3	–	–	3.4
Oleic	36.5	31.5	38.7	30.0
Linolenic	1.1	1.3	0.6	2.4
Linoleic	16.5	3.3	10.2	28.4
Palmitoleic	2.8	2.5	2.1	3.2
Total saturated	39.4	49.1	40.4	29.1
Total monounsaturated	39.7	41.0	47.1	33.2
Total polyunsaturated	20.9	10.0	12.5	37.6

treat or recycled into valuable products in order to lessen pollution (Mora et al. 2019, 2020). These fats consist of beef tallow, mutton tallow, pork lard, and chicken fats obtained from exposed fatty tissues of cattle, sheep, pigs, and blood, respectively (Sai Akhil and Alagumalai 2019; Barik and Vijayaraghavan 2020). The wet rendering process is carried out in which fats are separated from protein. Other fats are obtained from meat dealing out industries and by recycling of industrialized cooking trade. The recycled greases obtained through heating of animal fats from industrial and commercial cooking can be characterized into brown and yellow greases depending upon their free fatty acid (FFA) content (Banković-Ilić et al. 2014). Fatty acid composition of beef tallow, poultry fats, mutton tallow, and pork lard is given in Table 10.2.

Due to considerable proportion of saturated fatty acids, pig and ruminant fats are solids, while chicken fats are almost liquids (Öner and Altun 2009). So it can be stated that preheating at 45 °C is necessary to use the solid fats of animals for the principle of their utilization in biodiesel production (Cernat et al. 2019). More stable biodiesel production with high cetane numbers can be obtained by using the highly saturated fatty acids (Jayathilakan et al. 2012). In a study it has been stated that almost 81% lung and caul fat and 26% knob and channel, cod, and kidney fats from cattle are meant to be used for biodiesel production (Walsh 2014). Till 2019, more than 13 million tons of animal fats and vegetable oil feedstock was applied for biodiesel production in Europe from which 6% belonged to animal fats (Ramos et al. 2019; Flach et al. 2019). While in the USA, 8.4% of total feedstock belonged to animal fats like tallow, poultry fat, and white grease (Toldrá-Reig et al. 2020).

10.8 Biodiesel Production Via Transesterification by Using Animal Fats

The first step carried out for biodiesel production is pretreatment which is necessary to reduce the free fatty acids and water which are present in animal feedstock and can be reason to decrease biodiesel production and increasing the cost of production by separation and purification (Gebremariam and Marchetti 2018; Pinnarat and Savage 2008; Van Kasteren and Nisworo 2007). The main processes involved in production of biodiesel by using animal fat waste can be seen in Fig. 10.2.

Biodiesel is mainly produced through transesterification of fats with a short-chained alcohol in the company of a catalyst. There are many catalysts which are being used in production of biodiesel. Some of the traditional catalysts are stated in Table 10.3. Animal fat transesterification can be done at a speedy rate if alkali is used as catalyst which is 4000 times faster and easily available at low cost as compared to that of acid catalyst (Dias et al. 2009; Kirubakaran and Selvan 2018; Thangaraj et al.

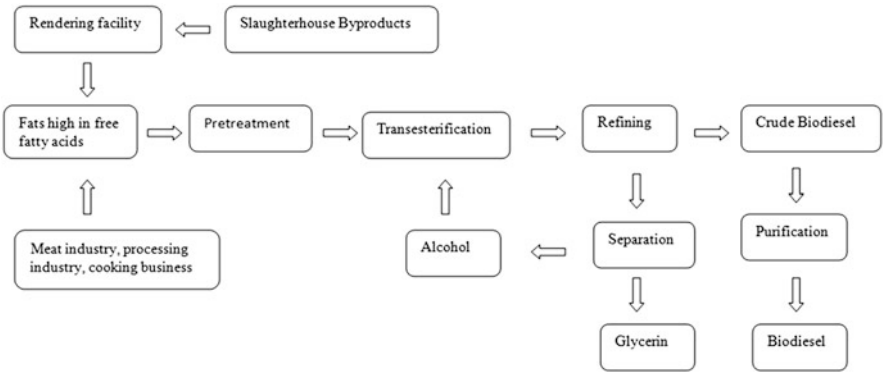

Fig. 10.2 Major steps used in biodiesel production by using animal fat waste. (Modified from Toldrá-Reig et al. 2020)

Table 10.3 Some of the traditional catalysts used for biodiesel production through transesterification (Ma and Hanna 1999)

Sr no	Catalysts	Types
1	Alkalis	Sodium methoxide, sodium hydroxide, sodium amide, potassium hydroxide, potassium hydride, potassium amide, and potassium methoxide
2	Acids	Organic sulfonic acid, sulfuric acid, phosphoric acid, and hydrochloric acid
3	Heterogeneous catalysts	Such as enzyme lipases
4	Complex catalysts	Zirconias, nanocatalysts, and silicates

2019). Methoxides can also perform in a good way, but it is economically non-suitable (Atabani et al. 2012). The employing of acid catalyst can slower the reaction speed and requires a large reactor which can cause corrosion (Canakci and Sanli 2008). The most frequently used catalyst is methanol because of its cost-effectiveness (Ramadhas et al. 2005). Transesterification in the company of alkaline catalysis is being preferred in biodiesel production at industrial scale because biodiesel producers have not yet adopted the technologies of using enzyme and heterogonous catalysts (Kristi et al. 2018).

Triacylglycerols are basically converted into diacylglycerols through transesterification by releasing one fatty acid molecule. Diacylglycerols are converted into monoacylglycerols later on by liberating another fatty acid molecule, and at last monoacylglycerols are converted into glycerol by releasing the one more fatty acid molecule (Van Gerpen 2005).

After the pretreatment the reaction efficiency is stands over some variables like temperature, time, fatty acid composition, catalyst amount, and many more listed in Table 10.4. Maximum alcohol to oil ratio for significant biodiesel production is reported as 6:1 while by any increase or decrease from this ratio can delay the separation of glycerol procedure (Musa 2016).

If alkali is used as catalyst, then a reduction in biodiesel production can be experienced due to soap formation resulted from free fatty acids and catalyst (Li et al. 2020; Talebian-Kiakalaieh et al. 2013). This all results in loss of ester product and catalyst in a massive cost and less biodiesel production with difficult purification (Lotero et al. 2005; Vicente et al. 2004; Anitha and Dawn 2010). Free fatty acid content in a range of 5–30% requires pretreatment (Mora et al. 2020). For efficient transesterification process, free fatty acid content can be suggested up to 1.0–1.5% (El-Mashad et al. 2008). To decrease the free fatty acid portion, feedstock can be esterified at high temperature (Encinar et al. 2011) or from neutralization and separation (Lee et al. 2002). So pretreatments are essentials to get rid of free fatty acids and excessive waster before transesterification. Some of these pretreatments are heat drying, calcium chloride, and silica gel otherwise anhydrous sodium sulfate to reduce moisture (Toldrá-Reig et al. 2020). At last suspended material can be removed through filtration in presence of vacuum or from cellulose filters (Felizardo et al. 2006; Predojević 2008; Toldrá-Reig et al. 2020).

10.9 Characteristics of Biodiesel Which Is Obtained from Animals Feedstocks

The American Society for testing Materials and European Committee of Standardization has given some policies for the biodiesel nature. There are many benefits that can be attained with biodiesel produced from animal fats like it has reduced release of the polycyclic aromatic hydrocarbons up to 75% to 90% (Carraretto et al. 2004; Mahlia et al. 2020). Sulfur dioxide and carbon monoxide emission is also reduced

Table 10.4 Different operating conditions for biodiesel production through animal fat waste from transesterification (Toldrá-Reig et al. 2020)

Animal fats	Catalyst	Reaction yield %	Weight (% to fat)	Operating conditions	Alcohol/ oil ratio	Reference
Beef tallow	KOH	90.8	0.8	60 °C, 2 h	6:1	Mata et al. (2014)
Pork lard	KOH	91.4	0.8	60 °C, 2 h	6:1	Mata et al. (2014)
Poultry fat	KOH	76.8	0.8	60 °C, 2 h	6:1	Mata et al. (2010)
Catfish fat	KOH	92.7	0.8	50 °C, 0.75 h	6:1	Huong et al. (2011)
Pork fat	KOH	97.3	0.5	65 °C, 2 h	6:1	Encinar et al. (2011)
Duck tallow	KOH	97.1	1	65 °C, 3 h	3:1	Chung et al. (2009)
Chicken waste	KOH	–	1	60 °C, 2 h	6.1	Lin and Tsai (2015)
Animal fats	Immobilized lipase from *Candida antarctica*	79	10	40 °C, 6 h	50:6	Pollardo et al. (2018)
Beef tallow	Immobilized lipase from *Burkholderia cepacia*	89.7	20	50 °C, 48 h	12:1	Da Rós et al. (2010)
Mutton fats	MgO-KOH	98.0	20	65 °C, 20 min	22:1	Mutreja et al. (2011)
Swine lard	KOH	98.0	1.1	65 °C, 3 h	7.4:1	He et al. (2020)
Chicken fat	KOH	82.0	0.8	60 °C, 1 h	8:1	Chavan et al. (2017)
Mutton tallow	H_2SO_4	93.2	1.25	60 °C, 24 h	1:30	Bhatti et al. (2008)
Chicken fat	NaOMe	88.5	1	60 °C, 4 h	6:1	Alptekin and Canakci (2011)
Chicken fat	Nano CaO	88.5	1	60 °C, 5 h	9:1	Keihani et al. (2018)

(continued)

Table 10.4 (continued)

Animal fats	Catalyst	Reaction yield %	Weight (% to fat)	Operating conditions	Alcohol/ oil ratio	Reference
Chicken fat	Composite membrane and NaOMe	98.1	1	70 °C, 3 h	1:1	Shi et al. (2013)
Chicken fat	$CaO/CuFe_2O_4$	94.5	3	70 °C, 4 h	15:1	Seffati et al. (2019)
Chicken fat	H_2SO_4	99.0	1.25	50 °C, 24 h,	1:30	Bhatti et al. (2008)
Lard	35% CaO/zeolite	90.9	8	65 °C, 1.25 h	30:1	Lawan et al. (2020)
Chicken fat	$AC/CuFe_2O_4$ encapsulated with Cao	95.6	3	65 °C, 4 h	12:1	Seffati et al. (2020)
Brown grease	ZnO/ZrO_2	78.0	0.8	200 °C, 2 h	1:1.5	(Kim et al. 2011)
Lard	Supercritical methanol	89.9	–	335 °C, 20 MPa, 15 min agitation 500 rpm	45:1	Shin et al. (2012)
Lard	Lipase from *Candida* sp	87.4	20	40 °C, 30 h	3:1	Lu et al. (2007)
Lard	Lipase from *Candida antarctica*	74	10	30 °C, 72 h	1:1	Lee et al. (2002)

(Shaghaghi et al. 2020). The cetane number of biodiesel is a sign of ignition character of biodiesel like the improved ignition quality can be linked with its high cetane number (Atabani et al. 2012). Biodiesel obtained from animal fats has greater cetane number than that of obtained from vegetable oil source because of the presence of maximum saturated fats and high oxygen portion (Cernat et al. 2019). The free glycerin percentage of biodiesel reveals the concentration of glycerol that is remained in final biodiesel yield. If free glycerin percentage is high, then fuel injection can be damaged (Atabani et al. 2012). Some of the main properties of biodiesel obtained from animal fat waste are given in Table 10.5.

The expenses of biodiesel obtained from animal's fat waste are reliant on the feedstock cost, free fatty acid proportion, the given pretreatment, operational conditions, and biodiesel purification (Rezania et al. 2019). Biodiesel obtained from vegetable oil can be easily purified as compared to that of produced from animal fat waste because of large amount of glycerin production from animal fats. This glycerin is produced during transesterification and can affect engine durability that's why its removal is essential (Sander et al. 2018). Almost 1 kg of glycerol is experienced while dealing with 10 kg biodiesel (Atadashi et al. 2010). The removed glycerin value is near to the ground due to its worldwide production, but it can be

Table 10.5 Characteristics of biodiesel produced from animal fat waste (Toldrá-Reig et al. 2020)

Properties	Limits	Pork lard Encinar et al. (2011)	Poultry fat Mata et al. (2010)	Beef tallow Atabani et al. (2012)	Beef tallow Mata et al. (2010)	Chicken fat Barik and Vijayaraghavan (2020)	Chicken fat Alptekin and Canakci (2011)	Mutton tallow Bhatti et al. (2008)
Density at 15 °C (kg/m^3)	860–900	870	877	–	870	830	883	856
Acid value (mg KOH/g)	<0.50	0.23	0.55	0.147	0.20	–	0.22	0.65
Cetane number	>51.0	56.9	–	–	–	50	–	59.0
Iodine value (g/100 g)	<120	66.7	78.8	–	44.4	–	–	126
Viscosity at 40 °C (mm^2/s)	3.50–5.00	4.74	6.86	4.82	5.35	3.5	4.94	–
Flash point (°C)	>120	175	172	>160	171	50	171.8	–
Water content (mg/kg)		500	1201		374		200	200
Free glycerin (%)	–	–	–	0.008	–	–	0.19	–
Pour point (°C)	–	–	–	–	10	−6	−6	−5
Cold filter plugging point (°C)	8	20 to 5	3	14	10	–	–	–

utilized in pharmaceutics, in fuel additive applications, and in personal care biopolymers (Faba et al. 2015; Rezania et al. 2019).

During current situation many developments are experienced in improvement of biodiesel production by using animal fat waste. China is the top of the list country highlighting the patents regarding biodiesel. In the USA, more than 50% patents focus on processing techniques, reactors technology, and pretreatment techniques (Mahlia et al. 2020). Various tools are developed in transesterification for the development of the processes like ultrasonic and microwave in order to increase the oil and methanol miscibility causing increase in transesterification yield thus leads to significantly improved biodiesel production (Shin et al. 2012; Stavarache et al. 2007).

Recently it has been studied that some adsorbents such as aluminum magnesium hydroxycarbonate and 3,5-ditertbuty l-4 hydroxybenzyl benzene can be significantly applied for retarding the degradation and enhancement of oxidative constancy of biodiesel and its combinations. The acid amount can be reduced up to 9%. Thus, these adsorbents can eliminate the originators of biodiesel aging by stabilization of free radicals and stopping them from startup of a new oxidation chain (Kpan and Krahl 2019).

Biodiesel obtained from vegetable oils has some steryl glucoside precipitates causing the filter blockage; this situation can be overcome by removal of these precipitates by adsorption with 3% silica at 112 °C temperature applied for 72 min (Saeong et al. 2017). Illustrations of some patents regarding biodiesel production are precisely given in Table 10.6.

10.10 Major Challenges and Future Prospects in Biodiesel Production from Vegetable Oil and Animal Fat Waste

The main issue which is being faced globally is energy demand. All global, economical, and social development requires energy. The major worldwide energy demand is being fulfilled through petroleum fuels currently. Till 2040 it has been suspected that global energy demand will increase up to 37% (Joshi et al. 2017). The depleting and limiting resources are pushing the researchers to find any alternative renewable fuel source to meet the energy demand. For this purpose a lot of methods and technologies are being practiced (Tomes et al. 2010; Kour et al. 2019). Biofuels are the type of fuels using renewable resources like lignocellulosic biomass and can meet the world's energy demand. These biofuels can be produced by using chemical resources obtained from biological means or by applications of the existing organisms like microalgae and bacteria (Rodionova et al. 2017). In the past few decades, plant biomass was being widely applied for biofuel production, but since some last year's algal biomass has replaced it and proved itself an emerging bioresource for biofuel production (Dragone et al. 2010). There is a lot of advancements in biofuel

Table 10.6 Some preferred patents for production of biodiesel by using animal fat waste (Toldrá-Reig et al. 2020)

Animal feedstock	Catalyst	Particular conditions	Biodiesel characteristics	References
Lard oil, fish oil, tallow oil	Pt and Pd plus an acidic component	Hydrodeoxygenation and hydroisomerization of the oil in a distinct step	Blend of C14 to C18 paraffins having a ratio of iso to usual paraffins of 2 to 8; less than 5 ppm sulfur; and tolerable lubricity	Herskowirz et al. (2006), Herskowitz (2008) and Helwani et al. (2009a, b)
Animal oil, lard, fish oil, tallow, rendered fats	Immobilized lipase	Surplus water removed by cross-flow filtration	Division of formed crude biodiesel and crude glycerol from the second reaction medium by means of a fourth cross-flow filtration cassette	Hoff et al. (2012)
Animal fats	ZnO, H_2SO_4	Degumming; physical cleansing (heating and vacuum pulling); and glycerolysis	Possibility of using a range of starting feedstocks with heat combination to reduce working costs	Lavella et al. (2014)
Animal fats including 10–20% free fatty acids	H_2SO_4 96%	Esterification in two steps	The amount of FFA in the blend is reduced to fewer than 3% by weight	Scott (2014, 2017)
Animal fats	KOH	Esterification reaction of free fatty acids if higher than a set value	Distillation to remove byproducts like glycerol and alcohol	Matsumura (2015)
Beef oil, pork oil, animal fats such as fish oil	KOH	Transesterification through lower alcohol content	Reducing expenses by producing glycerin and glycerin derivatives in high yield and purity	Matsumura (2015)

production by using microalgae and cyanobacteria (Demirbas 2009; Heimann 2016; Rodionova et al. 2017). Microalgae are a significant resource for biodiesel production due to its high productivity as compared to all other bioresources (Scott et al. 2010).

Regardless of all these profits, there are some major challenges needed to be tackled for biodiesel commercial production at a large scale which could sufficiently contribute in considerable biodiesel production to meet the energy demands of the transport region. The first and foremost arising issue is algal growing. It can be done by using open bioreactors which could be easily arranged, but the key step is contaminant removal by adventitious organisms. Furthermore, carbon dioxide and nutrient supply of culture is also a key factor. The principal condition is actually

stabilizing the release of oil with more cellular components like DNA or chlorophyll without any major contamination (Scott et al. 2010).

Some pretreatment like chemical, biological, physical, and physicochemical are required to improve the access of enzyme toward cellulose by removing the lignin, hemicellulose, and cellulose in the end following which saccharification and fermentation might be carried out (Wang et al. 2018). The chief disadvantages faced due to pretreatments are generation of a few inhibitors for microbes which could be levulinic acid, formic acid, aliphatic acids, and acetic acid (Zhang et al. 2016). Acetic acid presence in media can lead to reduce the biomass yield and growth speed of *Saccharomyces cerevisiae*. Recently a number of approaches are being experienced to improve the inhibitor tolerance of these microbes. These approaches are screening of genes which can be used for stress tolerance and metabolic and genetic engineering for enhancing the tolerance (Wang et al. 2018). At this moment it is almost impossible and very challenging for biofuel to compete the fossil fuel at commercial level, but the situation could be changed by developing novel strains having commercial prospective which can be developed by combing of many genetic engineering strategies so that maximum biofuel production can be attained (Rodionova et al. 2017). For achieving the sustainable biofuel economy in the future, it is necessary to understand the concept of how future environmental changes are going to affect the biofuel production. As a consequence, biofuels will replace fossil fuel by emerging as a sustainable energy supplier and will reduce the concentration of vehicle emissions as well.

10.11 Conclusions

Biodiesels obtained from algae and animal fat waste resources are being expended worldwide due to their biodegradable, sustainable, renewable, and sulfur-free nature. Biodiesel cost commonly depends on the cost of the feedstock. Generally some algae species can be utilized for biodiesel production after their alkaline pretreatment through NaOH. Animal fat waste feedstock leads to cheaper biodiesel fuel as compared to that produced from vegetable oil. Animal fat waste can be obtained from waste of the slaughterhouses and further can be applied as a feedstock in biodiesel production. Biodiesel production can be carried out through transesterification. For the purpose of pretreatment, alkaline catalysis is being preferred over acid catalysis in biodiesel production at industrial scale due to its faster rate and economic effectiveness.

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