

Interdisciplinary Biotechnological Advances

Maria del Pilar Rodriguez-Torres  
Claudia Martinez-Alonso *Editors*

# Biotechnology in the generation of biofuels



Springer

# **Interdisciplinary Biotechnological Advances**

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# Preface

Nowadays, the necessity for alternative energy sources has led to an increase in the research on topics such as solar, wind, geothermal energies and the like as well as of biomass. The biomass concept refers to renewable organic materials coming from plants and animals, and it is also fundamental when it comes to biofuels because it must be transformed in order to obtain them. The procedures to do this are mainly chemistry-based that can be tailored by including or adapting biotechnology.

This book, *Biotechnology in the Generation of Biofuels*, is intended to be introductory to the general outlook on biomass and biofuels starting from what they are, the different generations they are subdivided into, and the procedures used in general as well as with biotechnological interventions; hence, the reader can get the whole picture on the relevance and urge to make biofuels much more available as time passes by given the fact that fossil fuels are on the verge of disappearing as shown by yearly statistics worldwide. Additionally, advantages and disadvantages of biofuels are presented as well as information on LCAs, policies in some countries, professional opportunities, and support information on biotechnological methods to produce biofuels.

We think this volume can be quite useful to academia students and professors and people involved in industry or government, particularly because they are part of the whole assembly necessary for the development, fabrication, industry-scaling, and policy-making necessary for a smooth and gradual transition to the renewables domain.

We, the editors, thank all the authors who contributed to this book as well as Dr. Jayanta Kumar Patra, who besides, granted us the opportunity to participate in this project and aided with valuable comments and part of the edition.

The editors wish to thank Sudha Ramachandran for her support and professionalism during all the phases in the book processing as well as Haridharini Velayoudame for her intervention in the last production stage.

Dr. Martinez Alonso wishes to thank her husband and daughter for their support throughout the months the book was in its writing process and, also wants to dedicate the volume in memory of Dr. Victor Manuel Dominguez Marquez (Autonomous University of Guerrero @Iguala, Gro.).

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# Introduction



**Evelyn B. Díaz-Cruz, Jayanta Kumar Patra, Claudia Martinez-Alonso,  
and Maria del Pilar Rodriguez-Torres**

*I think the biggest innovations of the twenty-first century will  
be at the intersection of biology and technology. A new era is  
beginning.*

*Steve Jobs (February 1955–October 2011)*

**Abstract** This introductory chapter will present brief and elemental information, concepts as well as a summary of the general aspects that are related to the production of biofuels by means of the traditional methods and the biotechnology-based ones. Also, topics related to environmental impact like greenhouse gases (GHG), the depletion of fossil fuels, the evaluation of biofuels regarding their environmental issues, biofuels that are already in use, and necessary policies for biofuels implementations are covered. The aforementioned elements are relevant because as it happens with novel discoveries and technologies, risks and advantages must be taken into consideration. We want to clarify, too, that the book content is an effort, especially for the neophyte reader to visualize, in the first place a general overview on biofuels in a sequential manner that includes how they are defined and what the concepts such as biomass and feedstock refer to. Latterly, the role of

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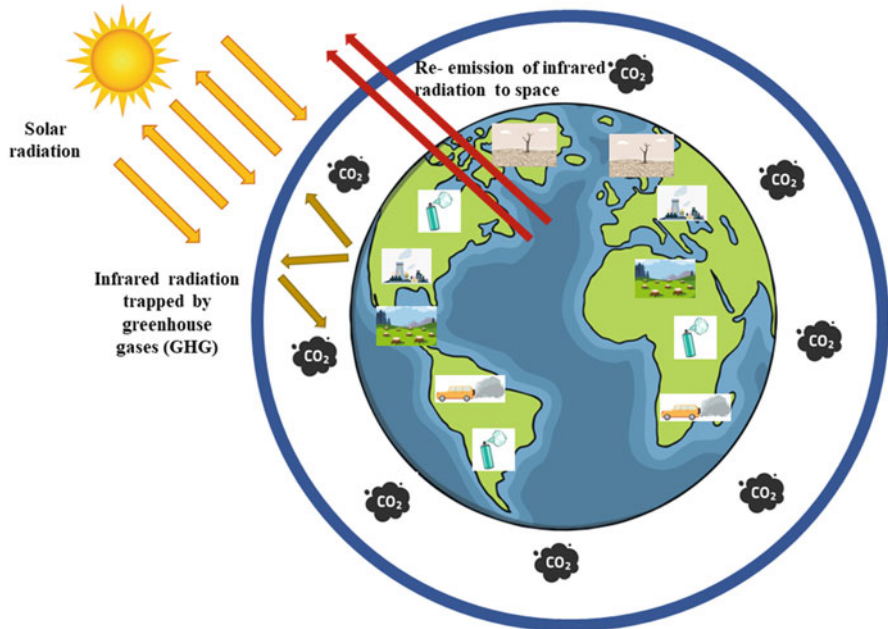
biotechnology starting with its basic definition and subdivisions is addressed along with the four generations of biofuels, from which, the third and fourth ones are related to biotechnology because of the tools they make use of like genetic engineering. It has to be noted, that at this time, solely biotechnology-based biofuels are not being implemented in the real world. They are being investigated and under development in research environments since proper biotechnological techniques are not used yet commercially but thermochemical or physical approaches. Even so, chapters on biofuels obtained by traditional methodologies have been written for further reference, for example, about pyrolysis and some of its subcategories, transesterification, and the like and then, including whenever possible wholly-based biotechnology methodologies to give support on these potential and benign processes. Therefore, this volume was done with the purpose of transporting readers from the established techniques to manufacture biofuels to the ones that are in the works and related directly to biotechnology, that have not have reached a scaled-up capacity yet, but that have an outstanding potential considering that relying completely on feedstock such as crops may not be the best option at the end of the day because of the risk associated to the costs competition between food production/supply and biofuels manufacturing. This mainly includes the involvement of micro-organisms as certain types of micro- and macroalgae. In the last chapter, conclusions are presented, future perspectives are given and even some discussions on industry global collaborations are mentioned with the intention of giving the book a frame of reference on the biofuels subject.

**Keywords** GHG · Biotechnology · Biofuels · Biomass

## 1 Insights

### *1.1 Energy and its Worldwide Exploitation*

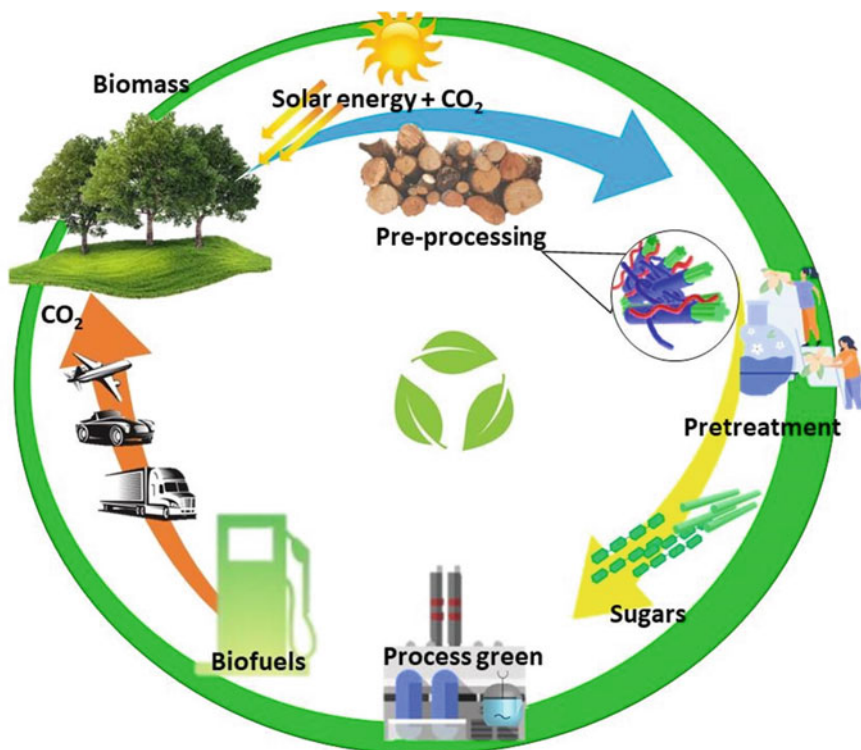
Climate change is one of the most serious problems humanity is facing nowadays. In 2006, Al Gore warned of a growing “planetary emergency” if global warming continued unchecked, including rising sea levels, coastal flooding, and climate refugee nations (Manuel 2017). The effects mentioned 15 years ago are evident today, with the increase in the average temperature of the planet even in zones that tend to be cold, the change in rainfall patterns which derives in floods in some cases, the increase in sea level, land exhaustion, and the frequency of extreme meteorological phenomena is confirming this argument and putting our model of life to the test, which will have to adapt to new climatic, social, and economic conditions. The most important cause of climate change is the warming of the planet, produced by the so-called Greenhouse Gases (GHG) and which are currently emitted in the industrial, agricultural, transport, and energy consumption processes that the current generated development model entails by human activity, even household activities such as the use of aerosols provoke environmental damage. The GHG layer, located in the lower area of the atmosphere, lets long-wave solar radiation pass toward the



**Fig. 1** Greenhouse effect

Earth's surface, which then heats up. However, when this heat is emitted back into the atmosphere, GHG do not let it escape and retain it, causing the planet to warm (Khanna et al. 2011). Figure 1 shows graphically how such a process occurs with GHG in the form of CO<sub>2</sub> (black clouds in the figure) emissions and depicting some of the polluting interventions that cause this.

The global energy demand has been increasing in a great manner throughout the last decades and it is expected that the tendency continues exponentially. This is greatly due to the increase in population which, in turn, contributes to the demand for all kinds of products and therefore leading to industrial growth (Zohuri 2020). Consequently, the need for alternative sources of energy that can potentially, in the beginning, if not suppress completely GHG, at least, be a barrier to their excess is a must. Furthermore, another factor to consider is the depletion of fossil resources, from which basically a great number of fuels come, e.g., gasoline, diesel, and natural gas. There are, additionally, other general issues such as supply security, and climate change problems; for which renewable energies in the form of biofuels represent a solution (Moriarty and Honnery 2016). Renewable energies are classified as wind, solar, geothermal, biomass, among others (Dogaru 2020). Biomass is a promising alternative instead of the raw materials used to produce fossil fuels and it constitutes the core of biofuel generation. One thing to clear out is that the concepts of biomass and feedstock are not far apart. Biomass is defined as a renewable organic material that comes from plants and animals whereas feedstock is a general term that refers to



**Fig. 2** Biomass processing

a material that is used to make fuels. In fact, they can be used interchangeably or even together, e.g., biomass feedstock (Bioenergy Technologies Office).

The conversion of biomass feedstocks into biofuels can be considered to some extent as a predominantly environmental friendly process, although that does not remove its industrial character totally. So is the use of biofuels for transportation. When we use bioethanol instead of gasoline, we help reduce atmospheric  $\text{CO}_2$  in three ways: first, we avoid the emissions associated with gasoline; second, we allow the  $\text{CO}_2$  content of fossil fuels to remain in storage; and third we provide a mechanism for  $\text{CO}_2$  absorption by growing new biomass for fuels. Due to their compatibility with the natural carbon cycle, biofuels offer the most beneficial alternative to reduce greenhouse gases in the transport sector, as shown in Fig. 2 (Khanna et al. 2011).

## 2 Renewable Energies and Their Relevance in Sustainability

Renewable energies also known to be clean and are defined as those that come from natural sources or processes that are constantly replenished. For example, sunlight or wind just to mention the most acknowledged ones. On the other hand, sustainability refers, in environmental terms, to the preservation of natural resources. However, there is a huge debate on whether renewable energies (usually generated from renewable resources) and sustainability go hand in hand, particularly considering that if the rate of use exceeds the rate of renewal, that is, the source is used more than its recreation, it may become unsustainable at some point. Despite this, interest in renewable energies has been sparking interest in many levels, scientific, political, and from the general public, since a long time ago (Owusu and Asumadu-Sarkodie 2016). And they have become an attention focus because of fossil fuels depletion in the long run.

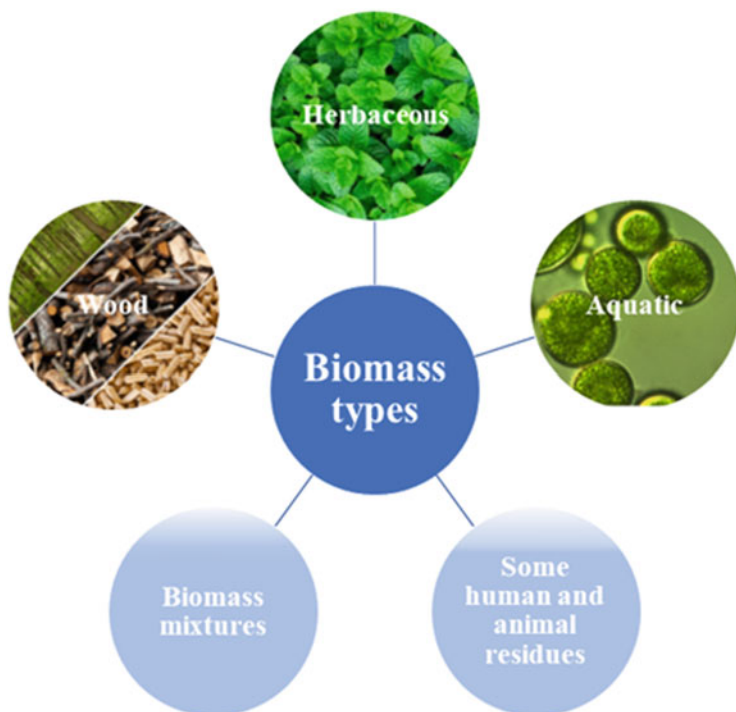
Another aspect to take into consideration is that the use of renewable energy sources and sustainable development are two variables that are closely related with each other because renewable energy resources appear to be the one of the most efficient and effective solutions to the current environmental problems, too, and not only to the depletion of nonrenewable resources (Dincer 2000). The difficulties that renewable energies implementation are facing have to do with the economy, mostly, for example, the solar industry employs more people than the one related to coal. Also, large-scale renewable energy projects require large amounts of capital to run and the lack of policies as well as regulations makes it difficult to minimize associated risks. In addition to this, the absence of infrastructure for distribution and trained personnel to work on it is slowing this advance, too.

There are also numerous life cycle assessment (LCA). LCAs are defined as systematic analyses about the environment during the whole life cycle of a product, material, process, or other measurable activities. These studies when it comes to biofuels take account of the climate change and other of their environmental impact factors. They are continuously published every year since a long time ago and, the main aspect that has been reported regarding biofuels is the achievement of the decrease in life cycle GHG emissions using the estimation of their potential repercussion on climate change, which is currently an issue of urgency. Nonetheless, their findings are often limited and inconsistent because they consider not global but very specific features, for instance, region, feedstock/biomass or biofuel kind (Jeswani et al. 2020). In addition to this, scientific literature is focused on too individual aspects in the same fashion as LCAs but more engaged to research on biomass/feedstock types and procedures to handle it, methodologies to produce biofuels, and so on. In like manner, some more details can be found on biofuels from government agencies, non-governmental organizations (NGOs) energy sector companies, but it is quite scarce (facts, statistics, initiatives, news on uses of some biofuels, and so forth). As a consequence, it is not likely that at this moment, a definite consensus on the real impact of biofuels can be achieved due to this information dispersion.

Still, efforts can be made progressively to surmount this, and it has to be considered that in several places in the world, some renewable energies have already been set, like in the case of solar panels (Østergaard et al. 2020). And as it has been mentioned in this chapter and will continue to be discussed throughout this book, some biofuels are already used especially in blends with common gasoline, for example.

### 3 Biomass Concept and Its Importance

Biomass is all that nonfossil organic material of natural origin, it has a great potential to provide energy through its conversion, therefore it is classified within renewable energies. Biomass can be classified in five types, as shown in Fig. 3. This classification is listed as wood/woody biomass (trees, barks, roots, leaves) as a source of carbohydrates and lignin; herbaceous biomass like crops and plants; aquatic biomass which encloses micro- and macroalgae; animal and waste biomass that includes bones and manure; and finally biomass mixtures (Tursi 2019).



**Fig. 3** Biomass general classification



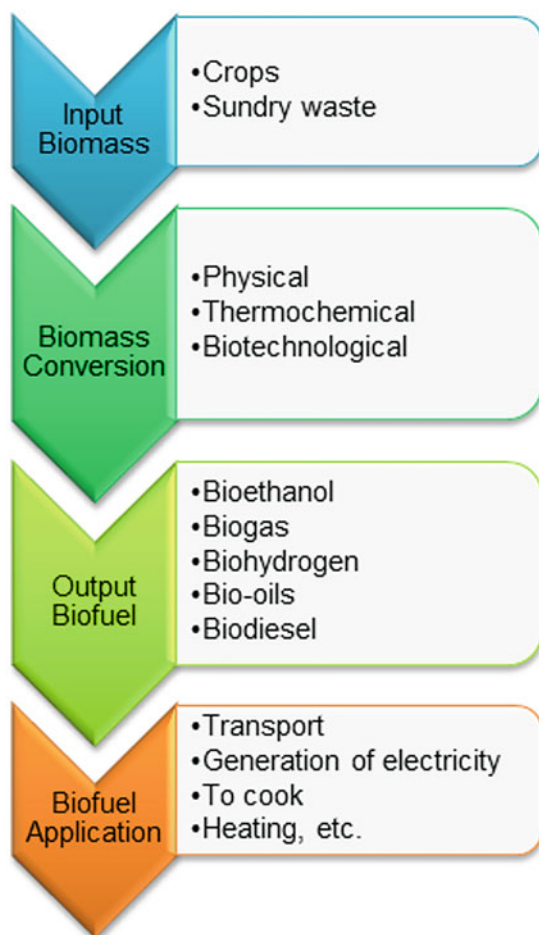
Biomass is considered the main emerging alternative to fossil fuel resources (oil, natural gas, and coal) (Awasthi 2020; Antar et al. 2021). Since it is readily available, it is abundant in nature and above all, renewable. Biomass is also classified into four generations (similar case to biofuels, which is discussed in chapter “Biotechnology and Its Connection to Renewable Energies: Biofuels Production”), these mainly include crops, sundry wastes (agricultural, forestry, domestic, industrial), microalgae, and genetically modified plants (Arpia 2021). Currently, the population’s food security is being sought, for this reason, the processing of biomass from edible crops has decreased considerably and lignocellulosic biomass (agricultural and forestry waste) has become very important. Through the processing of biomass, various fuels can be obtained, such as biogas, bioethanol, biodiesel, and bio-oils (Ong et al. 2020). The production of biofuels through biomass has variations between countries depending on the geography of the place, the availability of resources, biodiversity, technology, and the economy (Antar et al. 2021). On the other hand, depending on the biomass and the biofuel to be obtained, different methods are used, such as physical, thermochemical, and biotechnological methods, as shown in Fig. 4 (Arpia 2021). Finally, it is important to mention that biofuels present some problems such as high moisture content, low calorific value, composition, and variable properties (Ong et al. 2020). To minimize the aforementioned problems, they are refined or purified (Khalil 2020; Vignesh et al. 2020).

## 4 Biomass Processing to Obtain Biofuels

The transformation processes from biomass to biofuel are diverse, classified mainly in physical, thermochemical, and biotechnological methods, as mentioned above. In some cases, it is necessary to decompose the biomass through different pretreatment processes to separate the components and facilitate the obtaining of biofuel. There is high feasibility of replacing fossil fuels with fuels generated from biomass, attributed to the ease of using biomass as a raw material. Due to its abundance in nature and most importantly, it is classified as a renewable feedstock. The biofuels obtained from biomass are biogas, bioethanol, biodiesel, bio-oil, vegetable oil, and biohydrogen; and they have the potential to compete with and even replace fossil fuels (Arpia 2021). Each of the already mentioned biofuels is briefly discussed below.

Biogas is a flammable gas, very similar to natural gas. It is composed of a mixture of gases in different concentrations which depend on the feedstock used to obtain it (Hussein 2015; Arutyunov et al. 2020). Biogas is obtained by a biotechnological process called anaerobic digestion (AD), which consists of the decomposition of biomass in the presence of a consortium of microorganisms and absence of oxygen (Zheng et al. 2014; Khalil et al. 2019; Kainthola et al. 2019). AD consists of four stages (hydrolysis, acidogenesis, acetogenesis, and methanogenesis), each with its consortium of microorganisms that favor the formation of biogas. The biomass used to obtain biogas is diverse, crop residues, food residues, manure, wastewater, etc.

**Fig. 4** Conversion of biomass to biofuels



stand out (Lemma et al. 2020), the final properties of biogas depend on the biomass used. Finally, biogas is a biofuel with various applications, it is used to generate heat (cooking, heating, etc.), in transportation, like natural gas, etc. Biogas is considered a low-cost biofuel, as it uses waste biomass in its production process (Esteves et al. 2019). Through a purification process, biogas can be converted to biomethane and injected into natural gas networks for direct use (Arutyunov et al. 2020). A more detailed explanation is discussed in chapter “Biotechnological Formation of Biogas”.

On the other hand, bioethanol is obtained from fermentable sugars that are contained in the carbohydrates that make up the biomass. Therefore, it is very easy to obtain ethanol from edible sources containing mainly sugars and starch, which has generated growing concern over the debate on food production versus fuel production (Prasad et al. 2019). However, the competition between food and biofuels could

be resolved if bioethanol, in this case, was obtained from renewable resources, such as lignocellulosic biomass (LCB). This bioethanol is considered second-generation biofuels, and is also obtained from agricultural raw materials, forestry, agro-industrial solid waste and municipalities that do not interfere with world food production (Hassan Kamani et al. 2019). The use of biomass can have two main advantages, reducing the waste of resources and production costs, and on the other hand, mitigating serious environmental problems, reducing CO<sub>2</sub> emissions (Hou et al. 2020).

Biomass is composed of polysaccharides such as cellulose and hemicelluloses and an aromatic polymer called lignin, which combine to protect sugars that store energy and give plants rigidity. Therefore, to produce bioethanol from biomass, a complex conversion process is needed to decompose biomass into its components and take advantage of sugars, which has made it difficult to obtain it commercially (Limayem and Ricke 2012). These processes are described in more detail in chapter “Biotechnology in Bioethanol Generation” of this book.

Another biofuel is biodiesel; it is one of the most researched and used worldwide. It is obtained mainly from vegetable oils, waste oils, animal fats, and algae. The feedstock for obtaining biodiesel varies from one country to another and depends on the production and geographic location of the regions. The synthesis methods used to obtain biodiesel are blending, microemulsion, pyrolysis, and transesterification (Zhao et al. 2019; Chamkalani et al. 2020; Dey et al. 2021). The transesterification method is the most used to obtain biodiesel, although depending on the feedstock, the appropriate method can be chosen. The transesterification method consists of reacting the oil with short-chain alcohol in the presence of a catalyst, thus obtaining biodiesel and glycerol as a by-product. Currently, biotechnology focuses its attention on the catalysts used in the transesterification process, biocatalysts have been used that work the same as conventional (chemical) catalysts, but biocatalysts have the advantage of being friendly to the environment, non-toxic, easily degradable, and do not pollute water; resulting in an alternative to reduce the environmental impact of conventional catalysts. Finally, the main application of biodiesel is in vehicles with diesel engines. Biodiesel can be used directly or mixed with conventional diesel. Biodiesel is an excellent alternative to minimize the use of fossil fuels and is friendly to the environment (Raheem et al. 2020; Chamkalani et al. 2020; Singh et al. 2021; Dey et al. 2021). This topic is covered in more detail in chapter “Biological Methods for Diesel Formation”.

As for vegetable oils (VOs), these are not very popular as a potential biofuel, at least in the near future, due to its properties, especially, when considering its viscosity and difficulty to keep them stored (Altón 2001). On the other hand, they are quite useful when used as the basis for the production of biodiesel. In any case, vegetable oils are extracted by means of mechanical (pressing) and chemical (solvent) extraction, followed by chemical and/or physical refining to improve oil quality (Hognon et al. 2015; Dimitriadis and Bezergianni 2017). As for bio-oil, it is obtained by pyrolysis or hydrothermal liquefaction. It can be potentially applied in the operation of boilers, engines, and turbines to generate heat and power and it has an advantage when it comes to prices competitiveness (Esteban et al. 2011). This

topic is discussed in detail in chapter “Biotechnological-Based Production of Bio-Oil and Vegetable Oil”.

In the case of biohydrogen, there are many processes to manufacture it, among which dark fermentation is the one involving raw biomass. Although, until now, no raw material has been found that provides a high production performance of biohydrogen yield in this process. However, exists great interest in improving the low rates in the production of biohydrogen from lignocellulosic biomass. This has been achieved with the modification and combination of the different types of pretreatments since the objective of this is to destroy the lignin seal that protects the cellulose molecules and helps their release to the solution followed by the destruction of the crystalline arrangement of cellulose molecules and depolymerization to improve enzymatic digestibility and acidogenic fermentation in biohydrogen production (Mohammadi et al. 2011; Bundhoo 2017; Basak et al. 2020). The type of pretreatments used for substrates in the production of biohydrogen through dark fermentation are discussed in more detail in chapter “Biotechnology in Hydrogen Generation”.

The principal biofuels treated in this book are biodiesel, bioethanol, hydrogen, vegetable oil, and bio-oil. But biofuels. Additionally, other sustainable biofuels such as SAF (Sustainable Aircraft Biofuel) will be mentioned briefly as well.

## 5 Final Remarks

The objectives of the book have been outlined in this chapter along with the base concepts that are necessary for the understanding, discussion, research, and incorporation of biofuels as an alternative to fossil fuels. It is of crucial importance to point out that the definition of biomass and how it is classified will permit the reader to identify what biomass/feedstock is the most suitable to produce a certain biofuel and to know where and how to get it given the wide range of sources it can come from. Getting familiar with the information and research that can be found via search engines and databases will enable individuals to know how to handle what they find and apply it in the biofuels field as researchers, policy makers, feedstock suppliers, and staff related to sustainability topics. An outlook on the biofuels that will be reviewed will give the reader the opportunity to get familiar with their background, advantages, and disadvantages. Knowing about the general procedures, whether they are physical, thermochemical, or biotechnological, can be useful to decide on what the most suitable method will be to generate biofuels, to improve it, scale it up, and shift in the best way possible to more biotechnological approaches. This is mentioned due to even though biofuels come from biomass, which has a 100% natural origin, the procedures involved to process it do not necessarily involve wholly-based biotechnology-based steps. Most of them rely on more chemical and industrial processes except when procedures such as anaerobic digestion, the use of bioreactors, genetic engineering, and the general use of microorganisms are incorporated.

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# Biotechnology and Its Connection to Renewable Energies: Biofuels Production



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*Just as fossil fuels from conventional sources are finite and becoming depleted, those from difficult sources will also run out. If we put all our energy and resources into continued fossil fuel extraction, we will have lost an opportunity to have invested in renewable energy.*

*David Suzuki (March 1936-)*

**Abstract** Today, the increase in population and the indiscriminate use of fossil fuels are the main sources of pollution, jeopardizing the environment and human health. The excessive burning of fossil fuels contributes to the increase in greenhouse gases, causing an environmental lack of control, with the above, the need arises to propose alternatives for the solution to this problem. Also, there is the concern of depletion of the nonrenewable resources such as oil and natural gas, for example. In this context, use has been made of biotechnology and its different processes through which biofuels can be generated. The need to develop methodologies that allow for the obtaining of biofuels with the purpose that in the long run, they substitute the aforementioned nonrenewable sources of energy which could result in lowering costs and stopping pollution. This chapter will address the generalities of various biofuels: biogas, bioethanol, biodiesel, bio-oils, vegetable oils, and biohydrogen. As

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well as the biotechnology-based approaches used to obtain them, with a sustainable approach and without risking the environment.

**Keywords** Biofuels generations · Feedstock · Crops · Algae

## 1 Introduction

The term biotechnology was outlined for the first time by Károly Ereky, a Hungarian agriculture engineer, in the nineteenth century. The concept covers all technological applications that make use of biological systems, living organisms along with their byproducts and spinoffs to either create or modify products or processes for specific purposes (Bhatia and Goli 2018).

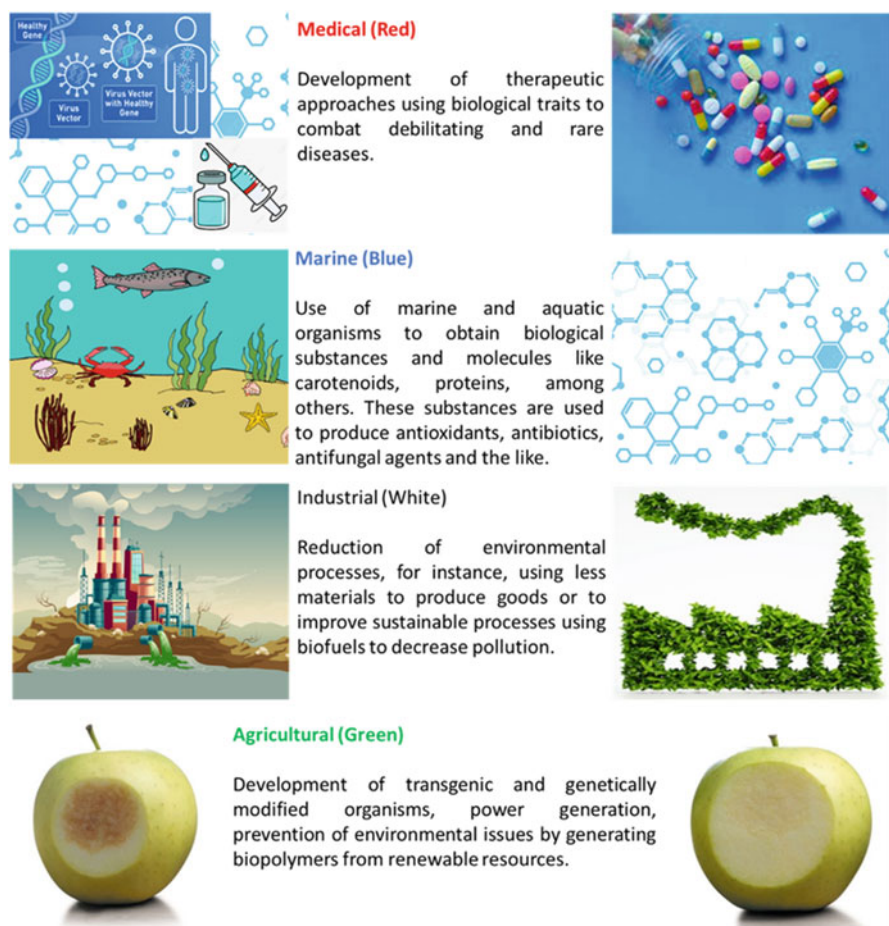
Biotechnology is subdivided into medical, agricultural, industrial, marine, food, and environmental, which can also be identified by colors as shown in Fig. 1. These biotechnology classifications have been proposed by different authors (DaSilva 2004). Particularly, industrial biotechnology is aimed at manufacturing biofuels, plastics, enzymes, and the like (Gupta et al. 2016). In the case of biofuels, there are two important concepts to be considered: green chemistry and biorefinery. The first one refers to the design and fabrication of chemical products and mechanisms that dwindle or eliminate the need for the use or generation of hazardous substances. On the other hand, the biorefinery concept refers to facilities that merge biomass conversion processes and equipment to generate biofuels, power, and chemicals out of agriculture, forest, microorganisms, waste feedstock, and the like. By means of the production of diverse goods products, a biorefinery can exploit the availability of different biomass streams and process intermediates and in order to boost the value coming from the feedstock (Rita et al. 2013). Regarding biofuels, biotechnology's intervention has to do with the procedures such as genetic engineering as it has already been noted to enhance, for instance, the production of oils and fatty acids and, in this way, it will be possible to get high yields of biofuels.

This chapter is devoted to cover general aspects related to the production of biofuels by physical and thermochemical methods as well as the emerging intervention of biotechnology in the production of biofuels, especially when fourth-generation biofuels are referred to and, the worldwide outlook and perspective on the use of biofuels in the near future.

## 2 General Biotechnology Role in Renewable Energies Development

Research focused on the production of biofuels has grown greatly due to the increment in energy prices and the reduction of fossil fuel sources (Rodionova et al. 2017). The methodologies used in biofuels production are based mainly on chemistry approaches, for instance, pyrolysis (Fernandez-Lopez et al. 2016),





**Fig. 1** Biotechnology subfields and some examples

thermochemical (Canabarro et al. 2013), and transesterification (Rezania et al. 2019) just to mention the most important ones. However, nowadays, biotechnology is being used to produce biofuels, too, also decreasing issues that their fossil-derived counterparts display, but it needs to be mentioned that they form part of biofuel production to some extent only, there is not actually a 100% biotechnology-based method. The extent of biofuel biotechnology comprises aspects such as feedstock chemistry, degradation processes, and even genetic engineering as well as transcription (Tseten and Murthy 2019).

Biotechnology's role in the field of renewable energies represents a wide range of potential developments and mediation (Kilbane 2nd 2016).

3 Overview on Biofuels

Bioenergy is the energy derived from biofuels and they stem from biomass. Still, it is worth mentioning that they have been around for a long, for example, wood (for domestic uses like cooking and heating). Figure 2 displays The inclusion of renewable energies is an important matter nowadays given the fact their positive impact in the long run, although their implementation still faces some issues related to reliability misunderstanding among the general public mostly, market entry, and initial costs among others. Biofuels are energy suppliers that come from freshly

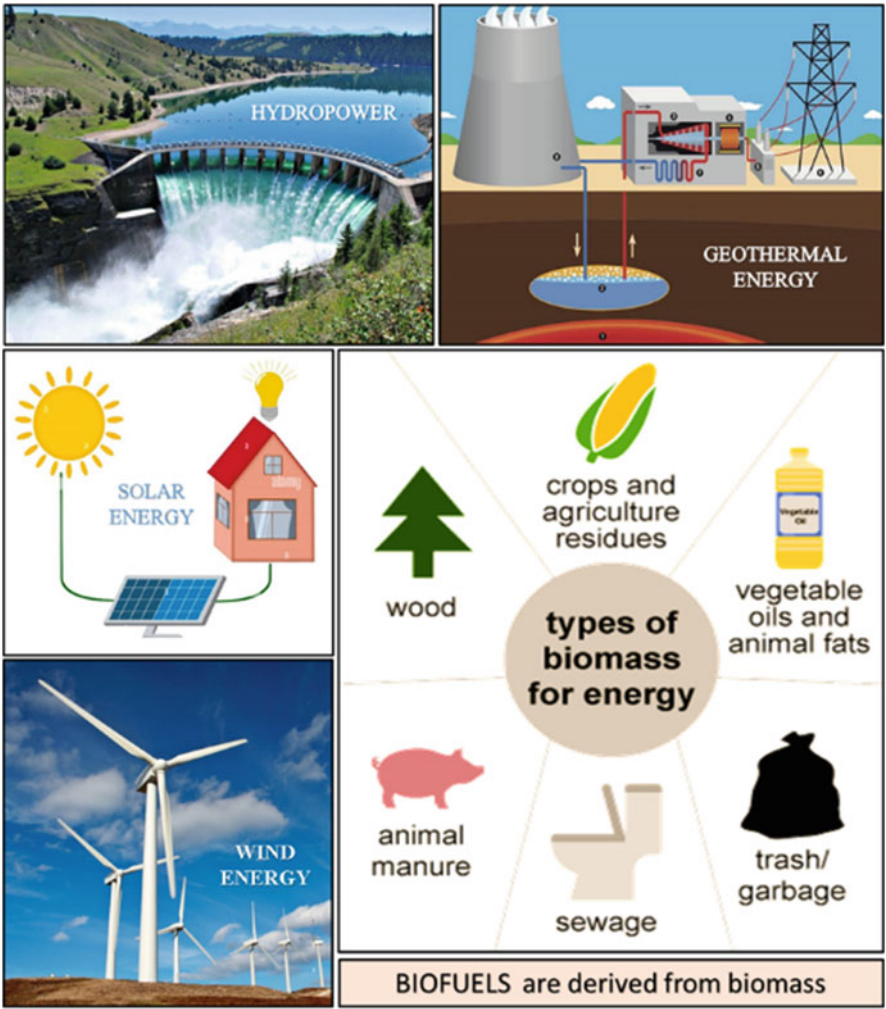
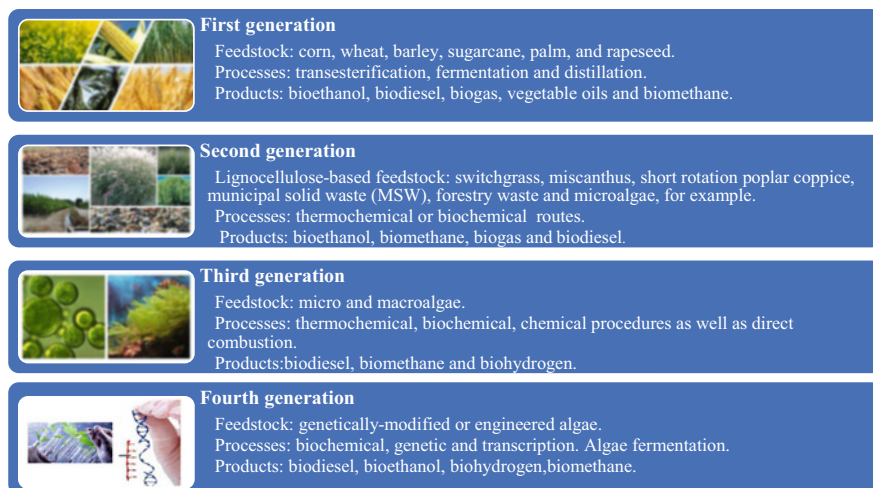


Fig. 2 Types of renewable energy technologies along with biofuels



**Fig. 3** List of the generations of biofuels so far and their general characteristics

grown biomass, and they can be manufactured in either liquid, solid, or gaseous forms. Fossil fuels like coal and petroleum as examples, also originate from biomass. However, in contrast to biofuels, their source materials took millions of years to be generated and, their demand is much higher because of their abundance and low cost. Biofuels are being considered as a replacement given the increasing oil prices, the decrease of fossil fuel reserves as well as the concern to have renewable and trustworthy, and lasting sources of energy and as an alternative to lessen and relieve the consequences of climate change. Biofuels are considered renewable resources due to their continuous replenishment. Their fossil counterparts, on the contrary, are not renewable given the fact it is mandatory for them to require really long periods of time to form. There are four categories of biofuels: first, second, third, and fourth generations. They are sorted according to the sources of biomass feedstocks they originate from, their restrictions as renewable sources of energy, and the process in their technology.

Figure 3 shows a general scheme where the subdivision of biofuel generation is displayed as well as some of their traits. As it can be observed from the listing, there is an evolution tendency in connection with the biomass used to produce a certain biofuel, for example, going from crops to algae. Also, it is obvious that one of the aims of biofuel production is to avoid exhausting crops in order to avoid competition with food production (mainly fruits and vegetables).

### **3.1 *First-Generation Biofuels***

Biofuels within this generation are also called conventional biofuels and come from sugar, starch, or vegetable oil (edible feedstock). All of them are manufactured by means of well-understood and established technologies and processes, for instance, fermentation, distillation, and transesterification. Sugars and starches go through a fermentation process to essentially produce bioethanol, and to a lesser extent, butanol, and propanol (Naqvi and Yan 2015) too. Bioethanol encompasses one-third of the energy density of gasoline although it is at present used in many parts of the world, including the United States and Brazil, as an add-on to common gasoline. An advantage of ethanol is that it tends to burn in a much cleaner manner than gasoline and in consequence, emits lesser greenhouse gases. Another first-generation biofuel is biodiesel, which is obtained when plant oils or animal fats are subjected to transesterification processing. Transesterification involves the exposition of oils to alcohols, like methanol, in combination with a catalyst (Fang et al. 2019). The distillation process involves then setting apart the principal product from the generated byproducts of the reactions. Biodiesel can be used instead of petroleum diesel in several diesel engines or in a mix of the two.

The first-generation biofuels represent a step in the direction of energy independence. They also could assist agricultural industries and country communities by means of the growing demand for crops. On this subject, first-generation biofuels also present drawbacks. They pose a threat to food prices since the biomass used are food crops such as corn and sugar beet (Naqvi and Yan 2015). First-generation biofuel production has been partially responsible for the recent increases in world-wide costs for food and animal feeds. There might likely be an unfavorable impact on biodiversity and the competition for water in some territories. Furthermore, biomass for first-generation biofuels demands huge extensions of land to grow, and this results in a restricted greenhouse gas reduction (Rulli et al. 2016). First-generation biofuels also only represent a small benefit over fossil fuels regarding greenhouse gases since they depend upon high amounts of energy to grow, collect, and process. Production practices in the present time use fossil fuels for power. First-generation biofuels are also a higher-priced option than gasoline, making it not really profitable and possibly disadvantageous in the long run (Thompson 2020). Finally, the main contribution of biotechnology to this generation of biofuels relies on the usage of transgenic crops (Martin 2010).

### **3.2 *Second-Generation Biofuels***

Second-generation biofuels undertake some issues having to do with first-generation biofuels since they do not portray a severe competition between fuels and food crops given the fact that they come from a different type of biomass. They also generate higher energy gains per acre than their first-generation counterparts. They

contemplate and permit the use of feeble quality land where food crops may not be able to grow (Naik et al. 2010). The technology associated with these biofuel generation is at some level still in the works, and as a result, it still has the potential to minimize expenses and increase production efficiency as scientific advances progress with time. Nonetheless, some biomasses for second-generation biofuels still represent a competition for the use of land since an amount of biomass pops up in the same climate and areas as food crops. This is a significant obstacle for farmers and policy makers with the strenuous determination of which crop to grow (Antizar-Ladislao and Turrión-Gómez 2008). Lignocellulosic sources (that is, those that are chemically composed of cellulose, hemicellulose, and lignin) that grow apace with food crops could be used as biomass feedstock, such as corn stover (leaves, stalk, and stem of corn) (Hirani et al. 2018). In spite of that, this would provoke the overabsorption of too many nutrients from the soil and would therefore need to be replenished with the aid of a fertilizer. In addition, the process to produce second-generation biofuels is more intricate because it requires pretreatment of the biomass to release the trapped sugars, by means of thermochemical and biochemical processes. This, in turn, requires more energy and materials usage and disposal (Datta et al. 2019).

The biomass sources for second-generation biofuels comprise wood, organic waste, food waste, and specific biomass crops (non-edible). Fast-growing trees, for instance, poplar trees need a pretreatment stage, which consists of a series of chemical reactions that break down lignin, the “glue” that holds plants together, for the sake of making fuels. This pretreatment step incorporates thermochemical or biochemical reactions that release the sugars embedded within the fibers of the plant. Following this phase, the process to generate bioethanol is quite similar to that of ethanol production. In a like manner, straw and other forest residues can undergo a thermochemical process that yields syngas (a mixture of carbon monoxide, hydrogen, and other hydrocarbons) (Sims et al. 2009).

Regarding biotechnology-oriented paths, microalgae are proposed as convenient biomass sources in the production of biodiesel, biohydrogen, and bioethanol (Schenk et al. 2008). For biohydrogen generation, there is the photobiological approach, for instance. In the case of biomethane, closed algal bioreactors are under research (Wagner et al. 2018). These microalgae-based methodologies started being investigated in the 50s and the 60s (20th century), but it is nowadays when they have gained importance with respect to biofuels production (Gilmour 2019).

### ***3.3 Third-Generation Biofuels***

Third-generation biofuels originate from engineered crops such as algae. These algae are usually in laboratories and then harvested to extract oil within them. The oil can later be transformed into biodiesel by a similar route to the one used for first-generation biofuels, or it can be refined into other fuels as replacements for petroleum-based fuels. Algae that are rich in carbohydrates, lipids, and proteins

are the most ideal candidates for biofuel production (Neto et al. 2019) due to the easiness to get higher yields.

These algae-derived biofuels are more energy robust than first- and second-generation biofuels per area of harvest. Algae are cultured since they represent an unexpensive, high-energy yield, and completely renewable source of biomass (Alam et al. 2015). They are advantageous for the reason that they can be grown in areas unsuitable for the crops used for the generation of first- and second-generation biofuels, which would help alleviate stress on water and the arable land used (Alam et al. 2015). Algae can be obtained using sewage, wastewater, and saltwater, such as oceans or salt lakes. As a consequence, it would not be required or mandatory to use water for human consumption (Sadatshojaei et al. 2020).

Notwithstanding, additional research still needs to be carried out to deepen the extraction process to make it financially competitive with petrodiesel and other petroleum-based fuels. The costs of cultivation in photobioreactors or open ponds, biomass harvesting, and oil extraction from algal biomass remain to represent an expensive investment (Chowdhury and Loganathan 2019).

### 3.4 *Fourth-Generation Biofuels (FGB)*

The last generation of biofuels, so far, makes use of genetically engineered feedstock with genomically produced microorganisms, like microalgae, which is rigorously related to biotechnology. This generation is not yet taken into account formally by some authors, though. This may be due to a fine overlapping with third-generation biofuels (Ale et al. 2019), but it is included for the reader to have an all-inclusive outlook on biofuels classification. It is relevant to mention that not all microalgae species are adequate to generate biofuels, especially biodiesel, this is because of lipids (polar or neutral) content and growth rate of microalgae (Dutta et al. 2014). The general procedure by steps for the generation of biofuels consists of first, the strain selection considering factors such as condition, growth rate, digestibility, and chemical composition. Then, genetic engineering techniques are used, but the microalgae's genomes must be known previously. This is where the contribution of biotechnology to biofuel generation is the most predominant in contrast to first and second generations.

Some of the most studied algae are *Eustigmatophyceae*, *Chlorophyceae*, *Bacillariophyceae*, and *Cyanophyceae* (Lü et al. 2011). When it comes to cyanobacteria (also commonly known as blue-green algae), another type of alga, they are particularly important because they represent an important source of atmospheric oxygen. Their use in biofuel production is attractive due to their plain and noncomplex genetic structure, low nutrient demand, and broad environmental unsusceptibility. The use of cyanobacteria in biofuels is targeted on ethanol, biogas, biohydrogen, and biodiesel (Farrokhi et al. 2019).

One aspect that needs to be worked on is the production of microalgae because it involves a big investment given the fact that they need to be grown, harvested, and



treated by means of genetic-based approaches for the production of biofuels. This generation is the one that is more involved with biotechnology (Abdullah et al. 2019).

Fourth-generation biofuels are not extensively studied but it is rather promising because it is not only intended for the production of biofuels but also for the generation of bioenergy and the capture and storage of carbon dioxide (CO<sub>2</sub>) during the production stages (Itskos et al. 2016).

### **3.5 Final Remarks**

It must be cleared out that basically the most important biofuels, or at least the ones that are given more priority are bioethanol, biodiesel, and biogas (Alalwan et al. 2019). Last but not least, there are others such as biomethane, biobutanol, and biohydrogen. Biofuels that belong to the first, second, and third generations to a great extent are mainly obtained by chemical-based approaches, and to a smaller one to either biological or biotechnology-related methodologies and the fourth generation is wholly related to biotechnological procedures. All generations display advantages and disadvantages, especially those associated with environmental impacts. Still, they portray a hopeful alternative for the generation of bioenergies that are clean and renewable (Malode et al. 2021).

## **4 Worldwide Perspective in the Production of Biofuels**

Day after day humanity has witnessed the growing demand for electricity and fuels that originates, mainly, from nuclear energy and fossil fuels (4% and 80%, as of 2019, worldwide) (Kumar et al. 2020). In the last years, the generation of greenhouse gas emissions (GHE) along with the depletion of the sources from which fossil fuels are obtained has caused a general concern because the demand for fuels and electricity has increased greatly (Jain 2019).

Hence, the necessity for the development of new forms of energy, clean ones, has become crucial. Biofuels have gained attention as sources of energy with a different origin, this is, not coming from the conventional fossil ones but from sources that can be reused not potentially causing environmental damage.

This section will cover some aspects related to biofuels and their importance, production, and use in developed and developing countries and at the same mentioning the influence their biomasses have on the environment.

## 4.1 Biofuels in Developed Countries

Developed countries have devoted budget to research of bioenergies, in general, and in some cases, they are already using biofuels but not to the same degree as the ones derived from fossils. It has to bear in mind that in countries such as Germany even though there are not wide pieces of land for crops or high sunlight irradiance, efforts have been made to implement bioenergies with good results. Regarding biofuels, efforts are mainly focused on biodiesel and bioethanol.

There are several reports in which it is stated that the most important countries that produce biofuels are the United States, Brazil, Germany, Argentina, and China (Rouhany and Montgomery 2019).

The United States statistics on biofuel production accounts for 44% of worldwide production, particularly aimed at biodiesel production from feedstock such as soybean. Figure 4 is an example of the application of soybean-based biodiesel used to power a bus (courtesy of the Nebraska Soybean Board).

Germany is the leading country in biofuel production in the EU, exporting its bioethanol production mainly to countries such as The Netherlands, France, Poland, and Austria. Also, it generated 3.2 million tonnes of biodiesel in 2018. Rapeseed and used cooking oil are the starting materials that are predominantly worked with in the production of this fuel (Achinas et al. 2019).

Regarding China, its bioethanol production increased from 2009 to 2019. It is considered the world's fourth-largest fuel ethanol producer and consumer right after the United States, Brazil, and the European Union, reporting production of 68,000 barrels/day in 2018 (Fang et al. 2019).

It is important to make reference to the fact that it has been considered that developing countries such as the ones located in Latin America contribute to biofuels



**Fig. 4** Soybean-based biodiesel used as a fuel in buses. Image credit: Nebraska Soybean Board



production by providing their extensive lands to get the necessary biomasses the developed countries need in order to equilibrate the exhaustion of their own land, even though when just very few countries actually contribute to biofuels production (Zuin et al. 2020), such topic will be covered in the following section.

## ***4.2 Biofuels in Developing Countries***

Developing countries are in a privileged position when it comes to renewable energies, this is because they are usually located in areas where natural renewable resources are abundant, especially talking about sunlight (Country classification, 2014). However, a serious inconvenience is the lack of the right policies that permit their implementation along with other factors such as corruption, the insistence on fossil fuels production and usage as well as the prejudice on the expenses/benefits related to renewable energies employment (Arndt et al. 2019). Despite this, efforts have been made since a long time ago to overcome these obstacles.

Brazil is the leader of developing countries when it comes to biofuel production, which consists of a 28% share of the worldwide one. Sugarcane is used for producing fuel ethanol whereas soybean is used for the production of biodiesel (Salina et al. 2020).

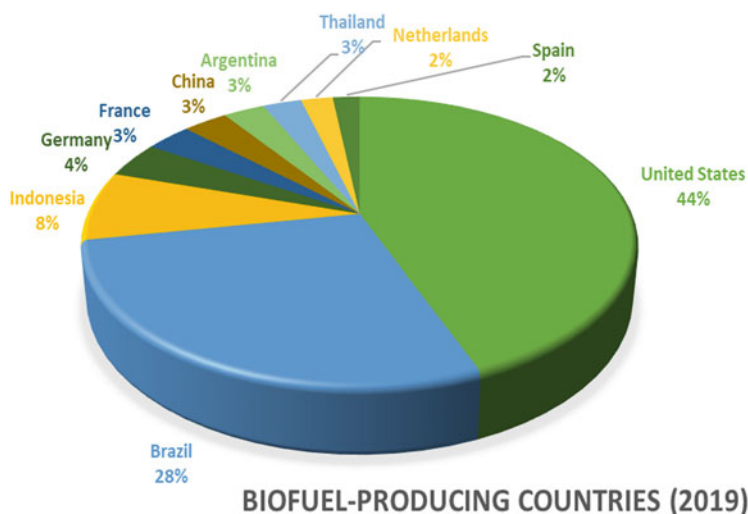
Nowadays, Argentina runs 19 bioethanol plants with a production capacity of 1.4 billion liters as of 2018, 13 of them use sugarcane as feedstock and the residual 6 opt for corn. It is reported that 70.6 thousand barrels/day of biofuels were produced in 2018, in this way representing 3% of the total production worldwide (Castelao Caruana 2019).

Many developing countries would be able to find funding from international financial institutions as well as regional and subregional development banks. Additionally, other research and development associates are also eager to participate in feasible projects. However, an essential first step will be government actions to devise an advantageous and promising environment for domestic, as well as international shareholders. Initiatives must build partnerships between the public and private sectors (Janssen and Rutz 2011).

Figure 5 shows a summary of the tendencies of biofuel generation (BP Statistical Report of Global Energy, 2020).

## **5 Conclusions**

It can be concluded that even though bioenergies, especially with respect to biofuels are still partially under development and with limited implementation, there is a huge potential for them to be applied at 100% in the near future. In the case of biofuels, overcoming obstacles such as the food crop-based biofuels production competition, biofuels upgrading, policy-making, and the like is a task that has been dealt with



**Fig. 5** Percentual statistics worldwide on biofuel production in 2019

since some years ago by experts in different areas. In any case, the fact that the raw materials to produce fossil-based fuels will be depleted over time, a gradual and smooth incorporation of biofuels is the most suitable solution to surmount this issue related to nonrenewable resources. Up to now, this has been accomplished by using biofuel–fossil fuels blends, even in some other cases biofuels are used directly in a successful manner but in very limited situations and circumstances as a result of the involved drawbacks like storage and the need to modify engines. The inclusion of biotechnology to produce biofuels besides the physical and thermochemical existing methods provides an alternative to expand the ways in which they are produced but with a greener approach, although this happens for now in laboratory settings and with the difficulty to scale-up such processes and the costs that are involved. Strictly speaking, biotechnology is used partially in third-generation biofuels and entirely in the fourth generation. Although the mentioned biotechnology-based methodology (involving genetically modified or engineered microorganisms) is new and still needing of improvement, in comparison with the already existing methodologies, it represents a big step in devising and developing an environment friendly approach to produce biofuels.

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# Biotechnological Formation of Biogas



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**Abstract** Biogas is an extensively studied biofuel, it is composed of a combination of gases, primarily of methane ( $\text{CH}_4$ ) and the rest of carbon dioxide ( $\text{CO}_2$ ) with minor impurities of nitrogen ( $\text{N}_2$ ), hydrogen ( $\text{H}_2$ ), ammonia ( $\text{NH}_3$ ), and hydrogen sulfide ( $\text{H}_2\text{S}$ ). It is similar to natural gas in terms of its composition, the main difference lies in its origin, natural gas is a fossil fuel while biogas is a biofuel. In this sense, biotechnology is widely used in the generation of biogas (also called biomethane), which is obtained by the anaerobic digestion of organic waste. The biogas yield process involves the use of organic waste to generate renewable energy. In addition to  $\text{CH}_4$ , all the other gasses present in biogas are contemplated as biogas impurities. There are several well-established physical–chemical technologies on the market related to “biogas cleaning,” involving processes of membrane separation adsorption and absorption. Biogas has been applied for heat production by direct combustion, heat-power, fuel for vehicles, injection to gas pipelines, and electricity production. This chapter will address in detail the several biological techniques of obtaining biogas, main feedstock characteristics, type of bioreactors used to obtain biogas, some techniques of cleaning biogas, as well as the applications of the biofuel obtained as an alternative to replace conventional sources.

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## 1 Introduction

The excessive use of fossil fuels has caused considerable damage to the environment and the health of living beings (Alavi-Borazjani et al. 2020; Yadav and Vivekanand 2021), due to the generation of greenhouse gases (GHG) from their combustion. Furthermore, overexploitation has caused their decline and it is estimated that fossil resources will only last for 60 years or less (Hidalgo and Martín-Marroquín 2020). Owing to this problem, renewable energy sources, where biomass is included, have taken strength. Biomass classifies waste materials derived from plants, animals, and generated by humans (Manyi-Loh et al. 2013; Zhu et al. 2019). Various biofuels are obtained from biomass, like biogas as an outstanding example. Biogas is a flammable gas very similar to natural gas, it is made up of a mixture of gases in concentrations that differ from each other depending on the feedstock used to obtain them (Arutyunov et al. 2021; Hussein 2015). The main gases that compose it are: methane ( $\text{CH}_4$ ) in 50–87%, carbon dioxide ( $\text{CO}_2$ ) in 13–50%, and minor impurities of nitrogen ( $\text{N}_2$ ), hydrogen ( $\text{H}_2$ ), ammonia ( $\text{NH}_3$ ), and hydrogen sulfide ( $\text{H}_2\text{S}$ ) (Aziz et al. 2020; Achinas and Willem Euverink 2020; Fu et al. 2021). Biogas is obtained by a biotechnological process well-known anaerobic digestion (AD), which involves decomposition of biomass in the presence of microorganisms and under oxygen-free conditions (Kainthola et al. 2019; Khalil et al. 2019; Zheng et al. 2014) it is considered that AD is a sustainable biotechnology-based route (Singh and Harvey 2010). The feedstock (biomass) used to obtain biogas is diverse: agricultural waste, manure, food waste, wastewater, among others (Lemma et al. 2021). On the other hand, this biofuel can be used as an alternative to natural gas for heating, cooking food, transportation, etc. It is considered a low-cost renewable energy source, since it is obtained from biomass (Esteves et al. 2019). Biomethane (analog of methane) can be obtained from biogas, but the biogas obtained in the first stage can be used as natural gas due to its similar characteristics (Arutyunov et al. 2021). Also, biogas can be subjected to a purification process to obtain biomethane. This chapter will address the biogas production process and its conversion to biomethane, its advantages and disadvantages, as well as its applications and its importance as a renewable energy source.

## 2 Feedstock for Obtaining Biogas

According to the feedstock used, the obtaining of biofuels has been sorted out by generations: first, second, and third (Hashemi et al. 2021). The first generation classifies agricultural feedstock (oilseeds, sugar, corn, etc.) (M. Song et al. 2015), but have the disadvantage of compromising food safety since the feedstock used can also be for human consumption. The second generation categorizes lignocellulosic biomass, as agricultural wastes (pastures, forest residues, wood, etc.) (Dong et al.

**Table 1** Variety of feedstock used in the production of biogas

Feedstock	Biogas yield (L/Kg)	CH <sub>4</sub> contained in biogas (%)	References
Agricultural waste	161	53	(Rafiee et al. 2021)
Food waste	160	62	(Bharathiraja et al. 2016)
Animal manure	40	60	(Bharathiraja et al. 2016)
Wastewater	380	65	(WBA 2019)
Microalgae	~ 417	60	(Zabed et al. 2020)

2020; Kapoor et al. 2020). The third generation involves microalgae biomass (González-González et al. 2018), in second and third generations, the food security is not affected. Biogas can be obtained from different types of biomasses such as agricultural waste (Aziz et al. 2019), food waste (Pramanik et al. 2019), animal manure (Esteves et al. 2019), wastewater (WBA 2019), industrial waste (Bhuvaneshwari et al. 2019), and microalgae (Thompson et al. 2019; Vij et al. 2021) resulting in optimal efficiency of biogas extraction due to the amount of nutrients that these feedstock present (M. Song et al. 2015). Each of the feedstock mentioned and their performance in the production of biogas are described below and shown in Table 1.

## 2.1 Agricultural Waste

Agriculture is an important part of the economy in developing countries. It is estimated that the yearly generation of waste from agricultural production is about 1680 million of tons (t) and a fraction of it is used in the production of biogas (Bharathiraja et al. 2016). Agricultural waste, for example, from sugar cane, rice, wheat, barley, oats, and corn, also known as lignocellulosic biomass, can be mentioned. The use of agricultural residues in the AD process would have the potential to generate 4000 terawatt hours (TWh) of biogas that can cover 6.5% of the energy demand of the world population. The use of these residues could mitigate 850–1100 t of CO<sub>2</sub> equivalent (CO<sub>2</sub> eq.) per year (Thiruselvi et al. 2021). As mentioned above, agricultural waste is part of lignocellulosic biomass, the composition of this biomass type involving of three main constituents: lignin, cellulose, and hemicellulose which often biodegradability reduce, which results in limited biogas yield (Dar et al. 2021). However, agricultural waste is a rich source of organic solid waste, which has good potential for renewable energy generation (C. Song et al. 2020). The composition of agricultural waste varies significantly depending on the type of crop; has a low moisture content, a high content of volatile solids (VS), and variable amounts of easily fermentable components (Bharathiraja et al. 2016). The characteristics mentioned above are found in agricultural waste of rice, corn, wheat,

among others. Some countries use cereals and canola (rapeseed) as feedstock for the generation of biogas. Among cereals wheat straw strands, represent the most generated agricultural waste worldwide. In general, the composition of agricultural waste creates a degradation deficit due to the lack of nutrients for the microorganisms and the unlimited buffer capacity for chemical reactions (Bharathiraja et al. 2016). This problem is solved with a codigestion process, generally with animal waste, which turns out to be a quality feedstock for the generation of biogas, due to its high nitrogen content that reduces the proportion of Carbon/Nitrogen (C/N) of agricultural residues and stabilizes the pH of the solution by the production of ammonia. Chemical, biological, mechanical, or thermal pretreatments are alternative to increase decomposition of lignocellulosic material (Bharathiraja et al. 2016; Kainthola et al. 2019; Yu et al. 2019).

## 2.2 Food Waste

Food waste includes food that is thrown away, not eaten, or wasted during stages of production, processing, transportation, and consumption of food feedstock (Pan et al. 2020). Food waste involves the loss of various resources closely related to food production (energy, land, and water) and not just the loss of food itself. In addition, this overexploitation of resources contributes to the generation of greenhouse gases, the cause of global warming. Therefore, the proper processing of food waste through AD mitigates the spread of diseases and odors, resulting in a healthy and hygienic way of cleaning (Pramanik et al. 2019). According to data from the Food and Agriculture Organization of the United Nations (FAO) in its report about The State of Food and Agriculture (FAO) (FAO 2019), 13.8% of the total food made in the world is lost between the agricultural exploitation and the retail trade. In the United States, 30% of food waste is generated each year. In Africa, 25% of what is harvested becomes waste. And in China, large amounts of food are wasted every year, such as 21 t vegetables, 23 t cereals, and 12 t fruits (Thiruselvi et al. 2021).

The generation of food waste depends on the different eating habits of the population in different countries. In addition, it varies in the components, which generally include rice, vegetables, meat, noodles, eggs, among others. Therefore, the composition of food waste is a heterogeneous mixture of carbohydrates, lipids, and proteins, representing a total solids (TS) value of 12.3–30.9%, VS of 11.6–26.3%, and rich in water about 70–80%. Due to the aforementioned, it is estimated that the composition of food waste causes a deficit of biodegradation in the AD process and consequently the generation of biogas is relatively stable. Another important point to consider in the AD process is that the pH of the food waste is low, which requires adequate monitoring to be successful in the generation of biogas (Zhang et al. 2019).



## 2.3 *Animal Manure*

Manure is the name by which animal excrement is known. Sometimes, manure is made up of more than one type of organic waste, such as animal excrement and litter remains, as is the case of straw. Animal manure is high in carbon, nitrogen, phosphorus, and potassium. This composition is varied depending on the type of animal, diet, and intestinal flora. Cow manure is normally composed of sugars, proteins, oils, and fats, for example. For this reason, optimal conditions are needed in the AD process, depending on the type of animal manure that is being worked, in order to optimize the production of biogas (Esteves et al. 2019; Zhang et al. 2019). Data from the World Biogas Association (WBA) (WBA 2019) in 2019 mention that with the manure of 22 billion chickens, 1.5 billion cows, 1 billion pigs, and 0.2 billion buffalo, processed by AD, 2600–3800 TWh of electricity can be produced. This would be enough to meet the energy demand of 0.33–0.49 billion people. And the purification of biogas to methane could meet the demand for natural gas in India and China. An encouraging fact is that the energy generated 2400 TWh from treated animal waste would supply 100% of the energy that is necessary for world agriculture (Thiruselvi et al. 2021). In general terms, animal manure is an excellent feedstock for AD, due to the moisture content that ranges between 75 and 92% and the VS that range between 72 and 93% of the TS present (Bharathiraja et al. 2016). In addition, animal manure works as a pH buffer in the digestion process, due to the ammonia generated. Another of its advantages is that, due to the diverse microbial flora existing in animal manure, the digester does not have to be inoculated, but just mixed with hot water. A possible disadvantage of this feedstock is the slow digestion, attributed to the hardly degradable organic matter; therefore, prolonged time can cause poisoning of methanogens by high concentrations of ammonia generated. To overcome this disadvantage, the codigestion process is a good alternative, using feedstock rich in carbohydrates and free or low in nitrogen (Li et al. 2021; Ma et al. 2020).

## 2.4 *Wastewater*

The WBA reports that 61% of the world population lives without adequate sanitation and it states that if the world wastewater was collected and treated using AD, 210–300 TWh of energy could be generated enough to provide 27–38 million people around the world. In addition, 75–100 t of CO<sub>2</sub> eq. of greenhouse gases per year would be mitigated (WBA 2019). Another important datum is that the sludge generated in the AD process can be used as fertilizer due to its high content of micro and macro minerals for plants. And as the main components of wastewater are proteins, fats, and carbohydrates, this organic matter provides nutrients to microorganisms during AD for the generation of biogas (Elalami et al. 2019; Elango et al. 2007).

## 2.5 Industrial Waste

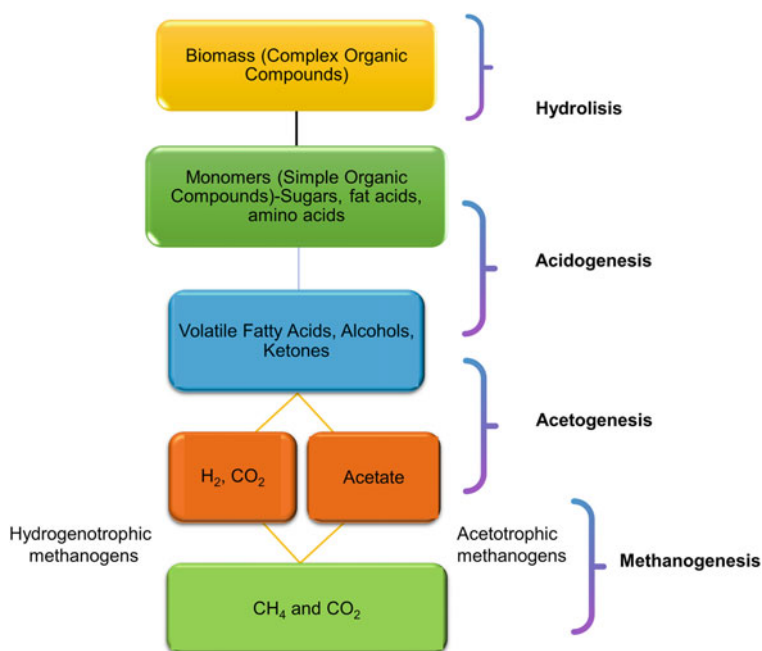
There is a wide range of industrial sectors that can contribute with their waste for the generation of biogas, for example, the dairy, distillery, cornstarch, and paper industries, as well as slaughterhouses, among others. The main characteristic is that they process waste in solid and liquid state and hence chemical waste generated by the various industries control the amount of biogas produced. To guarantee adequate AD, the feedstock must be free of trash and pathogens (Thiruselvi et al. 2021). In addition to thus guaranteeing the generation of biogas, quality organic fertilizers are obtained (Ravindran et al. 2021). The treatment of various industrial effluents by anaerobic digestion is carried out with the main objective of reducing the organic load and producing biogas (Gunes et al. 2019; Ravindran et al. 2021).

## 2.6 Microalgae

Microalgae are a diverse microorganisms group, most are unicellular and some are multicellular, such as prokaryotic algae (cyanobacteria) and eukaryotic algae (*Chlorophyta*) respectively, to mention some are red algae (*Rhodophyta*), and diatoms (*Bacillariophyta*) (Zabed et al. 2020). Microalgae have shown potential to produce biogas and have the advantage of being able to grow in wastewater throughout the year without the need to use arable land, or compromise food security. Furthermore, the strains can also be cultivated in fresh, brackish, and sea water (González-González et al. 2018). This group of microorganisms could better absorb nutrients and CO<sub>2</sub> than terrestrial plants, due to their growth rate is higher than other crops (Montingelli et al. 2015; Vij et al. 2021). The performance of microalgae in the production of biogas is higher than other feedstock, see Table 1, attributed to the fact that microalgae do not have lignin (or they have it in small quantities), which increases their biodegradability compared to lignocellulosic biomass (Hussain et al. 2021). In addition, microalgae in their degradation process release less H<sub>2</sub>S than other feedstock, due to their low amount of sulfur amino acids, and the effluent generated as waste after the production of biogas can be used as a nutrient for the microalgae themselves (González-González et al. 2018). H<sub>2</sub>S is corrosive and can damage biogas storage facilities, pipelines, and compressors. Also, their combustion produces sulfur dioxide (SO<sub>2</sub>), which is one of the main air pollutants (Nguyen et al. 2021). Although the production of biogas from microalgae has been investigated for more than 60 years, to date there are still some obstacles such as the C/N ratio, low biomass yield, and obstruction by volatile fatty acids accumulation in digester reactor; the success of the process depends on the correct selection of the microalgae strains to be used (González-González et al. 2018; Zabed et al. 2020).

### 3 Biological Process from Biogas Production

As mentioned previously, the production of biogas happens through an AD. The feedstock is transformed into biogas (rich in methane) as the main product and substances that are used as organic fertilizers as a by-product. It is a viable technology that enables energy production without compromising the environment. To start the AD process, the feedstock for digester goes through a pretreatment, depending on the feedstock to be used, the treatments are classified into chemical, physical, thermal, and biological methods (Gunes et al. 2019; Pramanik et al. 2019; Qyyum et al. 2020). Furthermore, it is essential to ensure conditions that favor the process and development of the microorganisms involved; optimal conditions such as temperature, pH, C/N ratio, an adequate load of the biodigester, mixing feedstock, hydraulic retention time (HRT), and organic loading rate (OLR) (Elalami et al. 2019; Kainthola et al. 2019). The AD process happens in a temperature range of 10–55 °C. Depending on the temperature range, the process can be classified as psychrophilic digestion under conditions 10–25 °C, mesophilic digestion under conditions 32–38 °C and thermophilic digestion under conditions 52–55 °C (Stolecka and Rusin 2021); and the proper choice of feedstock allows microorganisms (bacteria) to work correctly, to generate biogas (Alavi-Borazjani et al. 2020). Another important point is that, four fundamental stages stand out in the AD process (see Fig. 1): Hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Shah et al. 2021). It is



**Fig. 1** Stages of anaerobic digestion for biogas production

**Table 2** Chemical reactions in the formation of biogas

Biological processes	Chemical reaction
Hydrolysis	$C_6H_{10}O_5 + H_2O \rightarrow C_6H_{12}O_6$
Acidogenesis- acetogenesis	$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$ $CH_3CH_2OH + H_2O \rightarrow CH_3COOH + 2H_2$
Methanogenesis	$CH_3COOH \rightarrow CH_4 + CO_2$ (Acetotrophic) $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$ (hydrogenotrophic)

worth mentioning that each of the four stages of AD has its own microflora. The general chemical reactions are shown in Table 2.

### 3.1 Hydrolysis

Hydrolysis is the first stage in the anaerobic process, it is here where the breaking of chemical bonds occurs in the presence of water, obtaining intermediate products (Elalami et al. 2019). The organic matter is decomposed by the action of hydrolytic bacteria, for instance, *Cellulomonas clostridium*, *Bacillus thermomonospora*, *Ruminococcus bacteriodes*, *Erwinia*, *Acetivibrio microbispota*, and *Streptomyces* (Jain et al. 2015). Bacteria hydrolyze water-soluble macromolecules, including carbohydrates, proteins, and lipids, transforming them into monomers, such as sugars residues, fatty acids, and amino acids (Stolecka and Rusin 2021; Tabatabaei et al. 2020). Organic compounds are solubilized by enzymes excreted by hydrolytic bacteria that act outside the cell, which is why they are considered exoenzymes (Elalami et al. 2019). In this stage, the speed of the reaction is determined, it is the limiting step for difficult-to-digest biomass, like lignocellulose and waste rich in keratin (Patinvoh et al. 2017a, 2017b; Zheng et al. 2014). In general the pH at this stage is between 5.3 to 6.7, depending on the type of feedstock (percentage of lignin, carbohydrates, proteins, and fats) and the retention time the pH can drop to 4.5. Also, hydrolysis depends on temperature, hydraulic retention time, particle size, and the  $NH_4^+$  concentration (Adekunle and Okolie 2015).

### 3.2 Acidogenesis

The second stage of AD is acidogenesis, also called the fermentation stage. In this stage, *Salmonella*, *Streptococcus*, *Escherichia*, *Lactobacillus*, and *Bacillus* (Tabatabaei et al. 2020) are considered acidogenic bacteria they act by degrading the soluble compounds from hydrolysis, transforming them into volatile fatty acids with shorter chains (acetic acid and in smaller amounts butyric, propionic, and valeric acid), alcohols, some nitrogen and sulfur compounds, as well as hydrogen and carbon dioxide. In this stage, the pH can descend drastically due to the

accumulation of volatile fatty acids, because of the low buffering capacity of the feedstock and the high organic load rate. If this occurs, the methanogens will be inhibited, affecting the production of methane (Alavi-Borazjani et al. 2020; Khalil et al. 2019; Patinvoh et al. 2017b; Tabatabaei et al. 2020; Tyagi et al. 2021).

### 3.3 Acetogenesis

The third stage of AD is acetogenesis or also called dehydrogenation stage. Homoacetogenic bacteria (*Acetobacterium woodii* and *Clostridium acetivum*) are responsible decomposition of volatile fatty acids (VFAs) formed in acidogenesis into compounds of acetic acid, carbon dioxide, and hydrogen. The hydrogen formed in this stage, prevents the metabolism of acetogenic bacteria that is processed by methanogens microorganisms to converted into methane (Dar et al. 2021; Liew et al. 2020; Rajendran et al. 2020; Tyagi et al. 2021).

### 3.4 Methanogenesis

The fourth and last stage of AD is methanogenesis, here the transformation of hydrogen and acetic acid into methane and carbon dioxide is carried out with methanogenic archaeobacteries (Conrad 2020; Vij et al. 2021; Zamri et al. 2021). At this stage, two groups of bacteria are classified: hydrogenotrophic methanogens and acetotrophic methanogens. *Methanobacterium bryantii* and *Methanobrevibacter arboriphilus* are some examples of hydrogenotrophic methanogenic bacteria, responsible to converted hydrogen and CO<sub>2</sub> into methane, and acetotrophic methanogens (*Methanosaeta concilii* or *Methanosarcina acetivorans*) converted acetic acid into methane (Cazier et al. 2015; Fu et al. 2021). The acetogenic and methanogenic stages occur symbiotically between the microorganisms of both stages. The methane produced in this stage originated in a high percentage (70% approx.). By the decarboxylation of acetic acid and the remaining methane is formed from the reduction of CO<sub>2</sub> and the conversion of H<sub>2</sub>. The remaining acids (formic, propionic, butyric, and valeric) contribute less to the methanogens. Generally, the energy produced is used to power the system and a considerable amount is the final product (methane) for external use (Krzysztof Ziemiński 2012; Liew et al. 2020). As already mentioned, the performance of the biogas production process depends on the balanced relationship between microorganisms, the composition of the feedstock, and its biodegradability (Zamri et al. 2021).

## 4 Type of Biodigesters Used to Obtain Biogas

An anaerobic reactor or anaerobic biodigester is a container where the biomass (feedstock) is placed for their subsequent degradation in the presence of various microorganisms in an oxygen-free environment and where the main product is biogas (He et al. 2019; Khalil et al. 2019; Zabed et al. 2020). The anaerobic reactor is the heart of any biogas-generating plant. It should be noted that the anaerobic reactor must be easy to install, operate, efficient, and profitable in biogas production for the development of biogas technology to be on the rise (Patinvoh et al. 2017a).

Anaerobic reactor must be hermetically closed to prevent water or gas leaks, as well as to avoid the entry of ultraviolet light, chemical products, and so on. The selection of the appropriate reactor is essential to ensure optimal biogas generation. The biomass retention capacity is an important point for choosing the appropriate biodigester, since anaerobic microorganisms in the generation of biogas grow very slowly. Another important point in the selection of the biodigester is that its configuration isolates the hydraulic retention time (HRT) from the solid retention time (SRT). The uncoupling of these two factors contributes to maintaining a significantly high SRT/HRT ratio and favoring the speed of organic loading and the reduction of the size of the biodigester (Loganath and Senophiyah-Mary 2020). Currently, the technologies used in AD are classified into discontinuous or semi-continuous reactors and continuous reactors (Carlos-Pinedo et al. 2019).

### 4.1 *Batch or Semi-Continuous Reactors*

In this case, the reactor is first loaded and sealed with fresh feedstock inside and in the second step, the reaction and production of biogas are carried out without manipulating the feedstock. At the end of the process, the reactor valves only open to load and unload the reactor. The main advantages of these reactors are the simple design and easy control of the process, as well as the time of the process. Resulting in investment costs (e.g. operation, construction, production) in this type of reactors are lower than continuous stirred tank reactors (CSTR). Although it has the disadvantage of irregular biogas production (Carlos-Pinedo et al. 2019; Gunes et al. 2019; Kothari et al. 2014; Pramanik et al. 2019). In this type of reactor, the organic load rate is between 1 and 2 Kg COD/m<sup>3</sup>/day (where COD is chemical oxygen demand). These reactors are generally anaerobic ponds and lagoons and are used more frequently for waste storage (Loganath and Senophiyah-Mary 2020).

## 4.2 Continuous Reactors

It consists essentially of a continuously stirred reactor operating under mesophilic or thermophilic conditions, that keep the high-volume biomass inside the reactor, this type of reactor is the most favorable to produce biofuels. Within this type of reactor, the Continuous Stirred Tank Reactor (CSTR) stands out (Loganath and Senophiyah-Mary 2020). It is the configuration most used in biogas plants, about 90% of modern biogas plants have CSTR (Manyi-Loh et al. 2013). In this design, the reactor is continuously fed with the feedstock, mixed with the substrate, and the biogas production is constant. The organic load rate is between 5 and 30 kg COD/ m<sup>3</sup>/ day or even more and the THS must be maintained between 20 and 60 days. CSTR reactors have the advantage of being low in cost and operation, as well as being easy to use. Although they present some disadvantages such as the high residence time for good performance and the formation of foam (Carlos-Pinedo et al. 2019; Cremonez et al. 2021; O'Connor et al. 2021; Show et al. 2020).

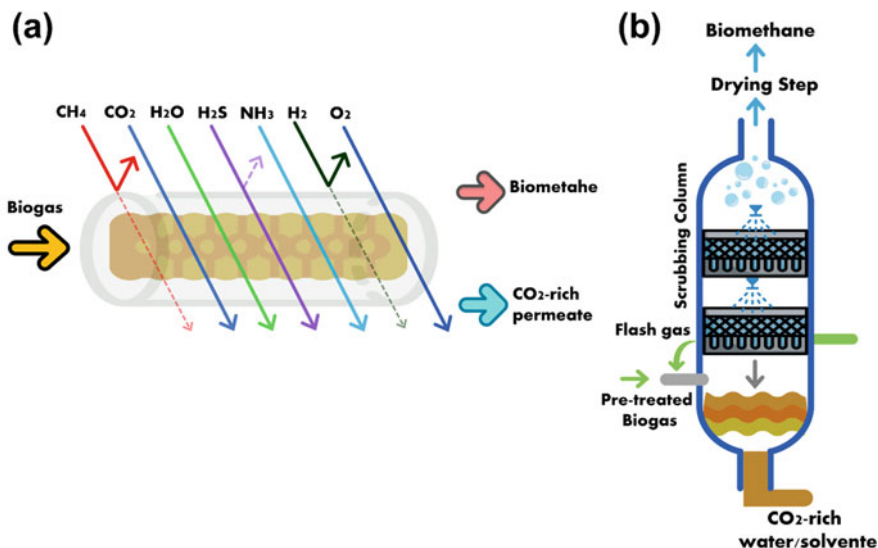
The types of biodigesters and their working technique are diverse, they are not a study object now. The most widely used is the CSTR mentioned above, it is usually connected with a secondary digester to increase the yield of biogas production.

## 5 Biogas Purification Processes to Obtain Biomethane

Currently, adsorption and membrane separation processes related to “biogas cleaning” are several physical–chemical technologies well-established on market to improve biogas (Angelidaki et al. 2018).

### 5.1 Membrane Separation Technology (MS)

It is a conventional technique based on absorption for the improvement of biogas. Fundamentally, its technological principle lies in the capacity of the membranes to carry out a selective permeability of the biogas separation in its components (Angelidaki et al. 2018). The membranes can be made of polysulfone, polyimide, or polydimethylsiloxane, which are integrated into a cylinder fabricated of stainless steel, and they are shaped of hollow fibers (Ardolino et al. 2021; Chen et al. 2015). The selectivity of the materials mentioned above is strong for the separation/transformation of CO<sub>2</sub> to CH<sub>4</sub>; the permeability mechanism is based on a flow that receives the name “permeate.” This flow is composed of CO<sub>2</sub>, H<sub>2</sub>O, NH<sub>3</sub>, and others that can penetrate the micropores of the membrane, while the gas rich in CH<sub>4</sub>, called “retained” is capable of crossing the membrane without changing its composition, as shown in Fig. 2a (Angelidaki et al. 2018).



**Fig. 2** Main physical-chemical technologies for cleaning biogas (a) Membrane Separation technology (MS) and (b) Water Scrubbing technology (WS)

## 5.2 Water Scrubbing Technology (WS)

The process is based on solubilizing  $\text{CO}_2$  in water according to Henry's law, which mentions that the solubility of methane in water at 25 °C is 26 times lower than  $\text{CO}_2$  (Ardolino et al. 2021). In a traditional configuration, the hydrogen sulfide is removed first from raw biogas by a treatment in a scrubber to form desulfurized biogas, this is brought to compression between 4 and 6.5 bar and is introduced on a washing column from the bottom, where from the top water is injected. The water absorbs the  $\text{CO}_2$  at the same time the  $\text{CH}_4$  leaves the column at the top through a drying and refining step through a filter composed of activated carbon. The scrubber with a rich amount of  $\text{CO}_2$  contains around 5–6% of methane in the compressed biogas while the saturated water comes out. After, the water is carried to a flash column that allows the separation and recirculation of methane at pressures of up to 2–4 bar; then, the water flow is then sent to an extraction column, where the  $\text{CO}_2$  is removed and can be reused in the process, as shown in Fig. 2b (Angelidaki et al. 2018; Ardolino et al. 2021).

## 5.3 Chemical Absorption Technology (CA)

The operating principle consists of the absorption of  $\text{CO}_2$  in respect of water through the use of amine solvents as selective scrubbers, which eliminate a greater amount of



CO<sub>2</sub> per volume unit (Patterson et al. 2011). Some commonly used organic amines are monoethanolamine (MEA), diethanolamine (DEA), methyldiethanolamine (MDEA), and diglycolamine (DGA) (Leonzio 2016). In a study configuration, raw biogas has a desulfurization process (same as MS and WS) and it is added to an amine solvent absorber (MEA), which absorbs CO<sub>2</sub> from the biogas. The exothermic process of the reaction elevates the temperature of the amine solvent which causes the biomethane come out from the absorber at the top entering a refining stage, followed by the last compression. First, the CO<sub>2</sub>-rich amine solvent is heated by a heat exchanger, then, it is fed to a separation column which ensures the heat necessary for the separation reaction by means of a provided reboiler. The obtained CO<sub>2</sub>-free amine solvent is returned to the heat exchanger (Ardolino et al. 2021). Traces of amine vapor and solvent are separated from the exhaust gas and then recirculated in the extraction column at the same time as the remaining part is liberated into the atmosphere. This remaining part is mainly composed of CO<sub>2</sub>.

#### **5.4 Pressure Swing Adsorption Technology (PSA)**

This technique utilizes a porous adsorbent medium that has the property to adsorb molecules from a mixture of gases and release them by the application of various pressure values (SGC 2013). The difference in molecular dimensions between CO<sub>2</sub> and CH<sub>4</sub> allows the retention of CO<sub>2</sub> through the use of a porous material that works as an adsorbent that has cavities of 0.37 nm, allowing CH<sub>4</sub> to flow without holding it (Patterson et al. 2011).

A case study configuration involves an activated carbon pretreatment stage to remove H<sub>2</sub>S with a drying stage to eliminate the water followed by a pressure increase to 4 bar into the PSA unit, which has four columns placed in series filled with a material that functions as an adsorbent, usually zeolite.

Different stages that compose the process are carried out in each column: initially, in a column in which the CO<sub>2</sub> is adsorbed, the compressed biogas is introduced, while the CH<sub>4</sub> penetrates without being adsorbed by the adsorbent material; a pressure drop allows CO<sub>2</sub> desorption in the second column; In the third column, residual CO<sub>2</sub> adsorbed material is cleaned by using biomethane by means of injection and is pressurized again in the fourth column. To remove Volatile Organic Compounds (VOCs) from biomethane, another activated carbon filter is used to later be compressed at 24 bar (Ardolino et al. 2021).

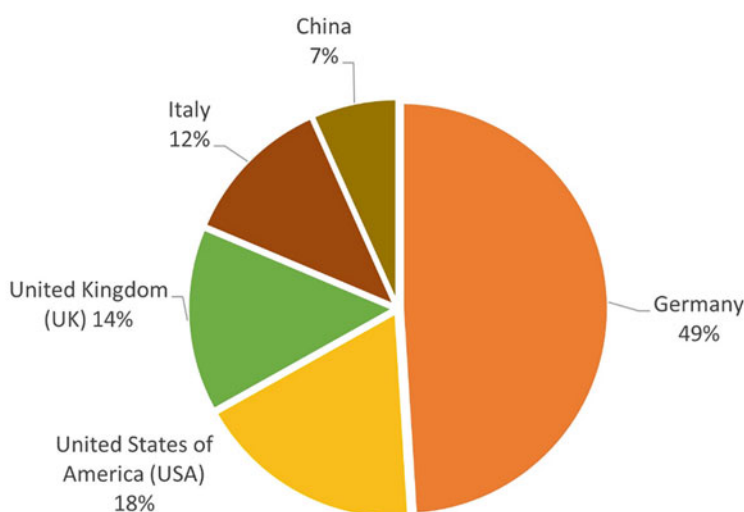
## 6 Applications and Importance of Biogas and Biomethane as a Renewable Energy Source

As already mentioned above, anaerobic digestion is a biochemical process that consists of degradation of organic matter in presence of a microorganisms group, in an oxygen-free environment, obtaining methane-rich biogas as the final product. The biogas obtained has a high calorific value that ranges from 17 to 25 MJ/m<sup>3</sup> and, can convert into heat when burned and release thermal energy or become electricity through internal combustion engines. In the biogas generation process, it is estimated that less than 25% of production may be employed for the operation of the plant of AD and the excess can be commercialized (Zhang et al. 2019).

According to the 2022 report from The International Renewable Energy Agency (IRENA), 96,565 GWh of biogas was produced worldwide in 2020 and the five countries where it is produced in the greatest quantity are: Germany with 49%, the United States of America (USA) with 18%, followed by the United Kingdom (UK) with 14%, Italy with 12%, and China with 7%, and values of 33,495, 12,218, 9907, 8166 and 4574 GWh, respectively, as shown in Fig. 3 (IRENA 2022).

It is worth mentioning that according to the report, Europe was the continent where the greatest amount of biogas was produced, of 96,565 GWh produced worldwide, Europe produces the amount of 67,000 GWh (IRENA 2022).

As already mentioned above, methane is the main component of biogas and its uses are diverse, highlighting the applications of low, medium, and high-quality gas. Biogas can be used for heat production by direct combustion, heat-power, fuel for vehicles, injection to gas pipelines, and electricity production; each application is described below.



**Fig. 3** Main producers of biogas

## 6.1 Heat Production

In the case of *heat production* by direct combustion, biogas can be burned in boilers or stoves. On a small scale, biogas produced is used in cooking food (Rajendran et al. 2012; Sun et al. 2015). Regularly, the amount of biogas used for cooking food ranges between 30 and 45 m<sup>3</sup> per month; a great quantity, compared to liquefied petroleum gas (LPG) whose consumption is between 11 and 15 Kg per month (Rajendran et al. 2012). Also, the heat that is generated can be used to heat spaces, heat processes, cook food, among others. It should be noted that the calorific value of the biogas used is low (Kadam and Panwar 2017; Rafiee et al. 2021).

## 6.2 Heat/Power

Diesel engines and Otto engines, as well as gas turbines can work with biogas. Biogas can be used in dual fuel engines. In the case of diesel engines, biogas and diesel are used to keep the efficiency of the diesel engine as high as possible. On the other hand, biogas has the potential to generate electricity on demand (Rafiee et al. 2021; Sun et al. 2015). Electricity contribution from renewable energies represents around 26.5% of total electricity in the world, of which only 2.2% comes from biomass (Safieddin Ardebili 2020). This technology is rare in most developing countries, but in industrialized countries like Germany it has become standard (Kadam and Panwar 2017; Rafiee et al. 2021). However, in 2019 the generation of electricity from biogas extended to more countries in the world, stand out Latin America, Middle East, Africa, and India. Some facts are mentioned below: In Maharashtra, India, a novel digester it feeds municipal and agricultural wastes was installed in a biogas plant that has the capacity to generate 4 MW of power. A combustion process was replaced by a electro-chemical process, in solid fuel cells that use the biogas generated to produce electricity (REN21 2020). Also, the first plant that works with pig manure started operations to produce biogas in Brazil's 18 large local piggeries provide the waste to this plant to make work two motor generators producing 240 kW which will power more than 70 buildings in Entre Rios do Oest municipality (REN21 2020). On the other hand, the United States is the largest user and producer of biomethane destined for transportation activities, while in Europe it is also used, where its use increase by 30% as transport fuel between 2015 and 2020. In the United States, biomethane use has increased ten-fold since 2014 (REN21 2021, 2022).

### 6.3 *Vehicle Fuel*

The purified biogas can power light or heavy vehicles such as cars, buses, and trucks. The use of biogas is considered the best way as fuel for vehicles to reduce the consumption of fossil fuels (Rafiee et al. 2021). Currently, more than one million vehicles run on biogas worldwide, demonstrating the ability of large-scale technology (O'Connor et al. 2021). Sweden, for example, uses half of the biogas produced as fuel for transportation, fueling light vehicles, buses, and trucks in quantities of 44,000,750, and 2200, respectively. Another example is Germany, which feeds with biogas to 96,000 light vehicles, 1700 buses, and 200 trucks (Nguyen et al. 2021). It is important to mention that the fuel used must be purified biogas (biomethane), of high quality to avoid damage to the vehicle's engine (O'Connor et al. 2021).

### 6.4 *Direct Injection into the Gas Network*

Recently, the importance of replacing natural gas with biogas has increased significantly due to the principal disadvantages of the first, such as its depletion and low quality. Certain product quality standards must be met, one of the main ones is the purity of  $\text{CH}_4$ , impurities must be eliminated of  $\text{H}_2\text{S}$  and water vapor, to generate the so-called biomethane. Biomethane can be mixed without any problem with natural gas in existing networks and is around 90% efficient in normal burners (Kadam and Panwar 2017; Ullah Khan et al. 2017). To prevent corrosion, Sweden, France, Switzerland, The Netherlands, and Austria have created standards to inject biogas into their natural gas networks. These standards consist of the concentration of methane and impurities allowed in it, in order to be injected into the network. For example, the percentage by volume (% vol) of  $\text{CH}_4 \geq 85$  for the Netherlands,  $\geq 86$  for France,  $\geq 96$  for Switzerland, Germany, and Austria. And impurities such as  $\text{CO}_2$ ,  $\text{O}_2$ ,  $\text{H}_2$ ,  $\text{CO}$ ,  $\text{H}_2\text{S}$ , and others have specific values for each country (Ullah Khan et al. 2017).

## 7 *Conclusions*

Currently, the use of renewable energies is a necessity, biomass allows obtaining various biofuels, as in the case of biogas by its easy obtaining. As mentioned in this chapter, the production of biogas through waste materials is undoubtedly an excellent alternative to a biofuel or energy generator that will have a favorable impact on the environment. Anaerobic digestion (AD) is the process through which biogas is obtained and is composed of four biological processes (hydrolysis, acidogenesis, acetogenesis, and methanogenesis), each process has its consortium of bacteria that favor the production of biogas. It is important to mention that there is a great variety

of biomass that can be processed to obtain biogas, biomass has been classified into generations, second- and third-generation biomass stand out, where the conversion of feedstock to biogas is higher, as well as the percentage methane generated is favored.

For each feedstock, the reaction behavior differs considerably. Finally, the biogas generated could be used directly to generate thermal energy, for applications in food cooking and as heating, another alternative is to subject the biogas to a purification process to obtain biomethane and be able to connect it to the natural gas network in the cities or being able to use it as fuel for cars. Without a doubt, biogas and biomethane are viable alternatives to reduce the greenhouse gases that affect the planet so much.

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# Biotechnology in Bioethanol Generation



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and Claudia Martinez-Alonso

**Abstract** The use of bioethanol from sugarcane and cellulosic corn feedstocks, such as crop residues and wood, has increased considerably. However, this biofuel considered the first generation, compromises the population's food security. For this reason, its production from lignocellulosic biomass, a material rich in carbohydrates, is evaluated with great interest. The process for obtaining bioethanol mainly comprises a pretreatment followed by the hydrolysis of the lignocellulosic structure to get fermentable sugars, fermentation, and finally, the distillation of the fermented product (considered the second generation). Other methods are also considered, such as microbial strains or their co-cultures, photosynthesis, and the fed-batch process. It has also shown a positive energy balance, implying that the process for its production does not require more energy than the one contained in the fuel itself. In this chapter, the biotechnological process for obtaining second- and third -generations ethanol will be described in detail, and its advantages–disadvantages and potential biofuel applications will be analyzed.

**Keywords** Bioethanol · Fermented product · Third generation-ethanol · Biotechnological methods

## 1 Introduction

Ethanol ( $\text{CH}_3\text{-CH}_2\text{-OH}$ ) generated from biotechnological methods is usually called bioethanol because it is produced from biomass. This biofuel is an eco-friendly liquid, and most importantly, it has a variety of uses because its obtaining is based on

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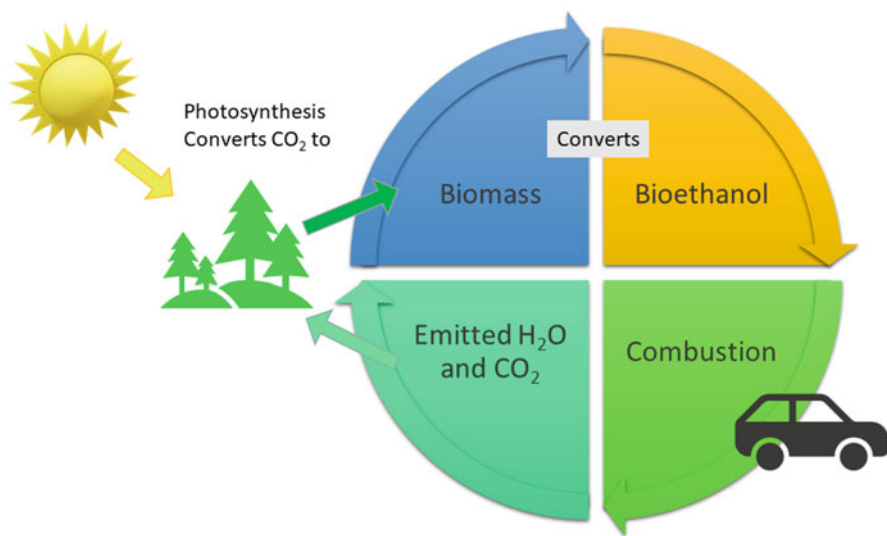
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green processes, it can serve as an alternative to nonrenewable energy (Taherzadeh et al. 2019). Since the 1970s, ethanol has been used as a fuel when Roberto Rodrigues, together with Victor Argolo and Agenor Pavan, developed a project to replace gasoline with sugarcane ethanol (Stolf et al. 2020), following it, in 1975, the Brazilian government decided to release “The National Alcohol Program” (Proálcool), which derived from the crisis of high oil prices, to eliminate all petroleum-derived fuels for automobiles gradually. The administration determined to use sugarcane due to its affordable. Hence, in 1979, many places began to supply only ethanol to vehicles in local subsidiaries. A few months later, the commercial distribution of cars running with E100 increased. So, in the 1980s, this country had more than four million vehicles. Nowadays, most of the vehicles in Brazil have flexible fuel systems, which can run on gas or 100% ethanol. In the beginning, the feedstock for bioethanol production was based on sugarcane, corn, and sugar beet (Nanda et al. 2018; Shokrkar et al. 2018). This is called the first-generation bioethanol; however, these products are not an option for bioethanol production as the human demand for food includes the same products, creating competition between biofuels and food production. Therefore, the need to search for new feedstocks that did not include food arose, leading to the second generation of biofuels, where the feedstock came from nonedible residues or lignocellulose such as agricultural biomass and forestry refuse (Prasad et al. 2019). Recently, a new raw material used for bioethanol production is alga biomass, which is attractive and promising, bringing about the appearance of the third generation of bioethanol (Naresh Kumar et al. 2020). Until today, there are three generations in ethanol production, where the feedstock used for their production sets the difference. Even though bioethanol commercialization predominately relies on the first generation (Hou et al. 2020), in this chapter, we will focus on bioethanol production of the second and third generations due to their sustainable and cost-effective approach essential an alternative as nonpetroleum-based and renewable energy resource. Besides, the topics covered here relate to biological methods because they are considered efficient, environmentally friendly, and cost-effective processes.

## 2 Bioethanol as Fuel

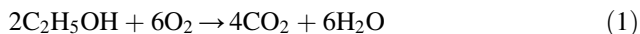
Bioethanol is alternative energy obtained from the fermentation of sugars and the starch components of vegetable by-products. Commercial bioethanol is obtained mainly from sugarcane and crops such as cereals, using yeasts. In some countries, it is also made with corn, potato, milk, rice, beet, grape, and banana. This depends on the agricultural production of each country.

Currently, bioethanol has many uses: the main one is based on making a mixture with gasoline to obtain a genuinely sustainable transport fuel, it is also used in cosmetics and other manufacturing processes, and for use in fireplaces and stoves, it has attracted much attention because it creates a burning and clean flame in our due



**Fig. 1** Bioethanol cycle

to its combustion. The combustion of ethanol, given by reaction of Eq. (1) (Campesi et al. 2012):



results in a clean emission: heat, water, steam, and carbon dioxide. Plants absorb CO<sub>2</sub>, which, as is widely known, with sunlight, plants photosynthesize to help them grow. Therefore, this limitless process of energy creation and combustion produces bioethanol, a carbon-neutral fuel source, as shown in Fig. 1.

## 2.1 Uses

The primary use for bioethanol is as a fuel for vehicles; it can be employed in different proportions: 5–10% for diesel motors; 10–85% for gasoline motors, to make combustion more efficient, and green as possible. In Brazil, the production of vehicles with this fuel is increasing year after year. It is the pioneer country in this biofuel, selling more than 400 thousand cars per year, and there are around 1.5 million of them circulating, of which the most are public transport. On other hand, in the United States, Europe, and Sweden, about 15 thousand of this kind of vehicle-powered by E85 (85% ethanol) (Nicola et al. 2011).

The denomination for the fuel mixture has numbers followed by the initial of the fuel, “E” for ethanol, and the numbers 0–100 (in percent) to indicate the fuel mixture. For example, E10 is 10% anhydrous ethanol and 90% gasoline. The E10 mix is the

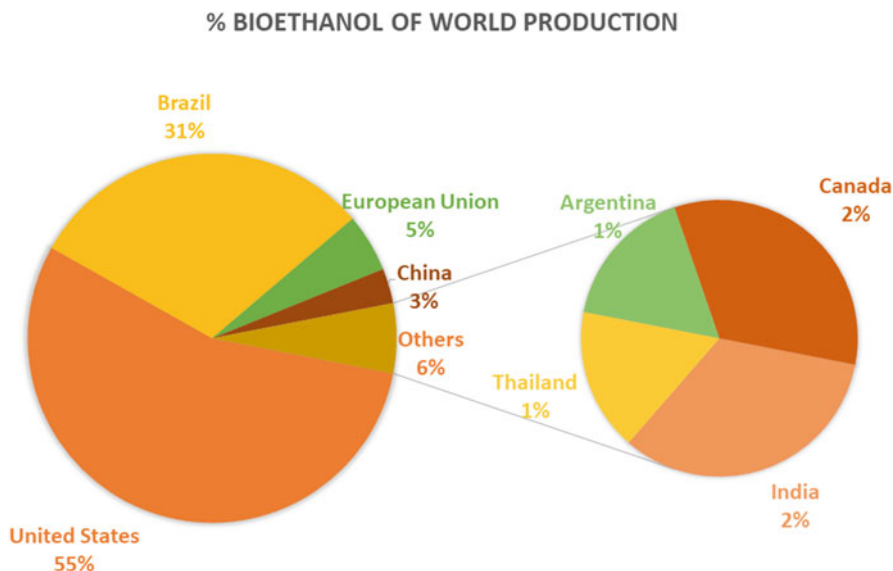
most commonly used worldwide; it is even utilized in more than 20 countries, where the United States is the leader, and ethanol represents 10% of the total reserve of gasoline fuels (Wayback Machine 2010).

Furthermore, Brazil has employed E20–E25 blends since the late 1970s. The United States and Europe commonly use E85 for flex-fuel vehicles. Nevertheless, Hydrated ethanol, or E100, is used in Brazilian pure ethanol and light flex-fuel vehicles, and hydrated E15, called hE15 for current gasoline vehicles in the Netherlands (Rico 2007).

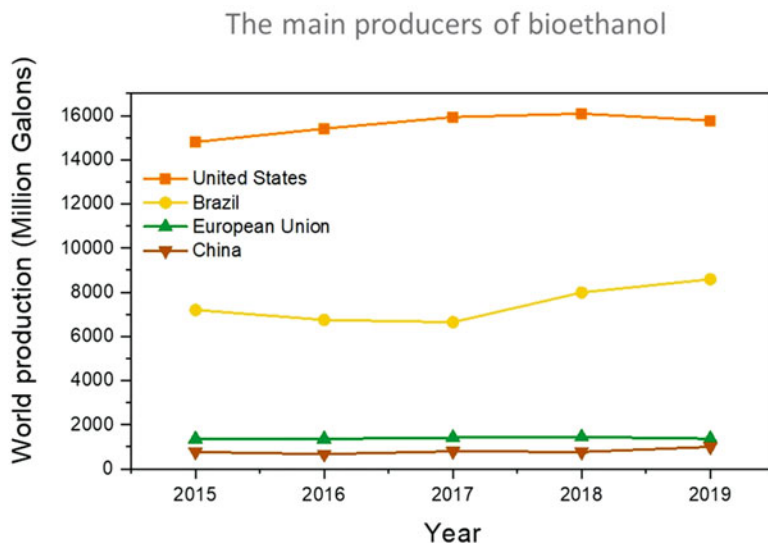
In its beginning, bioethanol has also been used with biodiesel produced from used cooking oils; these were mixed in a volumetric ratio of 20% and 80%, respectively. Cetane builder (di-tert-butyl peroxide) was added to the fuel mixture by volume at 1–2–3%. The mixtures obtained were used as fuel in the diesel engine single cylinder, direct injection, and four-stroke (Örs 2020).

## 2.2 World Bioethanol Fuel Production

It is known that the partial or complete replacement of gasoline with bioethanol is an excellent solution to reduce greenhouse gas (GHG) emissions. Every day, countries around the world are joining the use of bioethanol instead of gasoline; in 2019, the leading producers of bioethanol worldwide were the United States, Brazil, and European Union (EU), it is followed by India, Canada, Thailand, and Argentina with a minimal production (Nikkhah et al. 2020) shown in Fig. 2. However, more



**Fig. 2** % Bioethanol of world production



**Fig. 3** The leading producers of bioethanol in the last 5 years

and more countries are joining the production of biofuels. The highest production is done in the United States, then in Brazil. This last one is where biofuel development started, and the EU occupies the last place.

Bioethanol production has been increasing in the last 5 years, especially in countries with greater demand. In Fig. 3, are shown the four most important, United States, Brazil, European Union, and China, where the U.S. heads again as the country with increasing demand, with a production growth around of 2000 million gallons from 2015 to 2018. However, in 2019, the production decreased by 300 million gallons. A difference with Brazil, the second producer whose 2015–2017 production was kept constant at 7000 million gallons. But from 2017, the production growth reached 9000 million gallons. On the other hand, EU and China have remained under 2000 million gallons. It is worth mentioning that China overcame the production of EU in 2019, and it is probable that in 2020 this production will continue to increase since it is known that China has been betting on renewable energy (Annual Ethanol Production).

It is essential to mention that bioethanol production for the consumer is still from the first generation, in other words, from sugarcane and grain corn, due to the methodology for the production of big-scale bioethanol for the second generation being unsuitable. A third generation has taken importance by the efficiency in production and cost minor. In the following chapter, they will be detailed the biotechnological process involved in bioethanol obtention.

### 3 Ethanol Production from Lignocellulosic Biomass

Bioethanol is obtained from fermentable sugars contained in biomass as complex carbohydrates; these can be transformed into soluble sugars for possible fermentation. Therefore, it is evident that ethanol produced from alimentary sources is easy. However, raw material costs and growing concern over the dispute over food production versus fuel production have received increasing attention (Prasad et al. 2019). From here, the challenge is how this biofuel (bioethanol in this case) is obtained, so a possible solution is to employ renewable resources, such as lignocellulosic biomass (LCB). Bioethanol from LCB is part of second-generation biofuels. It is also obtained from feedstock such as agrarian (straws, sugarcane bagasse, corn stalks, and cobs), forestry (sawdust, bark, branches, etc.), agro-industrial and municipal solid wastes which do not interrupt global food production (Hassan Kamani et al. 2019). Furthermore, these feedstocks have extensive business in producing valuable products due to their large quantities, availability, and low costs (Bilal et al. 2017). The use of LCB can have two main advantages: reducing waste resources and production costs and assuaging the extensive disposal of biomass materials, leasing severe environmental problems, such as decreasing CO<sub>2</sub> emissions (Hou et al. 2020).

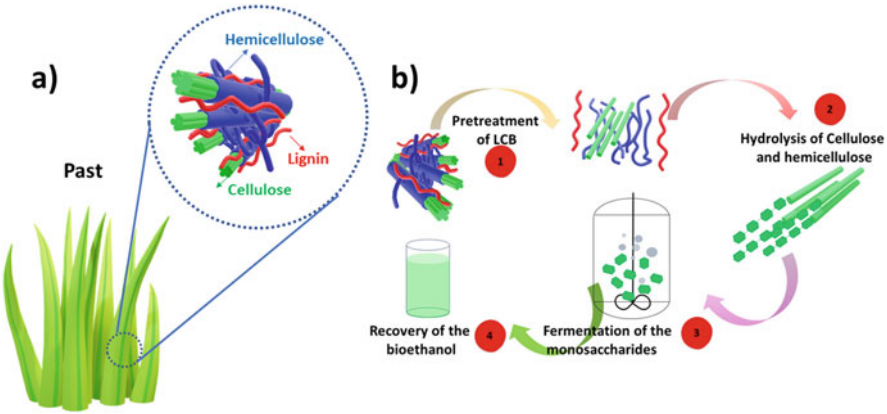
#### 3.1 Composition of Lignocellulosic Biomass (LCB)

To understand the process of conversion of lignocellulose to bioethanol, we will first focus on seeing how LCB is constituted. Lignocellulose biomass comprises polysaccharides (cellulose and hemicelluloses) and an aromatic polymer (lignin) that combine to protect energy-storing sugars and give plants cell wall strength and structure. Cellulose and hemicellulose are strongly connected with lignin via covalent and hydrogen bonds, making a complex lignocellulosic network tough to depolymerize. As a result, bioethanol production from LCB needs a complex modification process, making it industrially challenging to get (Limayem and Ricke 2012). The connection between the three main components in LCB changes drastically according to the biomass's supply and physical properties. Table 1 shows the potential lignocellulosic feedstocks and their composition.

**Cellulose:** It comprises  $\beta$ -D-glucopyranose units connected via  $\beta$ -(1,4) glycosidic bonds and uses cellobiose as a repeating unit. Besides, it represents 40–60% of the weight (Sharma et al. 2019). These create long chains called elemental fibrils (bars green in Fig. 4a), linked to each other by hydrogen bonds and van der Waals forces. Its structure is highly crystalline, aid to have tightly packed polymer chains, resistant to depolymerization (Hou et al. 2020). The capacity of polymerization (DP) of cellulose is associated with the quantity of glucose in the polymer, which takes an essential role in LCB recalcitrance. Hence, altering DP is always attended by modifications in structural parameters (porosity and crystallinity) (Zoghlami and Paës 2019). Nevertheless, disordered cellulose chains generate a small percentage of

**Table 1** Chemical composition of some LCB

Source	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Reference
Sugarcane whole	25	17	12	(Saxena et al. 2009)
Food waste	55.4	7.2	11.4	(Schmitt et al. 2012)
Office paper	68.6	12.4	11.3	(Mosier et al. 2005)
Leaves and grass	15.3	10.5	43.8	(Schmitt et al. 2012)
Coffee husk	43	7	9	(Gouvea et al. 2009)
Banana waste	13.2	14.8	14	(Monsalve et al. 2006)
Pineapple leaf fiber	70–82	18	5–1	(Reddy and Yang 2005)
Rice straw	28–36	23–28	12–14	(Reddy and Yang 2005)
Cotton waste	80–95	5–20		(Kuhad et al. 1997)



**Fig. 4** (a) Spatial arrangement of cellulose, hemicellulose, and lignin in LCB of the past and (b) Four steps of the bioethanol obtention from LCB

amorphous cellulose; therefore, the cellulose is more sensitive to enzymatic deterioration in this conformation (Pérez et al. 2002).

**Hemicellulose:** Represents 20–35% in weight (Zoghلامي and Paës 2019). Compared with the components mentioned above, this is composed of short, linear, and highly branched chains of diverse monomers (shell fibers blue in Fig.4a). Besides, it has an amorphous and random structure with little physical strength that includes five or six-carbon sugars that are suitable as potential suppliers to generate fermentable sugars (McKendry 2002; Rezania et al. 2017). Although the DP is much lower than cellulose, the hemicellulose hydrolyzes efficiently by diluting acids or bases (H. Isikgor and Remzi Becer 2015). The difference is that removing hemicelluloses through a pretreatment promotes cellulose conversion. However, the importance of hemicellulose on LCB conversion is still under development since studies suggest that hemicellulose removal is more efficient than lignin removal in enhancing the hydrolysis rate (YOSHIDA et al. 2008).



**Lignin:** The existence of lignin is the most critical barrier in producing biofuels because the availability of enzymes to cellulose is limited. It is a raw polymer with a complex 3D structure, highly branched and mononuclear aromatic, and it frequently covers the cellulose fibers to form a lignocellulosic complex (fibers red in Fig. 4a) (Drummond and Drummond 1996). It involves 15–30% of the lignocellulosic component (Hou et al. 2020). Lignin has the strong recalcitrance required to supply structural help and safeguard cells. Therefore, softwood barks have the highest level of lignin, between 30 and 60%, while fodders and agricultural remains contain the lowest level of lignin, 10–30% and 3–15%, respectively (Limayem and Ricke 2012). Several pretreatment methods have been developed to open their structure and remove lignin (Rezania et al. 2020).

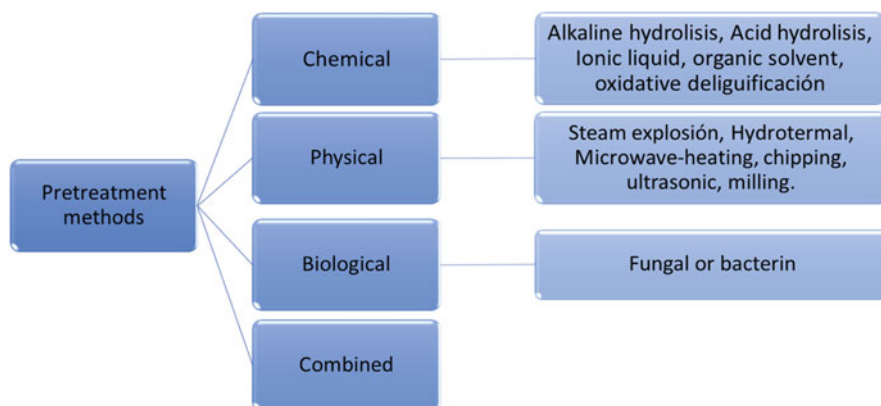
## 3.2 Bioethanol Production Steps from LCB

For the conversion of LCB to bioethanol, four steps are considered, as shown in Fig. 4b, (1) Pretreatment, (2) Hydrolysis, (3) Fermentation, and (4) Product recovery.

### 3.2.1 Pretreatment

It is the most complex and costly phase in bioethanol production (Hou et al. 2020). Nonetheless, it is the most important in the effective utilization of LCB as feedstock for bioethanol because it involves the separation of the principal biomass elements: cellulose, hemicellulose, and lignin (see Fig. 4b), thus that the macroscopic and microscopic dimensions, likewise the structure and chemical composition, are converted into smaller fragments so that fermentation of monomeric sugars can be achieved more rapidly and with greater yields (Mosier et al. 2005). Therefore, implementing the appropriate pretreatment methods can improve the whole process's efficiency by increasing fermentable sugars' concentrations (Maurya et al. 2015). There are different methods of pretreatments: chemical, physical, biological, and a combination of these, as shown in Fig. 5. The chemical methods consist of dissolving the LCB in different solvents, such as organic, acid, alkaline, or ionic ones. These methodologies are fast. However, they produce toxic substances and involve an extra cost for chemical recovery to sustain the system (Sharma et al. 2019). The physical methods cause less environmental pollution because they are used for the mechanical breaking of LCB, reduce its size and increase exposed surface area in further hydrolysis. Still, it needs high energy and power, increasing the production cost, therefore not cost-efficient (Chen et al. 2017).

The biological methods are characterized using bacteria or microbes to degrade LCB. In contrast to conventional chemical or physical pretreatment techniques, the biological is thought to be efficient, environmentally safe, and low cost (Kumar and Sharma 2017). Another convenience of these methods is that they are effectively



**Fig. 5** Pretreatment methods

degrading through the generation of enzymes such as peroxidases and laccases that decompose lignin (Kumar et al. 2011). In these methods, the fungi used frequently to degrade lignin are wood-rotting, including white-, brown-, and soft rot fungi (Chen et al. 2017). The fungi species usually used for pretreatment are *Phanerochaete chrysosporium*, *Ceriporia lacerata*, *Cyathus Stercorarius*, *Ceriporiopsis subvermispora*, *Pycnoporus cinnabarinus*, and *Pleurotus Astraeus*. Likewise, others include *Bjerkandera adusta*, *Fomes fomentarius*, *Ganoderma retinaculum*, *Irpex lacteus*, *Phanerochaete chrysosporium*, *Trametes Versicolor*, and *Lepista Nuda* for analysis of many lignocellulosic materials (Kumar and Sharma 2017). Among the advantages, they have presented mild conditions, low energy utilization, and the scarcity of contamination. Nevertheless, the rate of biological pretreatment is prolonged for industrial purposes, and the white-rot fungus uses part of the cellulose and hemicellulose in the growth process. Hence, the species of lignin-degrading microbes are insufficient and require high degradation (Chen et al. 2017). In general, microbial pretreatment presents several benefits; however, this method also presents some disadvantages of biological pretreatment that make it less suitable for the industry, including a long residence time of 10–14 days (Amin et al. 2017). Today, many efforts are focused on developing science and technology and bacteria genetic engineering, which will have a crucial position in the biological pretreatment of lignocellulosic biomass. Various technologies for the pretreatment of lignocellulosic biomass to improve bioethanol production have been described. As mentioned earlier, the main limitation of this technology is the presence of lignin, a significant cellulose blocker, and hemicellulose hydrolysis (Rezania et al. 2020). This opens extensive research in developing various pretreatment processes, considering physical, chemical, and biological principles. The use of these methods simultaneously is denominated “combined.” The chemicals and material mixtures are currently the most adequate and include the most encouraging technologies for industrial applications (Limayem and Ricke 2012). However, there is an important fact worth

highlighting: no treatment technology presents a 100% transformation of material to fermentable sugars until today (Hou et al. 2020).

### 3.2.2 Hydrolysis and Fermentation

Lignocellulosic enzymes, usually hydrolysis and fermentation, are carried out by yeast or bacteria. Hydrolysis contains the processing stages that transform carbohydrate polymers into monomeric sugars. Besides, it provides chances to enhance the technology, making biomass ethanol competitively analogized to other large-scale fuels. Many hydrolysis methods involve different processes described below and schematized in Fig. 6.

- (a) **Separated hydrolysis and fermentation (SHF):** This process consists of two stages, in which the hydrolysis and fermentation processes work differently. First, the lignocellulosic material is separated into monomeric (glucose and xylose) through enzymes; this is the hydrolysis stage. Subsequently, the sugar fermentation in ethanol remains. The principal benefit of this process is that both work to their respective requirements, which optimizes the process. However, the collection of sugars that inhibit the enzymatic action is still a great inconvenience affecting ethanol yield (Vohra et al. 2014).
- (b) **Simultaneous saccharification and fermentation (SSF):** In this process, enzymatic hydrolysis and fermentation are not separate locations. Both utilize the same reactor; therefore, the fermented microorganisms instantly transform the glucose released (by the cellulose) into ethanol. This results in the constant subtraction of glucose from the medium and has a corrective impact on general performance as a consequence of minimizing the final product inhibition on

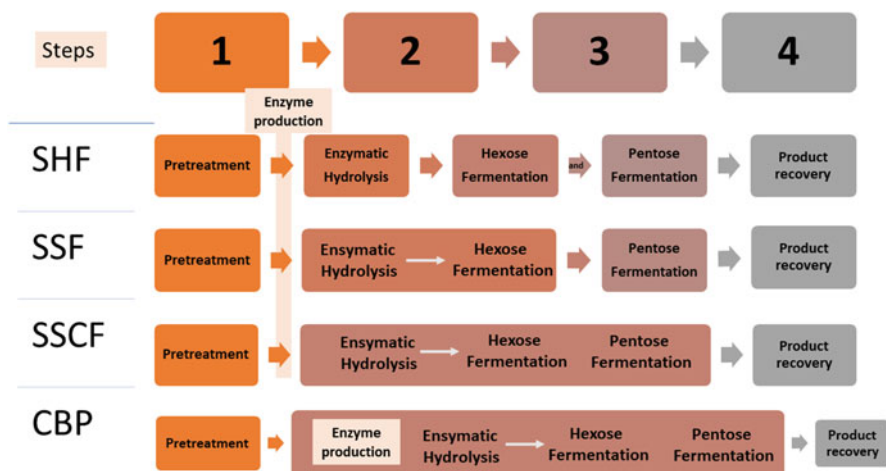


Fig. 6 The biological process according to the hydrolysis process

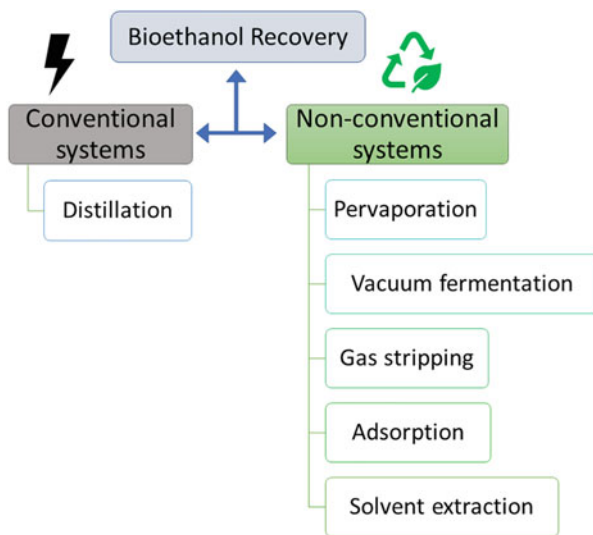
cellulolytic activities (Xiros et al. 2013). However, this process also has disadvantages, such as the temperature of enzymatic hydrolysis, which is commonly higher than fermentation (principally when this uses yeast). Hence, finding the optimal temperature in the SSF process is crucial. Also, an SSF process cannot reuse yeast because of yeast separation problems from lignin after fermentation. Thus, yeast represents a losing performance in an SSF process when they are obtained by carbohydrates and by external operation costs (Xu et al. 2008).

- (c) **Simultaneous Saccharification and co-Fermentation (SSCF):** This approach focuses on the microbial absorption of sugars discharged during the pretreatment and hydrolytic processes of LCB. The advantages of this method are cost reduction, operating time reduction, risk of contamination, and fewer blockage effects in enzymatic hydrolysis (Koppram et al. 2013). An option is using mixed yeast cultures that assimilate hexoses and pentoses. Nevertheless, the problem is that hexoses grow faster than pentoses, so there is a higher transformation of hexoses to ethanol. Another alternative is to use a single microorganism that assimilates both (hexoses and pentoses), permitting high ethanol production (Sánchez and Cardona 2008).
- (d) **Consolidated bioprocessing (CBP):** It is an alternative process incorporating specific phases of the LCB conversion process to reduce the production cost of ethanol from abundant lignocellulosic raw materials. Unlike traditional ethanol production, CBP integrates the stages of enzyme production, polysaccharide hydrolysis, and sugar fermentation in a unit procedure executed by one or more organisms (Mbaneme-Smith and Chinn 2015). This process has several benefits, one of which is that no capital costs are required to produce enzymes, the entire substrate is used to release sugars, and no part is used to produce cellulase. Furthermore, the enzymatic and fermentation functions are fully reconcilable. Thermophilic microbes have a clear benefit over traditional materials. Some inexpensive biomass feedstocks can be used directly and withstand high temperatures (Rastogi and Shrivastava 2017). Recently, various organisms can produce sorts of biofuels from LCB feedstocks. CBP organisms can be generated by improving the biofuel performance (natural cellulolytic microorganisms or changing methanogenic/ethanologenic microbes to be cellulolytic) (Parisutham et al. 2014).

### 3.2.3 Product Recovery

The recovery is the last stage of obtaining bioethanol, which is done through a conventional distillation (vapor/liquid mass transfer). Distillation permits the recuperation of dilute evaporative products, such as ethanol (Madson and Lococo 2000). During distillation, the mixture separates the ethanol from the other components. The ethanol obtained by fermentation is contaminated and in low fractions. The rectification allows reaching the concentration and purification of ethanol. However, dehydration is required to obtain pure ethanol (99.7%) (Robak and Balcerek 2018).

**Fig. 7** Bioethanol recovery process: distillation and non-distillation



On the other hand, it is known that distillation has some negative aspects, i.e., it is impossible to effectively dry the product without coupling in additional separation. This leads to high energy consumption and production costs (Vane 2008).

Nevertheless, given the disadvantages above to obtaining bioethanol in a green way of distillation remains in the last stage, it has led to a search for new separation techniques to improve the efficiency of biofuel production and reduce energy consumption during the purification process (Nigiz and Hilmioglu 2013). According to Serra et al., the bioethanol recuperation strategies from fermentation can be classified into traditional or modified traditional systems (distillation system) and nonconventional systems (non-distillation systems) (Serra et al. 1987), as shown in Fig. 7.

## 4 Ethanol Production from Algae Biomass

The previous section mentioned the disadvantages of producing ethanol with LCB due to its long obtaining times and high production costs, so this is not an excellent alternative to scale it at an industrial level. However, the accelerated growth of algae, microalgae, and cyanobacteria offers a significant advantage in producing biofuels compared to the superior first and second-generation bioethanol plants. Also, growing in different circumstances, including sewage, there is less necessity for water consumption or resources, and the possibility of not settling cultivation land (de Farias Silva and Bertucco 2016).

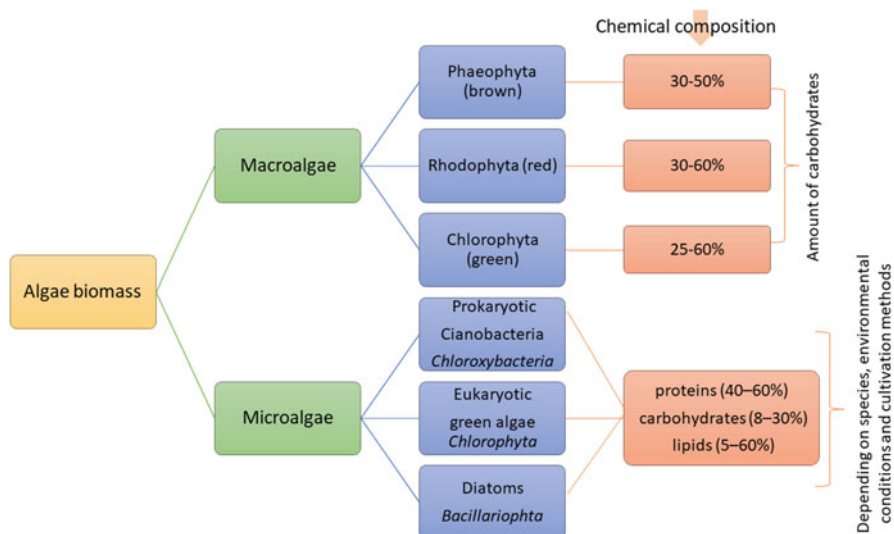
## 4.1 Classification of Algae

Algae are classified into two general groups: microalgae and macroalgae. Both classes consist of simple organisms that contain chlorophyll and can photosynthetically transform  $\text{CO}_2$  into metabolites and chemicals, such as proteins, hydrogen, polysaccharides, and lipids. Each group, in turn, is classified into subgroups, as shown in Fig. 8 (Sambusiti et al. 2015).

### 4.1.1 Macroalgae

Recently, macroalgae (photoautotrophic organisms) have taken on great importance as possible substrates for biorefinery due to their incredible capacity to generate and accumulate carbon resources using  $\text{CO}_2$  and  $\text{HCO}_3^-$  (Gao and McKinley 1994). Besides, they are the most noteworthy organisms in marine ecosystems to control eutrophication and pollution (Notoya 2010). Today, there are more than 10,000 species of macroalgae worldwide, although just a dozen species are grown commercially (Critchley et al. 2019).

Of all the macroalgae species, those considered suitable for biofuels are *Laminaria japonica*, *Eucheuma cottonii* (*Kappaphycus alvarezii*), *Undaria pinnatifida*, and *Gracilaria verrucosa*. Only two brown macroalgae are used, *U. pinnatifida* and *L. japonica*, and for the species of red macroalgae, *K. alvarezii*, *Eucheuma* spp., and *Gracilaria* spp. Nonetheless, the production of green macroalgae is low and can be assumed insignificant (Tan et al. 2020). So, to produce bioethanol, one can only opt



**Fig. 8** Algae classification: macro and microalgae

for red or brown microalgae. Goh and Lee have reported that if *Eucheuma* spp. is employed as a feedstock to produce bioethanol, calculated bioethanol products could achieve up to 110,000 tons per year (Goh and Lee 2010).

Macroalgae contain two monosaccharides with different proportions: the D-galactose (56.2%) and the 3,6-anhydrous galactose (43.8%) (Goh and Lee 2010); these require to be extracted by hydrolysis for their following fermentation. Due to macroalgae having a low lignin content, they do not require additional pretreatment, which is an expensive process, as was seen in the previous section (Meinita et al. 2012).

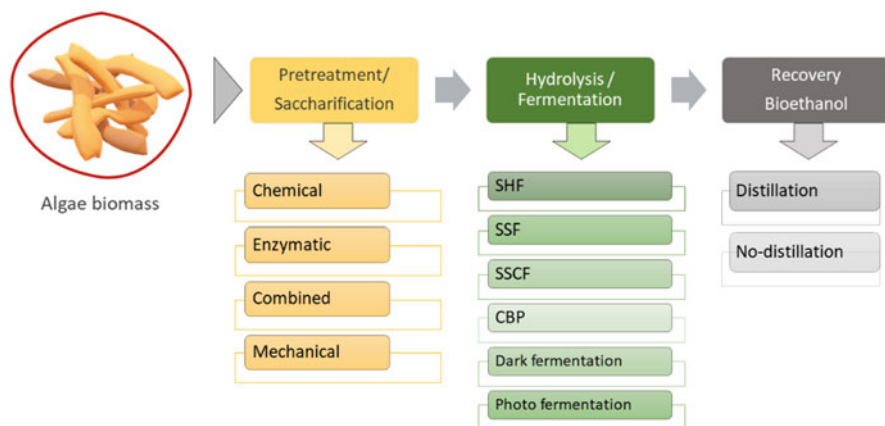
#### 4.1.2 Microalgae

They are single-celled or single-multicellular photosynthetic microorganisms generally found in marine and freshwater habitats. These can be grouped into procaryotic, eukaryotic, and diatoms (Sambusiti et al. 2015). One of the advantages of these microorganisms is that they can be grown in different environments, such as freshwater, seawater, and wastewater within open ponds (pipelines) and photobioreactors. Another meaningful advantage is that even microalgae can handle and adjust to diverse environmental conditions such as pH, light, and temperature. Therefore, there is no limitation to their production during the different seasons of the year (in terms of pH, temperature, and light.) (Uggetti et al. 2014). In general, microalgae represent a significant potential raw material for bioethanol production due to their low percentage of lignin and hemicellulose compared with other lignocellulosic plants as a substantial quantity of carbohydrates, galactose, and glucose (Domozych et al. 2012), which are energy-rich compounds.

Among the known microalgae species worldwide, the most habituated and most promising species to produce bioethanol are *Chlorella Vulgaris* and *Chlorococum* sp. The former can store up to 37% (dry weight) of starch, while the latter has shown good fermentation under different conditions (Nguyen and Vu 2012). There are two ways to obtain bioethanol from microalgae: i) directly from microalgae biomass and ii) from biomass depleted after lipid extraction. Even in the latter, they have found that the fermentation of the microalgae extracted from lipids produces bioethanol concentrations 60% higher than that obtained from the dry microalgae (Harun et al. 2010). The steps for getting bioethanol from algae are described in the following section.

### 4.2 Ethanol Production Steps from Algae Biomass

Three essential steps are required to obtain bioethanol from algae biomass: pretreatment, hydrolysis, and fermentation. These steps can be together or separately, as mentioned in Sect. 3.2.2. The same types of hydrolysis for lignocellulose biomass are used for algal biomass, and two other fermentation processes are used.



**Fig. 9** Process in the bioethanol obtention from algae biomass

Moreover, finally, the recovery of bioethanol is usually through distillation, but distillation is not considered a green process. Consequently, the ideal is to use another process that is not distillation in the last step. In Fig. 9, different stages in obtaining bioethanol through algae are described below.

#### 4.2.1 Pretreatment/Saccharification

As with lignocellulose biomass, a pretreatment is first required to access its glucose; something similar happens with microalgae. First, a pretreatment must be done to obtain their glucose. Like other plants, the rigid cell wall blocks the microalgae, preventing access to intracellular starch. However, because algae contain little lignin, this processing is a bit less expensive and more accessible. Even so, this step continues to be one of the principal restrictions for its large-scale presentation, like in the second-generation bioethanol (Souza de et al. 2020).

The methods are categorized according to their process to carry out cell disruption, as shown in Fig. 9. These can be chemical, enzymatic, mechanical, and non-mechanical. Chemicals focus on breaking alkaline cells with solvents, detergents, and acids. The mechanics consist of a homogenizer, ball mill, and ultrasound or combined, such as osmotic shock, freezing, and thawing. Corresponded to those used for the pretreatment of lignocellulose, these methods are much less expensive and easy to perform (Menegazzo and Fonseca 2019).

In comparison in the extraction of carbohydrates from *Chlorella* sp. by the standard solvent and assisted by ultrasound (Victor et al. 2015), the results demonstrated that the second one is more effective due to the glucose yield. A feasible production of bioethanol from hydrolyzed microalgae employing wet torrefaction by microwave method using dilute acids has also been reported (Yu et al. 2020).



#### 4.2.2 Hydrolysis/Fermentation

Hydrolysis of carbohydrates is required to expose them to the medium. This consists of breaking them to obtain their most straightforward sugars, such as glucose since yeasts ferment only simple carbohydrates (Mahdy et al. 2014). As shown in the scheme in Fig. 9, hydrolysis and fermentation can occur together or separately, as with lignocellulose from the previous Sect. 3.2.2.

Among the hydrolysis types, recent studies show that acid and enzymatic hydrolysis are the best in obtaining a more outstanding performance (Chen et al. 2013). Comparing these two, enzymatic hydrolysis has some advantages over acidic hydrolysis. Lately, a study for enzymatic hydrolysis of 92% compared to 76% for acid hydrolysis was even reported (Shokrkar et al. 2017).

On the other hand, fermentation is a metabolic conversion activity of monosaccharides in bioethanol and other by-products through fermentative microorganisms under specific conditions of temperature and pH (El-Dalatony et al. 2016), to name a few. In this process, the use of yeast or bacteria is widespread. In addition, when we talk about fermentation separate from hydrolysis, this is classified into two main types:

- (a) **Dark fermentation:** This process takes place under anaerobic states. Hence, the oxidative reaction of starch becomes insufficient in dark and anaerobic conditions. Microalgae can produce  $H_2$ ,  $CO_2$ , ethanol, lactic acid, formic acid, acetic acid, and others in variable proportions, depending on the microalgae used (John et al. 2011). Nowadays, a significant number of microalgae and cyanobacteria are known that can expel ethanol through the cell wall during the intracellular process without light, such as *C. reinhardtii*, *C. moewusii*, *C. Vulgaris*, *O. limnetic*, *O. Limosa*, *G. alpicola*, *C. littorale*, and *Synechococcus* sp. (Oncel 2013). Algae with a high concentration of carbohydrate content are more appropriate for the dark fermentation process. They are categorized into the classes of *Chlorophyceae*, *Prasinophyceae*, *Cryptophyceae*, and *Cyanophyceae* (John et al. 2011). In conclusion, the process of dark fermentation (microalgae) is not efficient for bioethanol generation because the reaction of starch becomes incomplete, and bioethanol production is poor (de Farias Silva and Bertucco 2016).

- (b) **Photofermentation.**

The photofermentation or “photofermentative” pathway (photanol) is a natural and efficient metabolic process where sunlight is converted into fermentation products (Hellingwerf and Teixeira de Mattos 2009). The essence of the photanol concept is to employ the strategies of synthetic biology to design and build a metabolic network that connects the phototrophic and fermentative metabolism in a new network that uses as raw material:  $H_2O$ ,  $CO_2$ , and solar energy to obtain a product of fermentation as an outlet and  $O_2$  as the unavoidable by-product of  $H_2O$  oxidation (Angermayr et al. 2009). Not only is photanol focused on ethanol production, but it is also used for many natural derivatives resulting from glycolysis fermentation (de Farias Silva and Bertucco 2016).

From this, photofermentation is an approach of increasing interest, mainly behind the statement of the induction of bioethanol manufacturing plants by altered cyanobacteria. The cyanobacteria are genetically modified by molecular engineering techniques where specific fermentation cassettes are introduced (Angermayr et al. 2009). Yet, given that this process involves a generic modification of microorganisms. The biofuels produced from this are called the fourth generation.

## 5 Conclusion

The transformation in the production of first-generation bioethanol for second or third generation is one of the significant engineering challenges. Even though the raw material of the second generation is much more viable, the production of lignocellulosic bioethanol needs advances associated with the pretreatment, enzymatic hydrolysis, and fermentation steps to improve the manufacture rentability and be able to scale it to an industrial scale. One of the main barriers to producing second-generation biofuels is biomass composition. Nevertheless, according to the advances in these procedures, it can be expected that these technical barriers could be overcome soon by improving the biochemical route. On the other hand, it has been found that microalgae solve the problems encountered with second-generation raw materials, due to the provision of numerous environmental and cost benefits, mainly in the pretreatment process, since microalgae by not have high resistance in its fibers, this process is more manageable and lowers production costs. Therefore, microalgae have the potential to be used as a substrate to produce bioethanol on a commercial scale while maintaining sustainability.

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# Biological Methods for Diesel Formation



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**Abstract** Diesel is a liquid hydrocarbon fundamentally composed of paraffin, produced conventionally from the fractional distillation of crude oil and used as a fuel in heating systems and diesel engines. In the case of biodiesel, it originates from natural lipids, which can be obtained by means of industrial processes from oil of vegetal origin and animal fat, being functionally identical to diesel. Biodiesel is one of the most widely investigated biofuels due to its excellent properties and good performance in engines. Its obtaining is carried out by means of transesterification, mainly. Advances in biodiesel research have led to classifying it into four generations based on synthetic and natural technologies: edible vegetable oils, nonedible feedstock, feedstock algae, and genetically modified feedstock, each one with its own advantages over the others, which provides certain versatility to this biofuel. Nonetheless, the biofuels industry is searching for alternative processes that lead to high production levels, minimum waste, and low cost. Biodiesel production using lignocellulosic biomass is quite flexible. Biomass is treated in order to degrade polysaccharides and accumulate lipids. These lipids are afterward extracted and transesterified to produce biodiesel. A similar process occurs with microalgae, which are treated to extract lipids and are subsequently transesterified. In this

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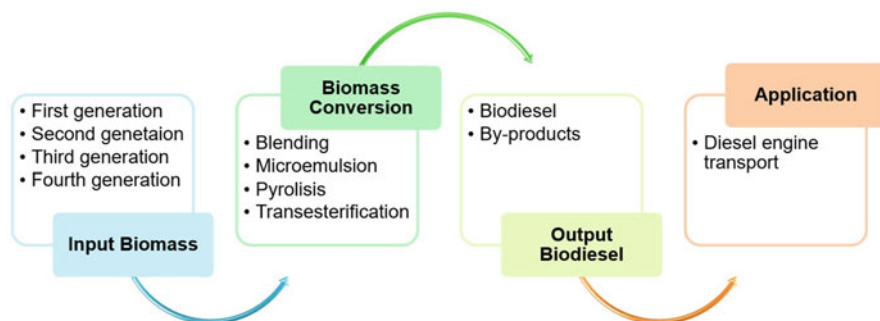
chapter, the four generations of biodiesel were addressed and subdivided into their principal feedstocks in order to encompass the most important. Also, the methods used for biodiesel obtention, the principal applications and its importance as a renewable energy source are explained to exhibit the impact of this biofuel on the way to the generation of a greener world.

**Keywords** Biodiesel · Biomass for biodiesel · Cetane number · Transesterification

## 1 Introduction

The global demand for fuels derived from petroleum or fossil sources increases considerably with the passage of time and in the near future, it may lead to the depletion of these resources. The transport sector is the largest consumer of fossil fuels, up to 60% with an annual increase of 0.7%. In addition, fossil fuels face the problem of polluting emissions at the time of their combustion, emitting hydrocarbons, carbon oxide, and nitrogen oxides, contributing considerably to global warming. In this context, the need arises to obtain fuels from renewable sources that can replace fossil fuels (non-renewable) (Ellappan and Rajendran 2021; Manojkumar et al. 2022; Singh et al. 2021a). A viable alternative is the use of biodiesel, which is a promising biofuel and a potential substitute for conventional diesel. The history of biodiesel began in 1900 when Sir Rudolf Diesel ran conventional diesel engines using vegetable oil, in 1930 and 1940 vegetable oil was used as emergency diesel fuel (Zahan and Kano 2018). The United States produces biodiesel to a great extent, making it one of the most important in the world. Currently, biodiesel is obtained mainly from animal, vegetable, and algae fats and oils. To obtain this biofuel there are various synthesis methods such as blending or dilution, microemulsion, pyrolysis or thermal cracking, and transesterification; each will be addressed later. It is important to note that no method of obtaining biodiesel is 100% biotechnological. Although, in the transesterification process, some stages involve microorganisms or biomass as catalysts. However, biotechnology in the process of obtaining biodiesel is closely related to the biomass used as feedstock (Chamkalani et al. 2020; Dey et al. 2021; Zhao et al. 2019). On the other hand, biodiesel has been classified into four generations by the European Academies Science Advisory Council (EASAC), according to the type of feedstock used, as shown in Fig. 1 (Cameron et al. 2014; D. Singh et al. 2020, 2021a). The type of feedstocks for obtaining biodiesel vary from one country to another and are highly influenced by the production and location of the regions (Alagumalai et al. 2021; D. Singh et al. 2021a). Various oil crops, for example, soybean, jatropha, among others, can be considered alternative fuel sources (Sitepu et al. 2020). Biodiesel is widely used in the automotive industry, it is a viable alternative to diesel engines, its application generates low polluting emissions, enhances performance of the engines, and a high cetane number, as well as being easy to degrade (Chamkalani et al. 2020; Dey et al. 2021; Raheem et al. 2020). Nevertheless, the high viscosity and the low calorific value are some disadvantages of biodiesel; but modifying diesel engines could solve





**Fig. 1** Generalities of obtaining biodiesel

the problem (Dey et al. 2021; Ellappan and Rajendran 2021). This chapter deals with an overview of biodiesel, the types of biomass and methods for obtaining it, as well as its potential uses as a biofuel.

## 2 Biomass for Obtaining Biodiesel

Lipids or fats are the main feedstock in biodiesel production (Alagumalai et al. 2021). As mentioned above, biodiesel is classified into four generations according to the feedstock used to make it. First-generation feedstocks are derived from edible oils, compromising oil crops for human consumption. The second generation classifies inedible oils, residual fried oils, and animal fat. The third-generation classifies algae that are abundant in nature. The fourth generation uses synthetic biology techniques to obtain feedstocks and subsequent production of biodiesel, in this last generation the research work is in the initial phase (Karmakar and Halder 2019). The fatty acid composition of vegetable oils differs according to the feedstock.

It is important to mention that vegetable oils differ in their fatty acid composition depending on the feedstock. Fatty acids have diverse carbon chain lengths and diverse degrees of unsaturation. Therefore, the properties of biodiesel can vary depending on the oil used as feedstock (R. Singh et al. 2021). Besides, vegetable oils have a higher amount of oxygen that ranges from 10 to 45% compared to hydrocarbons derived from petroleum, whose amount of oxygen is close to zero. The high amount of oxygen in vegetable oils allows complete combustion and low levels of carbon monoxide, although it has the disadvantage of affecting thermal stability and volatility (Demirbas 2009). It is important to mention that the fatty acids present in vegetable oils and animal fats are principally classified in two types: the saturated and the unsaturated fats. The animal fats are classified as saturated, and the fats of plants and fish oils are classified as unsaturated. The difference between saturated and unsaturated fats is in the chemical bonds founded their structure, finding single bonds at the saturated and double bonds at the unsaturated. Within unsaturated ones,

exist two types according to the number of double bonds, when there is only one it is called monounsaturated, and when it has more of one it is called polyunsaturated. Examples of saturated fatty acids are stearic, palmitic, and dihydroxy stearic acids; while examples of unsaturated fatty acids are oleic, linoleic, ricinoleic, palmitoleic, linolenic, and eicosenoic acids (Hamza et al. 2021). Each generation of biodiesel and the feedstocks involved are addressed independently below. One of the main properties of biodiesel is the cetane number. This number indicates the quality of diesel combustion and is established by the engine, where a high value carries out better internal combustion. Also, this value is higher for biodiesel than for conventional diesel, for this reason, biodiesel has a better combustion capacity than conventional diesel (Yesilyurt et al. 2020). The cetane number is based on the number of carbons, the amount of ester, and the molecular structure. Double bonds produce a reduction in the value of this number. According to European Committee for Standardization (CEN) with EN14214 and the American Society for Testing and Materials (ASTM) with ASTM D6751, biodiesel needs to have a value greater than 51 and 47 respectively, as shown in Table 1 (Noreen et al. 2021; D. Singh et al. 2019; Yesilyurt et al. 2020). Also, the biodiesel obtained from different biomasses has main properties, which are listed in Table 1, a comparison is made with the standards and with petroleum diesel.

## 2.1 First-Generation Biodiesel (Edible Vegetable Oils)

Currently, a high percentage (95%) of biodiesel is produced from feedstock from edible crops, which can result in a shortage of oils for human consumption and a negative effect on the economy (Athar and Zaidi 2020; Hamza et al. 2021). Mainly four types of seeds are used to extract their vegetable oil and produce biodiesel, which are, palm, soybean, sunflower, and rapeseed oils, contributing between 77 and 85% of vegetable oils to the market, and the remaining percentage is provided by feedstock from other generations (Athar and Zaidi 2020; Zahan and Kano 2018).

### 2.1.1 Palm Oil

This tree belongs to the Palmaceae family, it is a perennial plant constituting a group into the monocots. They are comprising two species, which are *Elaeis guineensis* and *Elaeis oleifera*, also known as *Elaeis melanococca*. The *Elaeis guineensis* has its origin in the tropical zone of Africa, but currently, it is present in the wild in tropical regions around the world, with a significant existence in Latin America, Asia, and Africa (Absalome et al. 2020). Among the four most used oils, palm oil stands out due to its high oil content (30–60%) and its low cost (Dey et al. 2021). In addition, the palm is an abundant resource and has a high production capacity, requiring a minimum area of plantation. Its approximated potential for oil production of 4000–6000 kg per hectare makes the palm the source with the bigger potential for oil production. Palm oil has a high amount of palmitic and oleic acids that have been

**Table 1** Biodiesel properties (Anwar 2021; Athar and Zaidi 2020; Chen et al. 2020; Dey et al. 2020; Gui et al. 2008; Raheem et al. 2020; Singh et al. 2021; Vignesh et al. 2021; Zahan and Kano 2018)

Properties	Petro-Diesel	Biodiesel Standards		Edible oil				Nonedible oil						Waste cooking	Animal fat (Tallow)
		ASTMD D6751	EN 14214	Palm	Soybean	Sunflower	Rapeseed	Jatropha	Karanja	Mahua	Castor				
Flash point min (°C)	68	100–170	≥120	164	178	183	170	58.50	160	208	160.90			161.70	157.20
Cloud point (°C)	–5	–3–12	–	13.0	1.00	3.40	–3.30	2.70	7.60	–1.70	–	13.40		5.30	16.0
Pour point (°C)	–20	–15–16	–	11.80	–3	–2	–9.70	–0.90	–	–	–32			–0.30	15
Kinematic viscosity at 40 °C (mm <sup>2</sup> /s)	2.6	1.9–6.0	3.5–5.0	5.70	4.04	4.44	4.44	4.80	3.90	3.98	15.25			4.75	4.82
Density at 15 °C (kg/m <sup>3</sup> )	795–840	820–900	860–900	876	884	880	882	879.50	880	850	899			880.60	874
Iodine number, max	–	–	120	52.70	128	102	109	99	85	184.50	82.50			85.10	54.50
Cetane number, min	43–65	47	51	62	49	51.25	54.40	57.10	55	56.90	42.10			47.7–59.8	60.90
Oxygen (wt. %)	0	11	–	11.27	10.95	10.98	10.93	12.21	11.51	–	–			–	–
Acid number, max (mg KOH/g)	–	0.50	0.50	0.24	0.27	0.52	0.30	0.40	0.62	–	–			0.59	0.21

characterized as suitable sources for the production of biodiesel; being used in the main countries of Asia (Chen et al. 2020; Zahan and Kano 2018). Indonesia and Malaysia are the main palm oil producing countries worldwide, producing 46,500 and 19,800 tons (t), respectively, followed by Thailand and Colombia with 3260 and 1838 t, until August 2022/23, considerably low amounts compared to the main producers (USDA 2022). Biodiesel produced from palm oil can guarantee energy supply, conserve the environment and favor the development of regions, mainly in countries with the highest production (Ashnani et al. 2014). Biodiesel obtained from palm oil has a deficient flow property in low temperatures, a good cetane number (62) attributed to the content of palmitic and stearic acid, contributors of saturated fatty acids, and an elevated oxidative stability. Furthermore, compared to conventional biodiesel, palm biodiesel has a major pour point due to its high saturated ester. In order to make it adequate to use in diesel engines, the palm biodiesel needs to be blended with diesel in various proportions to achieve the standard blend kinematic viscosity, cloud point, and pour point (ASTM D7467) (Ali et al. 2014; Athar and Zaidi 2020; Dey et al. 2021).

### 2.1.2 Soybean Oil

Soybean is an edible bean native to East Asia. Currently, the main producing countries are Brazil, the United States, and Argentina, with a production of 133,000, 112,549, and 48,000 t, respectively. Compared to other legumes, soybeans are more resistant to drought and can be grown in various types of soils. Soybean protein is heat stable, that is, its chemical composition does not alter at high temperatures; that is why soybean oil is the most used for cooking. The oil content ranges from 17 to 23%, mainly composed of polyunsaturated fatty acids, for example, alpha-linolenic acids, monounsaturated oleic, and saturated stearic and palmitic acids (Chen et al. 2020; Manojkumar et al. 2022; D. Singh et al. 2020). Soybean has a potential for oil production of around 400–600 kg per hectare (Chen et al. 2020). Biodiesel obtained from soybean oil has a cetane number of 49 (Ramos et al. 2009).

### 2.1.3 Sunflower Oil

The sunflower is a perennial plant with yellow petals and a brown center, where the seeds are located, from which the oil is extracted, it is native to North America and is cultivated to extract vegetable oil. The world production of sunflower oil is higher than 15.8 million tonnes per year. Ukraine and Russia are the main sunflower oil producers with a production of 5923 and 5203 t, respectively, followed by Argentina and Turkey with a production of 1225 and 1000 t, respectively (Manojkumar et al. 2022; Saydut et al. 2016). On the European continent, sunflower oil occupies the second place in biodiesel production, preceded by rapeseed oil (D. Singh et al. 2020). Sunflower seeds have a high oil content (25–35%), which is mainly

composed of linoleic acid in  $50 \pm 20\%$ ,  $28 \pm 13\%$  of monounsaturated oleic acid and lesser percentages of  $6 \pm 2\%$  and  $4 \pm 2\%$  palmitic acid and saturated stearic, respectively, is an oil very susceptible to oxidation (Manojkumar et al. 2022; Saydut et al. 2016; D. Singh et al. 2020). The oil from sunflower is a principal source to produce biodiesel. The cetane number of the biodiesel from sunflower oil is 51.25, being one of its main properties (Mujtaba et al. 2020).

### 2.1.4 Rapeseed Oil

Rapeseed is part of the mustard family; comes from Europe and Asia. It is considered the third largest source of vegetable oil production worldwide. And it is cultivated mainly to extract canola oil (Anwar 2021). In 2022, the main global producers are Canada, European Union, and China with 20,000, 18,000, and 14,500 t, respectively (USDA 2022). Comparing rapeseed to soybeans, the former has a greater capacity to produce oil in an equal area of land. Rapeseed has an oil percentage of 38–46%, which has 43.5%, 31%, and 20.3% of oleic, linoleic, and palmitic acids (Anwar 2021). On the European continent, rapeseed oil is used as a reserve to produce biodiesel, it represents approximately 80% of the feedstock (Chen et al. 2020). Among the properties present in the biodiesel produced from rapeseed oil, the high content of unsaturated fatty acids stands out (Gui et al. 2008), cetane number of 54.40 (Ramos et al. 2009), and best cold flow properties (Gui et al. 2008).

## 2.2 Second-Generation Biodiesel (Nonedible Feedstock)

Second-generation biodiesel involves feedstock that is not for human consumption, for what it contributes to solving the food shortage and the impact on the environment; the main benefits are the considerable reduction in the cost of biodiesel production, as well as the demand for agricultural land, since it does not require specific land for crops (Chamkalani et al. 2020). In addition, rural nonagricultural areas can be improved by the development of nonedible oil crops and sustainable biodiesel production can be helped (Ashraful et al. 2014). The disadvantage of second-generation biodiesel is the feedstock used for its production, classified mainly as non-edible oils, waste cooking oil, and animal fat, which are not available in the quantities necessary to ensure large-scale production (Andreo-Martínez et al. 2020; Aransiola et al. 2014; Chamkalani et al. 2020; Goh et al. 2020).

### 2.2.1 Nonedible Oils

Biodiesel obtained from this feedstock also complies with the standards established by current regulations (ASTM D6751 y EN14214). Regarding nonedible oils, they are mainly classified as *jatropha* (*Jatropha curcas*), *karanja* (*Pongamia pinnata*),

neem (*Azadirachta indica*), mahua (*Madhuca indica*), and castor (*Ricinus communis*) oils. The number of fatty acids and the content of free fatty acids (FFA) present in nonedible oils defines its feasibility to be used as feedstock in biodiesel generation (Arumugam and Ponnusami 2019; Ashraful et al. 2014; Hamza et al. 2021).

**Jatropha oil:** It is a relatively small plant that grows between 5 and 7 m high, it is part of the Euphorbiaceae family. It grows in tropical areas as well as arid and semi-arid. Currently, it is cultivated in the United States, Central America, India, and Africa. One year after planting, seed production begins and optimal production is reached after five years, the plant can live from 30 to 50 years. Jatropha has 20–60% by weight of oil and is a nonedible oil, which contains unsaturated fatty acids such as linoleic (31.4–43.2%) and oleic (34.3–44.7%), and some saturated species such as stearic acid (7.1–7.4%) and palmitic acid (13.6–15.1%) (Anwar 2021; Ashraful et al. 2014; Kibazohi and Sangwan 2011). Biodiesel obtained from jatropha oil can be used without problems in engines that work with diesel, and its cetane number is 57.1 (Chen et al. 2020; Ewunie et al. 2021).

**Karanja oil:** It is a leguminous tree belonging to the pea family and presents medium size, growing  $20 \pm 5$  m in height. Between three and four years after planting it blooms and the maturation phase is accomplished in 4–7 years. This tree is native to Asia, it has been successfully cultivated in countries such as India, China, Japan, Malaysia, Australia, and the United States. At present, Karanja seeds are considered a source of oil, it is estimated that a tree can produce between 9 and 90 kg of seeds, which contain between 25 and 40% by weight of oil. Karanja oil contains monounsaturated oleic, linoleic, and stearic acids in percentages 44.5–71.3%, 10.8–18.3%, and 2.4–8.9%, respectively (Ashraful et al. 2014; Y. C. Sharma and Singh 2010). Biodiesel obtained from Karanja oil has a cetane number of 55 (Anwar 2021).

**Neem oil:** It is a tree that measures 12–18 m in height, it is part of the Meliaceae family. This tree grows in all types of soils, its maximum productivity occurs 15 years after its planting, and it has a useful life that ranges between 150 and 200 years. Neem is native to India and Burma, currently, it is cultivated in various countries of south Asia and in the tropical areas of Australia. The oil contained in the neem seeds represents 20–30% of its weight, and the seeds contain 40–50% brown oil. Contains unsaturated oils such as oleic (25–54%) and linoleic (6–16%) and saturated oils such as stearic acids (9–24%) (Fazal et al. 2019; Kumar and Sharma 2011; Sanford et al. 2010). Biodiesel obtained from Neem seeds has a cetane number between 46 and 52 (Noreen et al. 2021).

**Mahua oil:** It is a large tree, grows up to 20 m in height, belongs to the Sapotaceae family, of forest origin. It produces seeds for 60 years after 10 years of being planted. It is grown in regions of India with high humidity. The Mahua tree produces 20–200 kg of oil seeds per year, according to their maturity. The seeds have an oil content of 35–50% by weight. Mahua seed oil contains 41–51% monounsaturated oleic acid, 20–25.1% saturated stearic acid, 16–28.2% saturated palmitic acid, and 8.9–18.3% polyunsaturated linoleic acid (Ashraful et al. 2014;

Balat 2011; Kumar and Sharma 2011). Biodiesel obtained from Mahua seeds has a cetane number of 56.90 (Gopinath et al. 2015).

**Castor oil:** It is an easy plant to grow and resistant to droughts. Its cultivation requirements are very similar to those of jatropha. The castor bean is part of the Euphorbiaceae family, it comes from Africa and India. Currently, in Australia and the United States can be found castor bean crops. Due to the similarity of cultivation with jatropha, castor can be cultivated by intercropping both crops (Gui et al. 2008). Castor beans have an oil content of 53%, with 89.5% ricinoleic acid. Biodiesel obtained from castor oil has a cetane number of 42.10 (Gopinath et al. 2015).

## 2.2.2 Waste Cooking Oil

Waste cooking oil (WCO) can be used as an alternative in the production of biodiesel, which is the oil left over after cooking, which contains accumulated free fatty acids, making it a good candidate to produce biodiesel (Goh et al. 2020; Wan Ghazali et al. 2015). There is a wide range of WCO and they have the advantage of being inexpensive and their use contributes to the sustainable generation of biodiesel, as well as reducing the competition between biofuel and food (Ambat et al. 2018; Khodadadi et al. 2020). The WCO has some disadvantages, such as the difficulty of the collection and the previous purification process, due to its composition (Karmakar and Halder 2019). The vegetable oil used for cooking gives specific characteristics of each WCO. Generally, WCO is made up of different types of vegetable oils, for example, oils of soybean, rapeseed, and palm (D. Singh et al. 2021b). It is essential to know the properties of the WCO before the production of biodiesel. Because the properties of WCO can vary considerably depending on how the oil is used for cooking (Goh et al. 2020). It should be mentioned that WCO has an elevated composition of free fatty acids (FFA) attributed to its degradation and high-water content. WCO, like oils of palm, pongamia, and peanut, has an elevated content of oleic acid and linoleic acid of 66.3% and 17.5%, respectively, of the total fatty acid composition. Besides, the cetane number of the biodiesel obtained from WCO is 47.7–59.8 (Singh et al. 2021b).

## 2.2.3 Waste Animal Fat

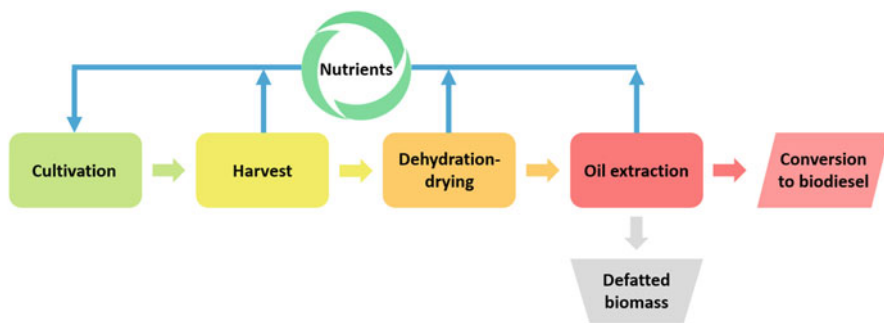
Waste animal fat (WAF) is a sustainable feedstock for the production of biodiesel due to its cost being relatively low or zero (Vargas-Ibáñez et al. 2020). It can be obtained from fish, cattle, chickens, and pigs (Rajak and Verma 2018). The use of WAF in the production of biodiesel also solves the problem of the final disposal of this organic waste. Today, animal fat is used mainly in the cosmetic and soap making industries; and it has not been widely studied in the production of biodiesel as in the case of vegetable oils. However, the properties of biodiesel synthesized from chicken fat conform to ASTM D6751 (Habib et al. 2020). Besides, it has been observed that the biodiesel obtained from WAF (such as beef tallow) has its cetane

number and oxidative stability is higher than that of biodiesel processed from vegetable oils, but the cold flow properties are better for the latter (Soares Dias et al. 2020). Attributed to these characteristics, biodiesel processed from WAF is more suitable in hot climate countries. Around 72% of beef tallow has been used for the generation of biodiesel (Vargas-Ibáñez et al. 2020).

### 2.3 Third-Generation Biodiesel (Feedstock Algae)

The third generation of biodiesel classifies the algae oil, which have turned out to be a promising feedstock (Balat 2011; Nautiyal et al. 2014). Algae are similar to terrestrial plants; both are photosynthetic beings but aquatic environments are the places where the algae grow (Hossain et al. 2020). Generally, algae are classified into green algae (*Chlorophyceae*), blue-green algae (*Cyano phyceae*), diatoms (*Bacillariophyceae*), and golden brown (*Chryso-phyceae*). Around 50,000 species of algae have been studied, which can be micro or macro. The oil composition in microalgae comprises  $45 \pm 25\%$  and they are easily cultivable. Also, it has a rich amount of polyunsaturated fatty acids with more than three double bonds. On the other hand, macroalgae are deficient in oil content, they contain around 1.3–7.8% by dry weight. Microalgae that have a high content of fatty acids can produce 12 kg or more of biodiesel/m<sup>2</sup> per year, this is a much higher amount than some vegetal oils, for example, the rapeseed oil that produces <0.1 kg of biodiesel/m<sup>2</sup> per year and palm oil <0.5 kg of biodiesel/m<sup>2</sup> per year (Athar and Zaidi 2020; Hossain et al. 2020; Mata et al. 2010). Large-scale algae cultivation is relatively new, it has the disadvantage of requiring a significant amount of fertilizers and water resources. The cultivation of microalgae such as *Chlorella* and *Spirulina* has grown to a large scale. The country with the highest large-scale production is China, producing 53.97% of the world's total (Kawai and Murata 2016). In addition, to the high performance in the production of oil that algae have, the low use of land in cultivation and food security, another of the main advantages is their potential for the treatment of wastewater, the bio-sequestration of CO<sub>2</sub> and a short growth cycle (Hossain et al. 2020; Karmakar and Halder 2019). Microalgae are produced from photosynthetic microorganisms with the help of water, CO<sub>2</sub>, and solar radiation, more efficiently than plants, that are why microalgae can solve the problem of food vs biofuel, their high performance is enough to satisfy the global demand for transportation fuel and its production is sustainable. The main drawback in obtaining diesel through algae is the high production cost, since algae with high oil yield are required, too much water, efficient and large bioreactors. However, microalgae can grow in combustion gases, they can absorb greenhouse gases, they can also be cultivated in seawater or polluted aquifers, without compromising water for human consumption (Arif et al. 2020; Jacob et al. 2021; Nautiyal et al. 2014). In general, biodiesel production process with algae as feedstock consists of the following stages: cultivation, harvest, dehydration-drying, oil extraction, and conversion to biodiesel, as shown in Fig. 2. It is worth mentioning that the defatted biomass can be processed in parallel, for the





**Fig. 2** Production process with algae as a feedstock

generation of other biofuels. During the cultivation stage light, CO<sub>2</sub>, water, and nutrients are required for the algae; the system can be open, closed, or hybrid. Since there is dense biomass, it is collected by different methods (coagulation, flocculation, flotation, sedimentation, filtration, or centrifugation). In the dehydration and drying stage, the excess water present in the algae is removed (the harvested algae contain between 85 and 95% water). Later the oil is extracted, it is necessary to overcome the cell walls for extraction with solvent. Finally, the extracted oil is gotten into the unit used for biodiesel conversion, generally by the transesterification method, and the by-product (glycerin) is collected. The defatted and dried biomass can be treated to generate biogas, bio-oil, electricity, heat, and biofertilizers. The conversion units of harvesting, oil, and defatted biomass have useful nutrients for the cultivate unit, so they can be used by reducing the supply needed for the algal biomass to grow (Chamkalani et al. 2020; Jacob et al. 2021; Mata et al. 2010). It is important to mention that biodiesel obtained from algae has better physicochemical properties than first- and second-generations biodiesel, one of the main properties is the cetane number, which is higher when microalgae are used as feedstock, it is worth mentioning the specific cases of biodiesel obtained from *Chlorella Vulgaris* and *Spirulina* with a cetane number of 60 and 70, respectively (Arif et al. 2020; Jacob et al. 2021).

#### **2.4 Fourth-Generation Biodiesel (Genetically Modified Feedstock)**

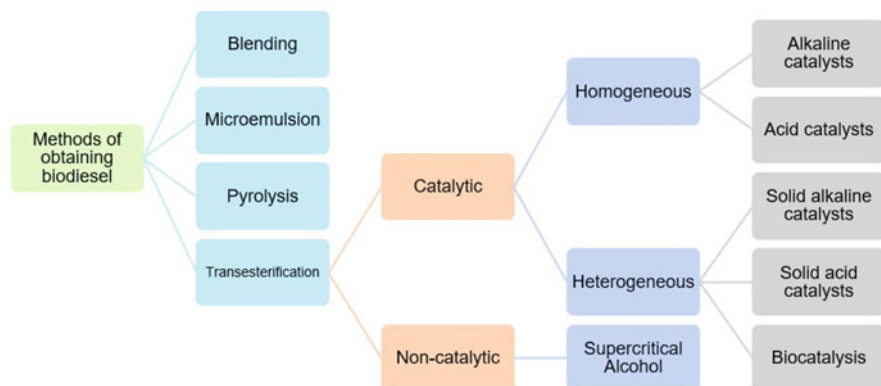
The fourth generation of biodiesel is focused on sustainably obtaining biofuel, at the same time that the process works to capture CO<sub>2</sub> and mitigate its harmful effect on the environment. The synthetic biology technologies have served as the basis for the development of fourth generation biodiesel; the feedstock for obtaining it is genetically modified algae and cyanobacteria, which convert solar energy into biodiesel through photobiological solar cells (Karmakar and Halder 2019; S. Sharma et al.

2020). The genetic modification of microalgae consists of improving the efficiency of photosynthesis, increasing the penetration of light, and reducing photoinhibition. The efficiency of photosynthesis can be improved by expanding the absorption spectrum of the feedstock. Reducing the size of the chlorophyll antenna allows for improved light penetration (Abdullah et al. 2019). The main advantages of the feedstock are high lipid content, higher CO<sub>2</sub> absorption, high energy content, and fast growth rate. And the disadvantages are the high initial investment and the recent creation of the research (Abdullah et al. 2019; Mohiddin et al. 2021). The production of biodiesel is given from the extracted oils, as it is done in other generations, various techniques are used such as transesterification, pyrolysis, dilution, among others. The biodiesel obtained complies with the established standards (ASTM D6751 y EN14214) (Cavalcante et al. 2021).

### 3 Methods for Biodiesel Obtention

According to the American Society for Testing and Materials (ASTM), biodiesel is a mono alkyl ester, produced from lipids, extracted from various feedstocks, such as vegetable oils, animals, and algae (Raheem et al. 2020; D. Singh et al. 2019). In general, the first step in the process of obtaining biodiesel begins with obtaining biomass. The second is oil extraction, there are various methods for this process, stand out extraction with a supercritical fluid, extraction assisted by ultrasound, mechanical screw press, and extraction with organic solvent (Mohiddin et al. 2021; Syafiuddin et al. 2020). The third step is the refining of the oil and the fourth and last step is the processing of the oil to obtain biodiesel (Kawashima et al. 2008).

As mentioned above, the methods for obtaining biodiesel are microemulsion, transesterification, thermal cracking or pyrolysis, and blending, as shown in Fig. 3



**Fig. 3** Classification of methods for biodiesel obtention

(Frkova et al. 2020; Raheem et al. 2020). Each of the aforementioned methods of obtaining diesel is briefly described below.

### ***3.1 Blending or Dilution***

This method consists of mixing conventional diesel with virgin vegetable oil in various proportions, reducing the viscosity of the second. The oils used for this method are mainly sunflower, rapeseed, and cottonseed oils (Yesilyurt et al. 2020). With this method there are drawbacks such as partial engine combustion, plugging of the injector, thickening of the lubricating oil, atomization difficulties, high emissions, etc. (Aghbashlo et al. 2021; Mujtaba et al. 2020; Tabatabaei et al. 2019).

### ***3.2 Microemulsion***

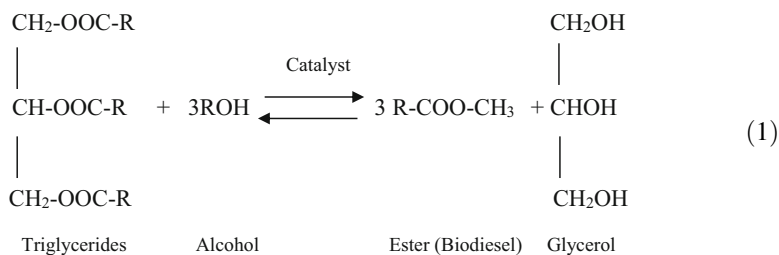
It is defined as the transparent and thermodynamically stable colloidal dispersion of a polar and a nonpolar phase, it is used for the production of hydrocarbon mixtures with the help of solvents such as ethanol and stabilized by a surfactant (Abrar and Bhaskarwar 2019; Ravi et al. 2020). This method mainly takes the biofuel viscosity to lower values, short chain alcohols can form microemulsions (Yesilyurt et al. 2020). Although this process is feasible and simple for the obtention of biodiesel, the cetane number decreases as has been observed when this method is used, it also involves several steps that increase the operating cost of this process (Ravi et al. 2020; Yesilyurt et al. 2020).

### ***3.3 Pyrolysis or Thermal Cracking***

Pyrolysis is a process by which hydrocarbons are produced by decarboxylation at temperatures above 350 °C, where a catalyst can or cannot be present (Ravi et al. 2020). It is a thermochemical process, which consists of a chemical simplification of organic material in simpler and smaller structures through the use of thermal energy without the presence of oxygen; this process is highly efficient (Yesilyurt et al. 2020). It is classified into three types: flash, fast, and conventional. Biodiesel from animal fats and vegetable, waste, and algae-based oils can be produced by using this method (Atabani et al. 2013). The decomposition process of oils for the production of biodiesel occurs as follows, it divides the oils into smaller molecules using thermal energy and the resulting liquid portion is biodiesel. The main disadvantage of biofuel production through pyrolysis is its high cost and sophisticated technology requirements (Ewunie et al. 2021; Jahirul et al. 2012).

### 3.4 Transesterification

Transesterification or alcoholysis involves the use of short chain alcohol to react the feedstock, be it oils or fats, generally methanol or ethanol, with a catalyst present (homogeneous, heterogeneous, or enzymatic), from the reaction described above, the first product obtained are fatty acid esters and glycerin is obtained as a by-product (Pinto et al. 2005). Currently, transesterification is the most extensively used to obtain biodiesel. The oil's viscosity decreases considerably with this method and is close to conventional diesel, in addition to maintaining the caloric value and cetane number, it is worth mentioning that this method is efficient and economically profitable (Arumugam and Ponnusami 2019; Mujtaba et al. 2020). When biodiesel is produced with the transesterification method, the concentration and type of catalyst are controlled, also the amount and type of alcohol together with the water content as well as the reaction time and the temperature; all these parameters influence the reaction yield. The biodiesel production process can be optimized by manipulating the aforementioned parameters (Arumugam and Ponnusami 2019). Technically, biodiesel results from the conversion of triglycerides that make up fatty acids into alkyl esters using short chain alcohol. Depending on the alcohol used, the final product can be a fatty acid methyl esters (FAME) (when using methanol) or fatty acid ethyl esters (FAEE) (when using ethanol), as shown in Eq. (1) (Hamza et al. 2021; Zhao et al. 2019).



On the other hand, catalytic and non-catalytic are the types of transesterification processes, where the first is composed of heterogeneous catalysis and homogeneous catalysis. Within the homogeneous catalysts, the acids (HCl and H<sub>2</sub>SO<sub>4</sub>) and the alkalines (NaOH and KOH) are classified. Heterogeneous catalysts are enzymes (mainly lipases) (Arumugam and Ponnusami 2019; Mujtaba et al. 2020).

#### 3.4.1 Non-catalytic Transesterification

It is a catalyst-free oil transesterification method and the existence of water and FFA does not affect the process. Also, is known as supercritical methanolysis, in which alcohol is used under supercritical conditions (high temperature and pressure), the alcohol-oil ratio is higher than that used in catalytic transesterification, and the

reaction occurs in a short time. With this method, the quality of the biodiesel obtained is preserved, as well as the conservation of the environment. It is a method that does not require washing steps or treatments with alkaline water, as is done to remove the catalyst from the product. However, the process requires more energy, thus increasing costs (Demirbas 2005; Román-Figueroa et al. 2016; Verma et al. 2016).

### 3.4.2 Catalytic Transesterification

Catalytic transesterification is classified as homogeneous, heterogeneous, and enzymatic (biocatalysis). In turn, homogeneous and heterogeneous catalysis are divided into alkaline and acidic.

*Homogeneous alkaline catalysis:* They have a low cost and a high catalytic activity, which increases the reaction speed and generates quality biodiesel as long as oils with a low amount of FFA are used. Alkali metal hydroxides, alkoxides, and carbonates are the usually employed catalysts. The alkaline catalysts par excellence in homogeneous alkaline catalysis are NaOH and KOH. These catalysts allow an increase in speed of the reaction at low temperatures and pressure, as well as being abundant and cheap (Narasimhan et al. 2021; Sivasamy et al. 2009). In addition to the above-described catalysts, sodium ethoxide ( $\text{NaOCH}_2\text{CH}_3$ ), sodium methoxide ( $\text{NaOCH}_3$ ), and potassium methoxide ( $\text{KOCH}_3$ ) are also widely used,  $\text{KOCH}_3$  and  $\text{NaOCH}_3$  generate a higher yield of biodiesel. The quantity of catalyst affects the biodiesel performance since if it is out of the required, it decreases. The lower the catalyst, the biodiesel yield is reduced or is not formed. The greater the amount of catalyst, a saponification reaction occurs, the viscosity increases and the yield of the biodiesel decreases considerably (Sivasamy et al. 2009). Homogeneous catalysis has the drawback that, attributed to the solubility of homogeneous catalysts, they are difficult to remove from diesel. In addition, a homogeneous catalyst can generate contamination of the water used during the process, which implies an additional treatment (Juan et al. 2011). However, the high catalytic performance and low cost of production make them an excellent choice as catalysts (Aransiola et al. 2014; Tan et al. 2021).

*Homogeneous acid catalysis:* Acidic homogeneous catalysts are less used to produce biodiesel in the transesterification process, attributed to their low reaction speed, their corrosive nature, and the high amount of alcohol that is needed for the reaction. However, they have a positive effect on FFA esterification, homogeneous acid catalysts do not favor soap formation, as occurs with homogeneous alkaline catalysts. For this reason, to treat feedstock with a high FFA content (tallow, used cooking oils, some vegetable oils, etc.) by transesterification, the use of homogeneous acid catalysts is recommended, to avoid saponification, as well as being economical. The acids most used in this process are sulfuric, hydrochloric, sulfonic, etc. These catalysts are less used to produce biodiesel in the transesterification process, attributed to their low reaction speed, their corrosive nature, and the high

amount of alcohol that is needed for the reaction (Aransiola et al. 2014; dos Santos et al. 2020; Tabatabaei et al. 2019; Vignesh et al. 2021).

*Heterogeneous alkaline catalysis:* Generally, the catalysts are solid and with a filtration process they can be separated from the biodiesel generated and can be reused, they have high hydrothermal stability, efficient regeneration capacity, otherwise than homogeneous alkaline catalysts, where the separation of the final product increases production costs (Aransiola et al. 2014; Juan et al. 2011). Heterogeneous alkaline catalysts are low cost, low corrosion, and environmentally friendly. Among the heterogeneous alkaline catalysts, the basic zeolites, alkaline earth metal oxides, and hydrotalcite stand out (Yesilyurt et al. 2020).

*Heterogeneous acid catalysis:* Heterogeneous acid catalysis is an alternative to the process of obtaining biodiesel, with these catalysts the transesterification and esterification reactions, can be catalyzed simultaneously (Maçaira et al. 2011). Another point to note is that with these catalysts the saponification that can occur when using homogeneous alkaline catalysts is avoided and that reduces the yield of biodiesel, in addition to that, homogeneous catalysts, produce wastewater. Easy separation of the heterogeneous acid catalyst from the final product is possible, reducing costs, they are less corrosive, less toxic, and friendly to the environment. The limitations of heterogeneous acid catalysis are the reaction temperature and energy required. Some heterogeneous acid catalysts are zirconium oxide, titanium oxide, tin oxide, heteropoly acids, acidic zeolites, and combinations (Amin 2019; Yesilyurt et al. 2020).

*Biocatalysis, enzymatic catalysis:* The deterioration of nature has led to research of more environmentally friendly methods that do not use chemical catalysts in the transesterification process. In this context, research on biocatalysis arises, which is a viable alternative in the transesterification process. Biocatalysis consists of the use of biocatalysts such as enzymes and microbial cells (Akoh et al. 2007; Robles-Medina et al. 2009; You et al. 2013). Biocatalysis requires less energy, produces less waste, and the catalyst is easy to recycle. Also, the reaction with enzymes as a catalyst does not depend on the amount of water or FFA in the virgin oil and does not generate saponification reactions (Thoai et al. 2019). Due to transesterification occur by hydrolyzing an ester bond in triglycerides, lipase (hydrolytic enzyme produced by microorganisms, animals, and plants) can be used as a biocatalyst in the production of biodiesel. Lipase can catalyze esterification and transesterification. The enzymes used as catalysts are classified into immobilized enzymes and soluble enzymes. The use of immobilized lipase is limited by its high cost, which does not allow their advantages to be used in obtaining biodiesel. Soluble lipase is less expensive, making biodiesel production viable. However, the use of biocatalysts in the transesterification of biodiesel is still under study, attributed to several problems, such as their low stability, they are not reusable, and the durability of the enzymes (Ismail and Baek 2020; Monteiro et al. 2021; Tan et al. 2021). Therefore, at present, transesterification with homogeneous catalysts is the most favorable for the production of biodiesel on a large scale and research and development in the field of enzymatic biocatalysts is necessary to reduce costs and increase the efficiency in the production of biodiesel (Tan et al. 2021).

*Biocatalysis, green catalysis:* Currently, there is research on other types of catalysts that replace commercial (chemical) catalysts with innovative green catalysts. Green catalysts are heterogeneous catalysts derived from waste biomass, significantly reducing production costs and biodegradability. Within this classification are ash catalysts derived from biomass (Pathak et al. 2018; Saputra et al. 2018), industrial waste ash catalysts (Ho et al. 2014; Xiang et al. 2017), mud catalysts (Wahyudi et al. 2017), catalysts based on animal bones (Corro et al. 2016; Obadiah et al. 2012), among others.

**4 Applications and Importance of Biodiesel as a Renewable Energy Source**

The implementation of biodiesel in diesel engines installed in trucks, buses, cars, and other vehicles, as well as in stationary heat and power applications, is its principal application (REN21 Renewables Now 2020). Biodiesel can be used directly in engines, or it can be blended with diesel derived from petroleum. Pure biodiesel (100% biodiesel) is commonly called B100 and blends of biodiesel with diesel derived from petroleum are named BXX, where XX represents the biodiesel proportion present in the blend, for example, B80 contains 80% biodiesel with 20% diesel (Mohd Noor et al. 2018). Sometimes engine little or no modifications are needed when using B100, but no modifications are needed when using biodiesel blends with petroleum-derived diesel (Demirbas 2009). As mentioned above, biodiesel can be obtained from various feedstocks. Rapeseed and soybean oils produce biodiesel in Europe and the United States. While in Malaysia, Thailand, Indonesia, Nigeria, and Colombia produce biodiesel from palm oil (Mohd Noor et al. 2018).

World biodiesel production in 2019 was 47.4 billion liters. The countries that lead the first five positions in biodiesel production represent 57% of world production. Being Indonesia the country that leads the world production with 17% of diesel produced, surpassing the United States with 14% of production, Brazil produces 12%, followed by Germany with 8% and France with 6.3% of world production, as shown in Table 2 (REN21 2020).

Derived from the COVID-19 pandemic in 2020 and 2021, world biodiesel production decreased to 46.8 and 45 billion liters, respectively (REN21 2021, 2022). In the last two years, Indonesia continues to lead the world ranking, followed

**Table 2** World biodiesel production in 2019

Rank	Country	Biodiesel produced (%)
1	Indonesia	17
2	United States	14
3	Brazil	12
4	Germany	8
5	France	6.3

by Brazil or the United States in second place, Germany and France have remained in fourth and fifth place, respectively (REN21 2021, 2022).

## 5 Conclusions

Today the population increase has caused the decline of fossil fuels, in this scenario biofuels emerge, with characteristics similar to fossil fuels but obtained from biomass. One of the most developed and researched biofuels is biodiesel. As mentioned in this work, biodiesel is an ester obtained from triglycerides (edible oils, inedible oils, microalgae, and genetically modified plants), by reacting with alcohol and a catalyst; the aforementioned reaction corresponds to a transesterification process. At present, research is being done on this and other processes for obtaining diesel, especially around biocatalysts (enzymes and waste biomass) that reduce the environmental and economic impact generated by conventional catalysts. In addition to transesterification, there are other methods for obtaining biodiesel such as blending, microemulsion, and pyrolysis. Depending on the feedstock and the method of obtaining it, the physicochemical properties of biodiesel may vary, always seeking to comply with the established standards (ASTM D6751 and EN14214). Finally, the biodiesel generated is used mainly in engines that work with diesel, its use can be carried out in a pure way (B100) as well as a mixture with petroleum diesel (B5, B15, B30, etc.). Biodiesel can contribute to reducing the environmental impact caused by fossil fuels, resulting in an environmentally friendly and sustainable fuel.

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# Biotechnological-Based Production of Bio-Oil and Vegetable Oil



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**Abstract** Bio-oil or bio-petrol complex composition contains lignocellulosic biomass chemical products. This is the direct result of the biomass thermal decomposition; its structure is very similar to the fossil-based petrol. On the other hand, vegetable oil possesses characteristics comparable to conventional diesel ones. Vegetable oils in diesel engines lead to significant reductions in sulfur dioxide, carbon monoxide, smog, and noise emissions.

For bio-oil and vegetable oil production microalgae-controlled cultures known as potential raw materials are used for biofuel production due to their lipid high content. By means of a photosynthetic process, microalgae produce sugars for its structure as well as vegetable oils, these last ones are leveraged as biofuels. In this chapter, processes related to the obtaining of bio-oil and vegetable oil, the processes stages, their advantages, disadvantages including their potential and real-life applications (airlines) as biofuels. This chapter also includes the processes under which cooking oil is subjected to provide insights on some efforts made these days to incorporate it into the biofuels market.

**Keywords** Bio-oils · Pyrolysis · Hydrothermal liquefaction · Seeds

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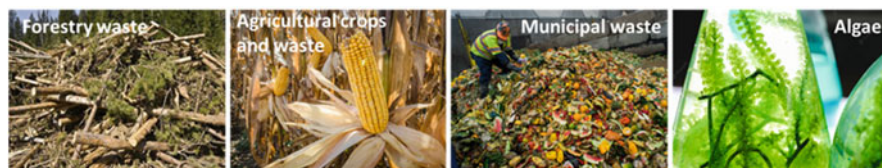


# 1 Introduction

Bio-oil is a type of liquid fuel derived from biomass, for example, agricultural crops, algae as well as municipal, forestry, and agriculture wastes (Xu et al. 2011) as shown in Fig. 1. Bio-oil is made up of a mixture of highly oxygenated compounds, acids, trace water as well as acids, alcohols, aldehydes, esters, ketones, and phenols. In appearance, it is dark brown, free-flowing fluid and with a characteristic smokey odor (Huber and Corma 2007). It is important to mention that it can be used as a substitute for conventional fuels after it is upgraded, which can be achieved under different schemes (Omar et al. 2019). This upgrading stage is a strict stage since bio-oil usually goes through several chemical reactions like polymerization which then induces a raise in viscosity when in storage (Yiin et al. 2014). It is also considered a green biofuel because of its biodegradability and neutrality when it comes to the generation of greenhouse gases, e.g., low CO<sub>2</sub> and even less than 50% nitrogen oxide emissions (Winjobi et al. 2016).

Along with bio-oil, there are also vegetable oils that are defined as a type of fats coming from some seeds, nuts, cereal grains, and fruits (Hammond 2003). Vegetable oils and other fat types are oleaginous materials that are used for human consumption, in animal feed, medicinal purposes, and technical applications (Arienzo and Violante 2007). Regarding vegetable oil as a biofuel, it is considered as an option in diesel engines and heating oil burners. Furthermore, when vegetable oil is used straightforwardly as a fuel, in either modified or unmodified equipment (engines), it is designated as straight vegetable oil (SVO) or pure plant oil (PPO) (Altin et al. 2001). Curiously, in the seventeenth century, Rudolph Diesel attempted the operation of biofuel-driven engines: the peanut oil engine run in 1900 and the vegetable oil run engine in the 1930s, whose fuel came from processed food crops (Ziolkowska 2020). On the other hand, vegetable oil wastes can further be used as a source of biodiesel (Selaimia et al. 2015) after undergoing processes such as transesterification (Yasvanthrajan et al. 2021). Hence, it is an oil that can be advantageous because of this duality. But the details of the vegetable oil-biodiesel topic will not be mentioned here because they are covered already in chapter “Biological methods for diesel formation”.

The aim of this chapter is first, to give an insight into the approaches used for the obtention of bio-oil and vegetable oil (in its two main forms) as well as the treatment of cooking oil to give the reader an outlook on the involved techniques and later, to jump onto perspective concerning their potential applications and future.



**Fig. 1** Biomass sources for bio-oil production



## 2 Bio-Oils: Generation and Potential Applications

### 2.1 Processes to Produce Bio-Oil

Bio-oil is also known as pyrolysis oil, pyrolytic oil and liquid wood (Czernik and Bridgwater 2004). Among some of its uses, we can mention energy generation and the production of chemical and carbon-based materials besides its use as a biofuel (Yasvanthrajan et al. 2021). Biomass in the form of agricultural crops, algae, municipal waste, and agricultural and forestry by-products can be used to manufacture bio-oil and there are two main procedures to transform it namely: pyrolysis and hydrothermal liquefaction (Xiu and Shahbazi 2012). Examples of feedstocks used in general are cellulose, hemicellulose, and lignin coming from the already mentioned biomass sources (Li et al. 2017).

Pyrolysis and hydrothermal liquefaction (HTL, also known as hydrous pyrolysis) are comparable technologies that have been under study for a long. Both can be used to treat any of the biomass types mentioned above. Table 1 is a comparison between the two methods and differences are noted in, for example, the oxygen and water content in the biomass used, pressures and temperature values under which both processes take place, and the degree of the obtained final biofuel product.

#### 2.1.1 Pyrolysis

Pyrolysis is a thermochemical approach that can be used on any organic material. It can be carried out on non-modified or pristine materials as well as on mixtures. First, the material is exposed to high temperatures, and then, in an oxygen-absent atmosphere it passes by chemical and physical separation into different molecules. This decomposition happens because of the short-term thermal stability of chemical bonds of the involved materials, which allows their disintegration by using heat. Thermal decomposition promotes the formation of new molecules. This, in turn, boosts the reception of products with a different, often more superior quality than the original residue (Al-Haj Ibrahim 2020). In the case of biofuels, this definition comes down to the thermochemical decomposition of biomass feedstock at medium (300–800 °C) to high temperatures (800–1300 °C) in an inert atmosphere (Zhang

**Table 1** Comparative between pyrolysis and hydrothermal liquefaction (HTL)

Parameter/characteristic	Pyrolysis	HTL
Biomass moisture content	< 10% mass fraction	>50% mass fraction
Temperature	350–550 °C	200–350 °C
Pressure	Atmospheric	2–20 MPa
Oxygen and water content	High	Low
Viscosity	Low	High

(Hognon et al. 2015) (Dimitriadis and Bezergianni 2017) → (Hognon et al. 2015; Dimitriadis and Bezergianni 2017)

et al. 2019). Depending on the operating condition, pyrolysis can be classified into three categories according to the operating condition used (processing temperature, heating rate, biomass particle size, and so on): slow, fast and flash depending on the time it takes for the completion of thermal decomposition of the feed materials (Jahirul et al. 2012). Pyrolysis takes place within reactors and, they are of different types, for example, fixed bed, batch or semi-batch, rotary kilns, fluidized bed reactors, microwave-assisted, and even some cutting-edge like plasma-based or solar ones, among others (Matayeva et al. 2019). The types of pyrolysis that are used for bio-oil generation are fast, flash, and to a lesser extent, slow one among others (Pawar et al. 2020).

With the purpose of achieving high yields in the pyrolysis procedures for bio-oil generation, it is necessary to implement a feedstock pretreatment in a way that it can promote the appropriate heat transfer rates, but it needs to be taken into account, that the type of feedstock used is an important factor. Regarding this topic, these treatments are thermal and go from range from simple drying to severe treatments; their purpose is the removal of moisture and some temperature-sensitive components before going through pyrolysis, at high temperatures, with or without the presence of an oxygen atmosphere, the most used pretreatment are drying and torrefaction (Yue and Mani 2018). The aspects that explicitly affect the pyrolysis process in the pretreatment stage are the following: particle size of biomass and alkali and alkaline earth metallic (AAEM) species presence because they accelerate the aging of the final product, therefore affecting its storage (Rezaei 2019); (Carpenter et al. 2014) → (Rezaei 2019; Carpenter 2014).

Fast pyrolysis is the most efficient and adequate method for the conversion of biomass into liquid products with an efficiency of even 80% (Shemfe et al. 2015). Hence, making it the most used technique for obtaining bio-oil as shown in Table 2, no matter whether the biomass used is a plant, agricultural, municipal waste, or algae. Besides the kinds of pyrolysis setups, the table also displays the types of biomass, bio-oil yields and for further reference the production yields of other by-products like bio-char, Diesel fraction, and generated waste. These parameters are of crucial importance because a perspective on the proper pyrolysis setup is useful to determine the type of biomass that can be treated.

For further reference, research works about other pyrolysis methods other than the fast one, used in bio-oil production can be consulted, for instance, solar (Rahman 2020), slow (Gerçel 2011), flash (Madhu et al. 2018), and the like. Additionally, researchers have also devoted time to the analysis of biomass and its influence on bio-oil properties (Gholizadeh et al. 2019).

### 2.1.2 Hydrothermal Liquefaction

Hydrothermal liquefaction (HTL) is the process by which thermal depolymerization process is carried out for the conversion of wet biomass into bio-oil, under a moderate temperature (200–400 °C) and pressure (5–20 MPa) scheme (Akhtar and Amin 2011). In more explicit terms, hot pressurized water as well as other solvents

**Table 2** Research works in which fast pyrolysis was used for bio-oil production

Pyrolysis procedure	Setup	Biomass type	Yields	Reference
Fast	Microwave assisted	<i>Vitis vinifera</i> (cv. Cabernet), crop	Bio-oil (34.9%) and gas (45.7%) or the production of high amounts of bio-char (up to 71.4%).	(Bartoli et al. 2018)
Fast	Vacuum ablative	Tobacco residues	All of them are variable according to the conditions of the torrifaction pretreatment	(Khuenkaeo et al. 2020)
Fast	Ablative hot surface	Straw and miscanthus	–	(Conrad et al. 2019)
Fast	Tube furnace	Greenhouse crop residue (GCR)	51.9%–79.8%wt (medium fraction, diesel) bio-oil, according to the different solid GCR material and to the temperature used	(Iáñez-Rodríguez et al. 2021)
Fast	Fluidized bed reactor	Cassava plants	62 wt.% and 65 wt.% on dry basis	(Pattiya 2011)
Fast	Custom-built auger reactor	Commercial <i>Chlorella vulgaris</i> and cultivated microalgae	47.7% wt at 550 °C	(Sotoudehniakarani et al. 2019)
Fast	Spouted bed reactor	<i>Nannochloropsis</i> (NC), <i>Tetraselmis</i> (TS) and <i>Isochrysis galbana</i> (IG) microalgae	NC or TC: Bio-oil (58%), bio-gas (13%), and bio-char (30%). IG: Bio-oil (66%) and bio-char (21%)	(Azizi et al. 2020)
Fast	Microwave-assisted	Soap stock and waste tire	Soap stock: 42.64% wt, waste tire: 28.74% wt	(Dai et al. 2017)
Fast	Microwave-assisted	Bamboo sawdust and waste tire	29.59 wt.% to 37.86 wt.% and 37.86 wt.% when bamboo sawdusts to waste tires ratio was varied	(Wang et al. 2017)

are used as the reaction medium and reactant, respectively. Then, biomass is depolymerized into bio-oil, bio-char, gases, and water-soluble components in an aerobic or anaerobic environment (Cao et al. 2016). Molecules hydrolyzed by hydrothermal liquefaction are quite reactive and repolymerize very fast to form oily substances (Marulanda et al. 2019). Among the advantages of this method its reliability, availability, and usefulness to treat biomass with high moisture content (such as wood-, waste-, and algae-based biomass) and its benevolence environmentally speaking (Gollakota et al. 2018) can be mentioned. The three main stages of HTL are depolymerization followed by decomposition and recombination.

**Table 3** Research works related to bio-oil production by means of hydrothermal liquefaction (HTL)

Biomass	Parameters	Yields	Reference
Coffee grounds	Different retention times (5 min, 10 min, 15 min, 20 min, and 25 min) and temperatures (200 °C, 225 °C, 250 °C, 275 °C, and 300 °C). Varied water/feedstock mass ratios (5:1, 10:1, 15:1, and 20:1)	Crude bio-oil yield: 47.3% at a 10-min retention, 275 °C reaction temperature, and a 20:1 mass ratio of water	(Yang et al. 2016)
Waste <i>Cyanophyta</i>	Reaction temperatures: 260–420 °C. Retention times: 5–75 min. Feedstock/water ratios: 0.02–0.3)	Bio-oil yield: 29.24% at 350 °C, 60 min, and 0.25 f/w ratio	(Song et al. 2017)
<i>Amphiroa fragilissima</i> biomass	Reaction temperatures: 220–340 °C Retention time: 60 min	Bio-oil yield of 28.9 wt% at 320 °C	(Arun et al. 2020)
<i>Nostoc ellipsosporum</i> in municipal wastewater	Reaction temperatures: 240–320 °C Retention times: 60 min	Bio-oil yield of 24.62 wt% at 300 °C	(Devi and Parthiban 2020)
Blackcurrant pomace	Reaction temperature: 300 °C Retention time: 60 min	Bio-oil yield: 28%	(Anouti et al. 2016)
Low-lipid <i>Scenedesmus</i> sp. microalgae	Reaction temperatures: 250, 300, and 350 °C Retention times: 7, 17, and 30 min	Bio-oil yield: 20–36 wt. %	(Wądrzyk et al. 2018)
Refinery wastewater was used to produce lipid-rich bacterial biomass	Reaction temperature: 250 °C Retention time: 60 min	Bio-oil yield: 26.5 ± 3.8% (w/w)	(Paul et al. 2020)

As for biomass intended to produce bio-oil, unlike pyrolysis, it does not need any drying pretreatment whatsoever. This is an advantage because it represents a lowering in production costs. However, both methodologies must be improved further, which will be discussed later on at the end of the chapter. For the moment, as for research works related to HTL, a list in Table 3 is presented the same way as in Sect. 2.1.1 to give the reader an insight into the type of biomass (mostly related to moisture content) used. From this table, the most important parameters are the retention times, temperatures, and the water to feedstocks ratios.

### 3 Potential Applications of Bio-Oil

It has been suggested that the potential of bio-oil as a biofuel is aimed at its use in boilers, engines, and turbines to generate heat and power. They have also been evaluated for electricity and heat production in boilers, furnaces, and combustors (Pattiya 2018). Even though bio-oil is considered a renewable feedstock for sustainability applications, there are some drawbacks that have impeded so far, its whole commercialization like poor fuel properties such as low pour point, flash point, and high viscosity. On the other hand, there are advantages associated with it, for example, its comparable competitiveness with fossil fuels when it comes to direct combustion without the need for any additives, engine combustion efficiency increases when bio-oil is co-fired with fossil fuels.

### 4 Vegetable Oils: Processing to Produce Bio-Oils

Vegetable oil, when used as a fuel directly without any kind of modification is also known as straight vegetable oil (SVO), pure vegetable oil (PVO), or pure plant oil (PPO). Vegetable oils have been investigated for their use as biofuels, mostly in diesel engines, and as sources of biodiesel by undergoing esterification.

#### 4.1 Production of Vegetable Oil as a Biofuel

Straight vegetable oil (SVO), pure vegetable oil (PVO), and pure plant oil (PPO) refer to oils from plants and seeds that have not been modified chemically and whose purpose is its application as a fuel in diesel engines. SVO/PVO/PPO has the advantage of being produced at relatively low costs. From Fig. 2, it can be observed that they can be obtained from biomass as different as edible or nonedible fruits,



**Fig. 2** Some biomass sources for vegetable oil

seeds of varied crops, like palm, nuts, peanuts, cotton, sunflower, jatropha, soybean, rapeseed, groundnut, among others (Nde and Foncha 2020).

The methods used to modify the biomass are mechanical (pressing) and chemical (solvent) extraction, followed by chemical and/or physical refining to improve oil quality (Cheng et al. 2019). In the first one, an expeller press is used to exert pressure on the biomass to cause the oil to flow. There are plenty of factors that influence oil extraction efficiency, for instance, the biomass type, cleaning, temperature, and press configuration. The mechanical pressing method normally produces yields of only 65–70% of the oil whereas 80% is considered a favorable alternative for oil extraction. Most large commercial operations use a gentle pressing operation to extract 60–70% of the oil, while the remaining is solvent extracted (Alonge and Jackson 2019).

On the other hand, chemical solvent extraction is a process involving the extraction of oil from the biomass by means of treatment with a low boiler solvent. This method recovers almost all the oils and leaves behind only 0.5%–0.7% residual oil in the untreated material. The solvent extraction method can be utilized directly for any low-oil content raw material. It can also be used to extract pre-pressed oil cakes obtained from materials that have large oil contents. Because of the high percentage of recovered oil, solvent extraction has become the most attractive method of extraction for oils and fats (Karmakar et al. 2017).

All impurities have to be removed from the raw oil, which can be done by any of two pathways: one is by decantation in consecutive tanks, or by direct filtering (Patel and Shrivastava 2017).

As a final remark on vegetable oils, it has to be noted that little is mentioned on either microorganisms or any algae type in the literature regarding biofuels production. For example, it has only been reported that microbial-based oils from oleaginous microorganisms serve as a potential alternative to generate biofuels because of the similarity of their fatty acid composition to the one found in vegetable oils (Christophe et al. 2012) (Matuszewska 2016) → (Christophe et al. 2012; Matuszewska 2016).

## ***4.2 Potential Applications of Vegetable Oil***

It has been reported that even though vegetable oil, as a biofuel (SVO/PVO/PPO) might act as a potential energy source instead of fossil fuels because of its properties similar to those of diesel, it still bears some drawbacks that can be reduced as time passes by.

Its viscosity is a limitation for use in diesel engines because it can cause incomplete combustion and carbon deposition within the combustion chamber, also related to low net calorific value and low Cetane number. The cloud point tends to be high, too. Contaminants, like phosphorous traces, are also present in unrefined PPO/PVO/SVO. Oxidation stability and iodine number, acidity, and carbon residue are also relevant issues regarding PPO/PVO/SVO quality. Hence,

the solution to this, is blending it with other fuels such as diesel or ethanol, for example, and by modifying the engines to overcome this (Che Mat et al. 2018) (Russo et al. 2012) → (Che Mat et al. 2018; Russo et al. 2012). In fact, this has been assessed successfully, by diverse research groups and companies in different parts of the world, using vegetable oils from rapeseed, jatropha, and coconut among others, alone or mixed with other fuels (Ramadhas et al. 2004). The Bioenergy and Food Security (BEFS) Approach (FAO) has reported more applications than those related to diesel engines, e.g., the potential one to develop electricity generation systems for supply in rural areas without this service, by using SVO as the fuel source. These systems are evaluated by comparing three typical power generation capacities (10 kW, 40 kW, and 100 kW).

In May 2021, Air France-KLM announced that an Airbus A350 flew from Paris to Montreal fueled by cooking oil produced by Total at its Oudalle plant near Le Havre as well as La Mede, a refinery located in southern France converted to biofuels in 2019. This is very good news since it is an application that supports that vegetable oil can run a plane (Air France-KLM press area, May 2021).

## 5 Remarks on Pyrolysis and Hydrothermal Liquefaction Related to Biotechnology

Pyrolysis and hydrothermal liquefaction have already been defined and mentioned as two procedures to obtain either bio-oil or vegetable oil, but they are not biological routes, even though they can be used for microalgae conversion. Thus, a more oriented biotechnological perspective on biofuel production must be given, too. Both techniques are potentially adequate to work synergistically with biotechnology-based approaches for the improvement or upgrading of obtained products, e.g., biofuels, especially due to the presence of excess moisture.

In the case of pyrolysis oil or its fractions, biotechnological approaches can aid in the treatment of low molecular weight molecules found in the hydrophilic fractions of products and hence, improve final products. For instance, screening and strain engineering can be carried out for the improvement of efficient biochemical conversion of oils obtained by means of pyrolysis. Another alternative is the conversion of pyrolysis oil into microbially utilizable feedstocks (Arnold et al. 2017).

Concerning hydrothermal liquefaction (HTL), it can be coupled to methodologies such as recirculation (Chen et al. 2020), supercritical gasification (Okolie et al. 2019), anaerobic digestion (Salman et al. 2019), and papermills (Vaez and Zilouei 2020).

All the aforementioned processes are still under exhaustive study to the current difficulties for scaling to industrial levels and their relation to biotechnology relies on the fact the source to obtain fuels is biomass.

## 6 Cooking Oil, Lastly but Not Least: Processing and Opportunities

Cooking oil was not mentioned explicitly as vegetable oil, but it is. It is not used directly as a biofuel but as a source to produce other ones in the form of its waste product known as waste cooking oil (WCO) which is also considered municipal solid waste (MSW). This represents a sustainable opportunity not only to manufacture biofuels but also to recycle waste to manufacture another product. To get a bigger and clearer picture, for instance, in the European Union (EU) it has been reported that approximately between 100,000 and 700,000 tons per year of WCO are generated (De Feo et al. 2020), whereas in South Africa 200,000 tons per year were reported. Additionally, similar massive amounts (also in tons per year) are reported for countries such as Canada (120,000–135,000), Japan (60,000), China (45,000), and Malaysia (60,000). This WCO comes from homes, bakeries, restaurants, food stands, and the like, basically from cooking (Awogbemi et al. 2019). With reference to these data, the reader can comprehend the enormous field there is for the generation of biofuels and the benefit of handling waste products to avoid water contamination, for example, and thus, working on sustainable alternatives to partially take care of the planet.

### 6.1 *From Waste Cooking Oil to Other Biofuels*

This is by far, the first example of how to take advantage of WCO. As mentioned in Chap. “Biological methods for diesel formation”, biodiesel is clearly the top bio alternative to diesel. The process to go from WCO to biodiesel is that of transesterification which consists of the WCO reacting with short-chained alcohol, methanol, or ethanol, in the presence of a catalyst to yield fatty acid alkyl esters (Topi 2020).

Another biofuel that can be obtained from WCO is the biojet fuel (it can also come from other biomass such as vegetable oils, sugars, animal fats, and even waste materials). The method used for this is the hydrotreatment of esters and fatty acids that allows for the conversion of triglycerides and unsaturated fatty acids to biojet fuel by means of deoxygenation, isomerization, and cracking (Goh et al. 2020).

There is also sustainable aviation fuel (SAF) used in air crafting. It has similar properties to those of the usual jet fuel, but it leaves a much smaller carbon footprint. SAF can be obtained from a variety of feedstock that also includes WCO. To start with, it has to be mentioned that there are seven ASTM-approved types of technologies to produce SAF, but only two require WCO as feedstock are Hydroprocessed Esters and Fatty Acids (HEFA) and Catalytic Hydrothermolysis Jet fuel (CHJ). Concerning the HEFA process, it involves the deoxygenation and hydroprocessing of WCO in order to manufacture a pure hydrocarbon-based fuel blending material.



On the other hand, as for, CHJ, the hydrothermal liquefaction approach is used. HEFA was ASTM-approved in 2011 while CHJ was in 2020 (IATA 2019).

Other techniques to obtain WCO-based biofuels are pyrolysis, fast, and catalytic pyrolysis (bio-oil) which were already covered in this chapter, hydrocracking (bio-oil, biodiesel, and biokerosene) as well as gasification (syngas H) (Nascimento et al. 2022).

## 7 Conclusions

Bio-oil is a biofuel that will continue to be investigated for its further use and the methodologies to produce it are well-established, pyrolysis and hydrothermal liquefaction. Also, there is a great area of opportunity for them to be coupled to biological routes in order to improve and purify bio-oil due to issues such as polymerization when in storage. In the case of vegetable oil, very few approaches to obtain it are covered in literature, and they are quite simple. Also, biotechnological routes used, or at least, coupled, for the generation of vegetable oils are not covered either, which can be attributed to the fact, that in general, vegetable oil is treated to generate biodiesel, but it is promising if more research on it is done.

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# Biotechnology in Hydrogen Generation



Evelyn B. Díaz-Cruz and Maria del Pilar Rodriguez-Torres

**Abstract** Hydrogen is environment friendly, it does not generate polluting emissions during its combustion. Currently, most of the hydrogen production comes from petroleum-derived fuels where industrial processes intervene to obtain gaseous hydrogen, some of them include reforming with natural gas, partial oxidation of methane, and coal gasification. Other processes involve the thermochemical splitting of water. Other processes involve thermochemical water-splitting, photoelectrochemical water-splitting, and electrolysis of water. Nevertheless, biological approaches are being investigated by hydrogen production (biohydrogen). Such methods include biomass-derived liquid reforming, biomass gasification, photobiological processes, and microbial biomass conversion, which are biotechnology oriented. This chapter will deal with biohydrogen production from biomass, the benefits, drawbacks involved, and applications.

**Keywords** Hydrogen · Biomass feedstocks · Biological processes · Hydrogen

## 1 Introduction

In the universe hydrogen ( $H_2$ ) is the heartiest element. However, on our planet, this element does not exist in gaseous form therefore, gas hydrogen must be fabricated from raw materials that contain  $H_2$  compounds, being considered as an energy carrier, rather than a primary power source. In the energy crisis of the 1970s, the

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use of hydrogen as a source of energy became famous, at which time hydrogen was touted as the “fuel of the future” (Benemann 1996). However, to this day all hydrogen produced comes from hydrocarbon feedstocks such as natural gas, coal, and oil, therefore  $H_2$  is currently not renewable or carbon neutral. Besides,  $H_2$  production has a large carbon footprint (Lee et al. 2010). Beginning of the 1800s, recent research proposed that algae and bacteria had the potential to produce hydrogen (Grimes et al. 2007). However, it was not until the early 1970s that the biological production of hydrogen was first considered a practical possibility. As a result of several meetings sponsored by the National Science Foundation (NSF, Washington, DC) on biological hydrogen production (Gibbs et al. 1974).

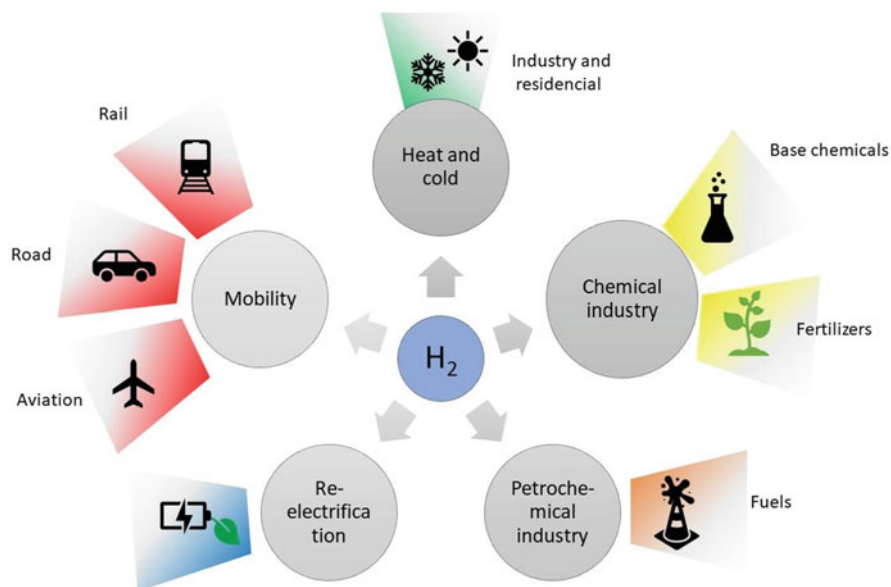
Hydrogen production, become interesting again in the 1990s when it became apparent the atmospheric pollution by nonrenewable fuels and the so-called global warming emerged, therefore, biologic hydrogen manufacturing turns a focus of governmental support, principally in Germany, Japan, and the United States (Levin et al. 2004). There are two main routes to produce hydrogen, based on the methodology used, the first being by physicochemical methods and the second based on biological processes. Physical–chemical processes use fossil fuels and water as feedstocks, while biological processes use organic compounds (biomass) as feedstocks (Guo et al. 2010). Since this book deals with biotechnology, herein we will focus only on the biological processes for hydrogen manufacturing, achieved by photosynthesis, fermentation, and microbial electrolysis cells (Lee et al. 2010).

## 2 Hydrogen as Fuel

One of the biggest challenges of the 2020s is to change common habits to stop climate change. The key to achieving this challenge is to significantly increase renewable energy sources as well as integrate these into a network that can work between different types of renewable energies, achieving their autonomy, on the other hand, a coupling between the infrastructures industrial and mobility with the renewable energy is required.

Hydrogen ( $H_2$ ) is a very valuable energy source, which has a high energy content of 142 kJ/g (Das and Veziroglu 2008). Since the decade of 2000, its use as an energy carrier has been especially inviting due to its applications as a source of power for chemical fuel cells (Singh Yadav et al. 2018). In the industrial sector,  $H_2$  has various applications for example for the hydrogenation of products, such as heavy oils in the production of gasoline, food, and ammonia for fertilizers (The Future of Hydrogen—Analysis).

However, hydrogen has unprecedented momentum, due to its “green” production and is presently earning great help from the government administration and entrepreneurs throughout the planet, generating and updating strategies and plans fast-enlarging.  $H_2$  as a fuel has been predicted to have great potential to meet global energy demand, including the capacity for renewable energy storage. It also attempts to replace the current nonrenewable energies of the sectors including long-haul



**Fig. 1** Hydrogen as the energy source of the future

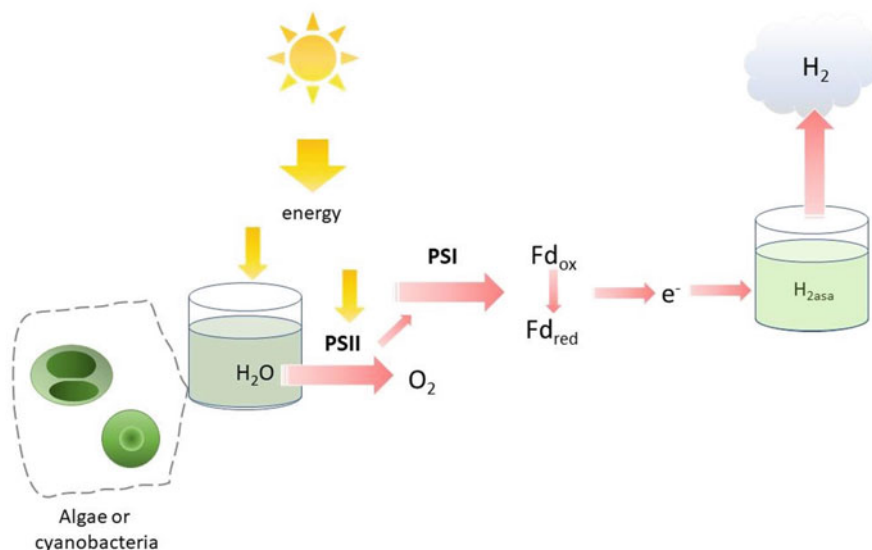
transport, the chemicals industry, and re-electrification, among others. With all the above, you can contribute to significantly improving the quality of the air we breathe, and therefore, this leads to increased energy security (The Future of Hydrogen—Analysis). In the scheme of Fig. 1, can see the applications different that hydrogen as fuel can offer.

Hydrogen as a source energy of itself is a storage medium because due to its gaseous aggregation state, it can be stored in large underground storage facilities, transported to the end user by a pipeline of economic infrastructure, and even facilitates transportation on long-distance due to its physicochemical properties.

### 3 Hydrogen Production Processes

There are several ways to obtain hydrogen, and these can be classified into two main groups such as observed in Fig.2: Physical–Chemical process and Biological processes, within this general classification there are other subclassifications, which are dependent on the feedstock that is obtained. Physical–Chemical process two raw materials are used: fossil fuels and water, however, when  $H_2$  is obtained from organic compounds, large amounts of  $CO_2$  emissions are produced (Mackenzie 2019). On the other hand, in biological processes the raw material is biomass, this fact facilitates the use of non-aggressive catalysts in the environment, for example, it is known that some kinds of microorganisms are usually used to decompose biomass





**Fig. 2** Direct Bio-photolysis pathway in green algae and cyanobacteria

under standard conditions of pressure and temperature, however, a disadvantage of these processes is that they are for small-scale production and must also be carried out in places where biomass is an available resource (Das and Veziroglu 2008).

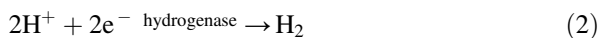
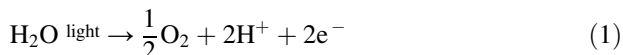
In this chapter, we will focus on biological processes, that are largely affected by operating conditions, such as temperature, pressure, pH, type of reactor, substrate, among others, and of course, some other process conditions such as the retention time, the microbial activity and the final yield of the product will depend on the type of microorganism used as a catalyst agent in the decomposition of the biomass (Wang and Yin 2019). The biological processing of hydrogen can be divided into photosynthetic, fermentative, and hybrid production routes. In all cases, hydrogen is the product of the elimination of the reducing agent of hydrogenase or nitrogenase activity (Lee et al. 2010).

### 3.1 Hydrogen Production by Bio-Photolysis

The process in which the visible spectrum of electromagnetic radiation intervenes as a catalyst in the reaction is called bio-photolysis, two types of direct and indirect bio-photolysis are known (Akhlaghi and Najafpour-Darzi 2020).

### 3.1.1 Direct Bio-Photolysis

When the energy from the solar spectrum is converted directly into hydrogen through photosynthesis reactions, it is called direct bio-photolysis. This normally happens in photosynthesis with some substrates such as algae. The simplicity of this process makes it highly attractive since only an easily accessible substrate is required. (McKinlay and Harwood 2010). The overall reactions in this process are described as follows:



In this type of bio-photolysis, photosystem II (PSII) absorbs energy from the visible spectrum, and the electrons photogenerated in this process are transferred to ferredoxin (Fd) using the energy absorbed in the photosystem I (PSI) process. Subsequently, hydrogenase ( $\text{H}_{2\text{ase}}$ ) participates, which is an enzyme that catalyzes the reversible oxidation of molecular  $\text{H}_2$  and has a key part in microbial digestion. The  $\text{H}_{2\text{ase}}$  then accepts the electrons directly from the reduced ferredoxin to produce  $\text{H}_2$  (Das and Veziroglu 2008), as illustrated in Fig. 2.

To carry out the direct bio-photolysis process, only two groups of organisms green algae and cyanobacteria have been reported. Under anaerobic conditions, when illuminated green algae can produce hydrogen or absorb it through the process of fixing  $\text{CO}_2$  in the dark (Kim and Kim 2011). On the other hand, cyanobacteria belong to the group of prokaryotes that have the oxygenated photosynthetic capacity, they are the only prokaryotes that carry out this type of photosynthesis, which is why they are also called oxyphotobacteria. On the other hand, cyanobacteria can fix nitrogen under certain conditions. (Lucia Ghirardi et al. 2009). The following describes the bio-photolysis process with each of the mentioned organisms:

1. **Green algae:** In general, through the process of photosynthesis with algae, water-splitting can be achieved into  $\text{H}^+$  and  $\text{O}^{2-}$  ions, and when the hydrogenase enzyme is used, it is possible to transform  $\text{H}^+$  ions into gaseous  $\text{H}_2$  (Singh Yadav et al. 2018). One of the algae best known as a hydrogen producer is *Chlamydomonas reinhardtii* (Ghirardi et al. 2000).  $\text{H}_{2\text{asa}}$  activity has also been found in certain species of green algae such as *Scenedesmus obliquus* (Florin et al. 2001), *Chlorococcum littorale* (Ueno et al. 1999), *Playtmonas subcordiformis* (Guan et al. 2004), and in *Chlorella fusca* (Winkler et al. 2002a). Previous studies have compared the  $\text{H}_{2\text{asa}}$  activity of different types of algae, a study in 2002 reported that the enzymatic activity of *C. reinhardtii* (200 nmol / g Chl a.h) is higher than *Scenedesmus* sp. (150 nmol / g Chl a.h) (Winkler et al. 2002b). The water and the electromagnetic spectrum radiation as the chief sources of energy for the biocatalyst with green algae are very attractive for the generation of hydrogen since water is the source of electrons, and no carbon emissions are emitted; second, water is an available and affordable

resource; third,  $\text{CO}_2$  is reduced because is consumed as a precursor in this process (McKinlay and Harwood 2010). However, the production of hydrogen with algae has faced some drawbacks such as sensitivity to oxygen and competition between hydrogenase and other electron transport pathways (Lucia Ghirardi et al. 2009). Another problem within these systems is the low light efficiency in the depth of the crop, known as the self-shading phenomenon (Akhlaghi and Najafpour-Darzi 2020). Another central challenge is that  $\text{O}_2$  inactivates hydrogenase and nitrogenase. Different studies have revealed that  $\text{O}_2$  has effects that greatly inhibit the ideal production of  $\text{H}_2$  by direct bio-photolysis (McKinlay and Harwood 2010). So, genetic engineering plays an important role at this point to overcome these challenges. With genetic engineering, the utilization of *Chlamydomonas reinhardtii* with the mutation of the Moc 1 gene has been patented (Allakhverdiev et al. 2010). With this patent,  $\text{H}_2$  production was increased up to four times compared to the wild strain, since an electron transfer was achieved through the respiratory electron transfer chain (Plummer and Plummer 2012). Another important patent that emerged is genetically modified green algae for the reduction of the expression of  $\text{SO}_4^{2-}$  permease (Lucia Ghirardi et al. 2009), where hydrogen is produced in a sustainable way, because of the elimination of the ability of chloroplasts to ingest sulfate. However, given the drawbacks that this method presents regarding the penetration of light, it is necessary to design and implement a photobioreactor with a large and highly efficient area for the cultivation of algae, and thus improve the penetration of light in the medium (Akhlaghi and Najafpour-Darzi 2020). On the other hand, the inhibitory effects of  $\text{O}_2$  could be mitigated by means of a continuous bubbling of an inert gas (argon); but this is not profitable for the sustainable fabrication of  $\text{H}_2$  extensively. Therefore, the utilization of genetically modified strains is being widely used to improve oxygen-tolerant hydrogenase (McKinlay and Harwood 2010).

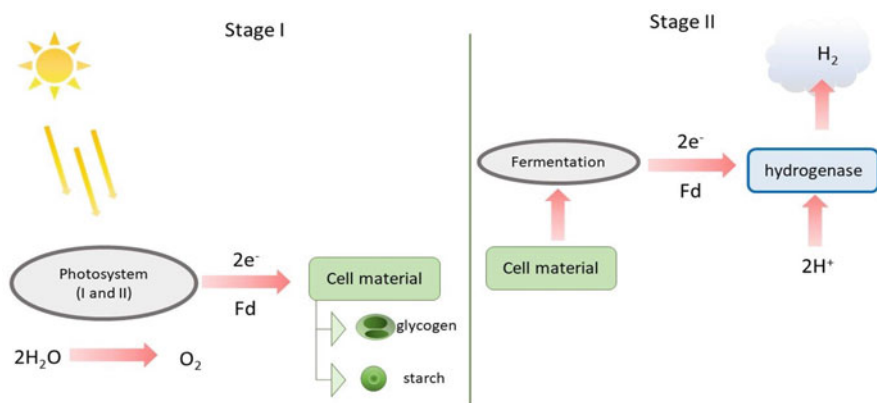
2. **Cyanobacteria:** Bacteria capable of oxygenic photosynthesis (blue-green algae). These microorganisms belong to the group of prokaryotes that have the oxygenated photosynthetic capacity (Lucia Ghirardi et al. 2009) and they are considered the microbial species that can fabricate  $\text{H}_2$  by means of direct bio-photolysis. These organisms are formed by enzymes that are directly involved in the metabolism of  $\text{H}_2$  (Ghiasian 2019), nitrogenase, hydrogenase uptake, and bidirectional/reversible hydrogenase. Although cyanobacteria have  $[\text{NiFe}]\text{-H}_{2\text{asa}}$  which are not as sensitive as  $[\text{FeFe}]\text{-H}_{2\text{asa}}$  (green algae hydrogenase) to oxygen (Lucia Ghirardi et al. 2009), in the production of  $\text{H}_2$  it is very important to consider the environmental conditions under which the reactions are carried out since the yield of  $\text{H}_2$  production depends on them, therefore, these conditions are key in scaling up to produce large quantities of this biofuel. It is known that the production of gaseous  $\text{H}_2$  free of  $\text{N}_2$  provides a high rate of hydrogen yield (Demirbas 2009).

The  $\text{H}_2$  production using cyanobacterial occupies very simple nutritional conditions, which is why it has turned out to be a well-studied method. In addition to this, these organisms have some characteristics that are responsible for protecting  $\text{O}_2$ -sensitive enzymes from oxygen evolved from photosynthesis. Several

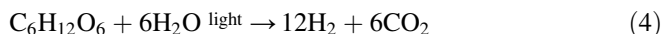
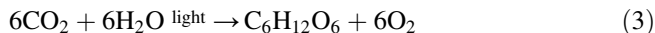
nitrogen-fixing cyanobacteria manage to carry out cell differentiation by which they can physically separate oxygenated photosynthesis and nitrogen-fixing enzymes through heterocyst cells, these are normally found in most plant cells (Khetkorn et al. 2017). Genetic engineering has managed to make the production of  $H_2$  more efficient by means of microorganisms, for example, the cellular modification of the heterocyst has achieved the incorporation of internal and external signals through the vegetative filaments (Kumar et al. 2010), hence, characteristic can eliminate the hydrogen oxygenation stage or else incorporate it during nitrogen fixation, therefore, the yield of the process would increase because  $H_2$  produced by  $H_{2asa}$  will behave as a product (Akhlaghi and Najafpour-Darzi 2020). Previous studies report that the use of an engineered bacteria of Nostoc filamentous heterocystous cyanobacteria without any  $H_{2asa}$  intake function, under aerobic or anaerobic conditions in a photobioreactor with visible electromagnetic radiation energy conversion efficiency of 4.0%. The highest yields in obtaining  $H_2$  with this method were 700 and 1700  $cm^3H_2/m^3h$  with and without oxygen presence, respectively (Nyberg et al. 2015).

### 3.1.2 Indirect Bio-Photolysis

By using indirect bio-photolysis in the manufacture of  $H_2$ , hydrates of carbon such as starch and glycogen in microalgae and cyanobacteria, respectively, can accumulate in the photosynthesis stage during  $O_2$  production and  $CO_2$  fixation, subsequently, in a second stage, the accumulated carbohydrates are used to produce  $H_2$  under anaerobic conditions, and with the release of  $CO_2$  (Hallenbeck 2012). In Fig.3, the indirect bio-photolysis process is shown, which includes two stages: photosynthesis for the accumulation of carbohydrates (I) and dark fermentation (II) of the carbon reserve for the production of  $H_2$  (Ghiasian 2019). The sequence of photochemical reactions in the typical indirect indirect bio-photolysis process is shown in Eqs. (2) and (3) (Das and Veziroglu 2008):



**Fig. 3** Indirect bio-photolysis



In indirect bio-photolysis for  $\text{H}_2$  production, both microalgae and cyanobacteria can produce nitrogen-fixing enzymes. However, since cyanobacteria's hydrogen-producing yield is much higher than microalgae, it is preferred over cyanobacteria (Akhlaghi and Najafpour-Darzi 2020). This is because, during the production of  $\text{H}_2$ , they carry out photosynthesis in stages or with time separations (Ghiasian 2019). With cyanobacteria, in step I,  $\text{CO}_2$  is fixed to produce cellular materials and saccharide deposits, to successively in step II produce hydrogen from these deposits under an unlit anaerobic state (Gouveia and Passarinho 2017). To do this, photosystem II uses the energy from the visible electromagnetic radiation of the solar spectrum to remove the electrons from the water molecule. During the oxidation of  $\text{H}_2\text{O}$  some electrons are released, these electrons are transferred to the protein ferredoxin in the reduction step of photosystem I.  $\text{H}_{2\text{ase}}$  accepts the electrons when ferredoxin is reduced and donates them to two protons to generate a molecule of  $\text{H}_2$ . To carry out this process, the differentiation of two plant cells is used that, on the one hand, carry out normal photosynthesis and, on the other, provide nitrogen fixers to the reducing agent required by  $\text{H}_{2\text{ase}}$ . Bidirectional hydrogenases and nitrogenases can be used in this two-step process (Ghiasian 2019).

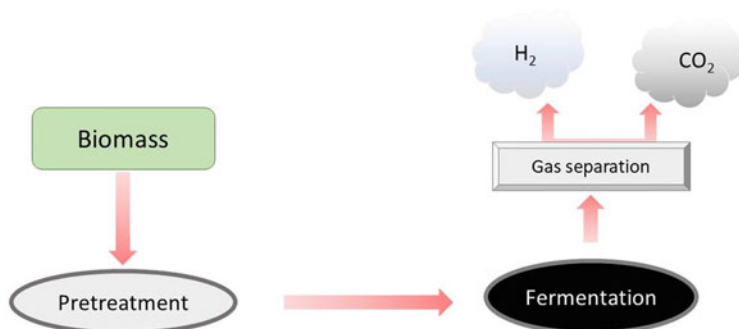
### 3.2 Hydrogen Production by Fermentative Process

Lignocellulose biomass (LCB) in its different conformations such as trees, plants, and other natural vegetation, is the second largest component of our planet (Mora-Pale et al. 2011). More than 200 billion tons of LCB are produced globally each year, which has the possibility to become biofuels, including hydrogen (Sharma and Arya 2017). Since 2014, it was considered that the production of hydrogen from LCB (biohydrogen) could be regulated to the current energy needs and mitigate global heating (Liu et al. 2014). Previous studies affirm that the use of LCB to produce biohydrogen is performed all over the globe (Kumar et al. 2015).

The methods of biohydrogen production extend as a sustainable and environmentally safe procedure in which microorganisms produce  $\text{H}_2$  sustainable from LCB through fermentation (Basak et al. 2020). Depending on its operating conditions, it can exist in two forms in the absence of light (dark fermentation) and in the presence of light (photo-fermentation).

#### 3.2.1 Dark Fermentation

Unlighted fermentation is one of the most tested procedures to produce hydrogen through biological perspectives (Bundhoo 2017a). Is the acidogenic decomposition



**Fig. 4** Dark fermentation process

of organic material in dark conditions and without oxygen to produce biogas from biohydrogen,  $\text{CO}_2$ , and a mixture of metabolites of the final product, such as acetic acid, butyric acid, and ethanol, among others (Azwar et al. 2014). The production of biohydrogen by unlighted fermentation occurs through a chronological sequence, as illustrated in Fig. 4.

Fermentation is present only in some microorganisms, and most of them use glucose as the starting carbohydrate, although some can break down other carbohydrates. The first stage of carbohydrate metabolism is the degradation of the initial molecule to pyruvic acid or its derivative. If the initial molecule is glucose, the process is called glycolysis (Łukajtis et al. 2018). After the pyruvate is obtained, it gives acetyl-CoA and reduced ferredoxin (PFOR), or via formate (PFL). There are organisms that have a limited production of  $\text{H}_2$  (2-mol  $\text{H}_2$ / 1-mol glucose) since they cannot way in an NADH to manufacture  $\text{H}_2$ , these organisms are those that only have the PFL pathway, whereas organisms using the POR pathway may derive some  $\text{H}_2$  beginning with oxidation of NADH using one or more of several  $[\text{FeFe}] \text{H}_{2\text{asa}}$ . Therefore, depending on the NADH-dependent  $\text{H}_{2\text{asa}}$ , Fd-dependent with ferredoxin reduced by NFOR (NADH: ferredoxin oxidoreductase), or the bifurcating NADH-Fd-dependent  $\text{H}_{2\text{asa}}$  (Hallenbeck et al. 2012). The subproducts of fermentation are acetic acids (theoretical maximum of 4-mol  $\text{H}_2$  / 1-mol glucose) and butyric (theoretical maximum of 3.4-mol  $\text{H}_2$  / 1-mol glucose), with which the practical hydrogen yields in unlighted fermentation are around 2-mol  $\text{H}_2$ /1-mol glucose (Levin et al. 2004).

Products generated from pyruvic acid that result from fermentation depend on several factors such as the feedstock from which the fermentation will be generated, the type of pretreatment to which the feedstock is subjected, the microorganisms involved in fermentation, and reactor operating conditions, therefore all this affects the actual yield of hydrogen production are described below.

1. **Feedstock:** To produce biohydrogen to be a sustainable process, the substrates used must meet certain criteria. Substrates must be produced from renewable resources, be available in sufficient quantity and/or concentration so that fermentation occurs efficiently, and hydrogen is produced in an energetically favorable way (Guo et al. 2010). As well, for uninterrupted manufacture of hydrogen, renewable products and preferences that are the second generation are required, which, as we have already mentioned throughout this book, have abundant residues of LCB or carbohydrates, which cannot be used as livestock feed (Hawkes et al. 2007). The use of problematic residues such as organic residues from various sources, whey protein, oily wastewater, fatty acid methyl esters (FAME) production, and manure, appear to be promising in dark fermentation (Łukajtis et al. 2018). Recent research has focused on the manufacturing of  $H_2$  on a large scale through the dark fermentation of these types of primal matter: (a) Agricultural waste, (b) industrial waste and effluents, (c) waste of animal origin, and (d) organic municipal waste.
  - (a) *Agricultural waste:* The crop residues are the more abounding, inexpensive, and most easily accessible biological trash to be converted; these include straw, stubble, husks, ears, stalks, and bagasse, among other LCB debris (Łukajtis et al. 2018). Biohydrogen manufacturing with biomass remnant of the harvest is restricted by the hydrolytic activity of microorganisms that participate in the biological transformation of the diversified and microcrystalline structure of the lignocellulosic parts and in the festering of cellulosic blend into dissoluble sugars (Guo et al. 2010). For this reason, appropriate pretreatment steps are often required for the raw material that can promote hydrolysis. The chief pretreatments are found in mechanical, physical, chemical, and biological methods (Mtui 2009). According to current information on the production of biohydrogen by dark fermentation with different raw materials, the hydrogen production yield depends largely on the pretreatment carried out on the raw material, for example, an investigation on the production of biohydrogen from the waste of corn stalk, showed that after an acidification pretreatment coupled with a heat pretreatment, it obtained a maximum surrender of  $H_2$  post-processing with 0.2% HCl, that is, 50 times the original value, which gives an idea of the effectiveness of the acidification pretreatment phase (Zhang et al. 2007). Therefore, more achievements are needed for a deeper knowledge of the efficiency of the processes for obtaining biohydrogen, such as the compositions and quality of organic substrates. The crop residue pretreatment procedure also requires deeper analysis because the origin and composition of organic substrates determine which specific pretreatment is the most acceptable (Guo et al. 2010).
  - (b) *Industrial waste and effluents:* Industrial wastes and carbohydrate-rich effluents such as wastewater from oil mills (Ntaikou et al. 2009), effluents from palm oil mills (Mohammadi et al. 2011), and scraps from the milk and beer industries (Kargi et al. 2012), they can be substrates for dark fermentation. For example, in 2012 biohydrogen was obtained from a solution of powdered

they cheese by thermophilic dark fermentation (Kargi et al. 2012). It has also been possible to produce biohydrogen from the unlighted fermentation of lactose-rich cheese whey effluent using a mixed culture under above 40 °C and mesophilic conditions (Azbar et al. 2009). Research has also recently been conducted on substratum that excludes carbohydrates. Such is the case of glycerol, a remainder in the obtention of FAME, and it is hard to use in big bundles (Nakashimada et al. 2002). In 2005 the transformation of glycerol into hydrogen through unlit fermentation was reported (Ito et al. 2005). The hydrogen yield was 6.69-mmol H<sub>2</sub>/ 1-g glycerol, which was greater than the yield of simple hydrates of carbon, such as glucose (1.97-mmol H<sub>2</sub>/1-g glucose), galactose (1.90-mmol H<sub>2</sub>/1-g galactose), and fructose (2.17-mmol H<sub>2</sub>/1-g fructose) (Ito et al. 2005).

- (c) *Waste of animal origin*: Animal waste can be classified into three main groups: liquid manure, such as bird feces or cattle urine; solid muck or farmyard muck and effluent, which may contain a range of agricultural waste including runoff from farmyards, silage juices, gauzes, disinfectants, among others (Burton and Turner 2003). Animal waste can cause serious environmental problems, with more than 1.5 billion tons of animal dung produced each year, including 1.284 million tons of bovine dung and 295 million tons of pig dung in the 27 member states of the European Union (Holm-Nielsen et al. 2009). And if dung is not managed or treated, is considered a problem because generating the emission gases of harmful gases, mainly derived from elements S, N, and P into the atmosphere, and can cause air, water, and soil contamination (Burton and Turner 2003). For manure or its derivatives to be useful in obtaining hydrogen from dark fermentation, the residues must be pretreated with one of the different techniques that exist such as high temperature, microwaves, ultrasound, with pH to eliminate methanogenic bacteria, among others. One more drawback of using dung as raw material is the dense accumulation of N-compounds that in turn can transform into ammonia during fermentation and ammonia at a definite quantity inhibits unlighted fermentation, therefore, H<sub>2</sub> production ceases fast when the NH<sub>3</sub> is produced in excess (2 g-N/dm<sup>3</sup>) (Łukajtis et al. 2018). The efficiency of the processes in obtaining biohydrogen from livestock waste is lower as opposed to biohydrogen obtained from crop scraps. The highest yield was reported in research work on the hydrogen production potential of heat-pretreated cattle dung. This high yield was likely the result of the use of fresh dung, since when stored the ammonium concentration was multiplied by three with respect to the initial value due to the decomposition of organic matter (Kotsopoulos et al. 2009).
- (d) *Organic municipal waste*: This classification groups together all those easily biodegradable organic wastes that come from the food industry, homes, schools, and restaurants, among others. This type of waste has taken great importance due to the biochemical composition of the material, since it is made up of polysaccharides, such as starch, cellulose, and hemicellulose, as well as proteins, lipids, and simple carbohydrates (Shin et al. 2004). On the



other hand, these wastes cause that release methane gas into the atmosphere in its decomposition, contaminating the groundwater, when the wastewater that is created in the landfills during the festering of the biological matter leaks into the aquifers. Thus, the requirement to soothe the negative effect of such residues encourages the scientific global and the industry to study its application routes as raw material in unlighted fermentation (Kim et al. 2004). The average production of biohydrogen from this raw material is considerably higher than the values gotten from crop and livestock residues (Guo et al. 2010).

2. **Pretreatments:** According to studies in the field of biohydrogen production through dark fermentation, there is no feedstock capable of producing this biofuel with a high yield so far. However, many efforts have been focused on improving the yield of biohydrogen production from LCB material. One of the keys to this objective is the pretreatments that are carried out on the substrates, because the release of the cellulose depends on this, which will later be destroyed to obtain its molecules and achieve good depolymerization, to improve the enzymatic digestibility and the acidogenic fermentation, all these factors have a direct impact on the final product yield of biohydrogen (Mohammadi et al. 2011; Bundhoo 2017b; Basak et al. 2020). Besides, a series of pretreatments have also been carried out on the cultures of various microorganisms (inoculum) to increase the yield of the final product of biohydrogen. The purpose of this type of pretreatment is to destroy the bacteria that consume  $H_2$ , such as hydrogenotrophic methanogens, homoacetogens, and sulfate-reducing bacteria, among others. On the other hand, the pretreatment of microorganisms also tries to strengthen the bacteria  $H_2$  produces, and this way, the production of  $H_2$  will be increased. (Saady 2013). Currently, there are several technologies to carry out pretreatments with the aim of increasing the biohydrogen yield of LCB and can be classed as (a) physical, (b) physicochemical, (c) chemical, and (d) biological (Bundhoo et al. 2015; Wang and Yin 2018).
  - (a) *Physical:* The procedures for the pretreatment of microorganisms based on physical methods are the following: thermal, which includes freezing and thawing, and radiation with different wavelengths, the most common being GAMMA ( $10^{-17}$  m), UV ( $10^{-9}$  m), IR ( $10^{-5}$  m), and MW (10 mm–1 m). On the other hand, the physical pretreatments focused on the substrates are mechanical methods, which involve the structural modification of the material, reducing the size mainly to achieve better hydrolysis, there are also thermal (freezing and thawing) and radiative treatments with large MW wavelengths and US (less than 10 mm) (Taherzadeh and Karimi 2008).
  - (b) *Physicochemical:* There are several physicochemical technologies for the pretreatment of LCB, however, to produce biohydrogen through fermentation in the dark, only two of these techniques have been applied: water steam explosion and ammonia explosion (Bundhoo et al. 2015). In water steam explosion or autohydrolysis, LCB is put through to saturated steam at high temperature (433–533 K) and constant pressure (0.69–4.83 MPa) for a few

seconds (30–1200 s) (Hendriks and Zeeman 2009). In ammonia explosion, liquid  $\text{NH}_3$  hot and high pressure is employed to pretreat the LCB material for a specified time before the pressure is released suddenly, resulting in depressurization biomass, generating the delignification of LCB, maintaining cellulose and hemicelluloses are unstirred (Taherzadeh and Karimi 2008).

- (c) *Chemical*: The techniques based on chemical methods for the pretreatment of both the microorganisms and the substrates are the following: through ionic liquids (IL), modifying the pH, ozonolysis, shock waves, methanogen inhibitors such as linoleic acid, carbon dioxide, and potassium nitrate, among others. (Bundhoo et al. 2015). One of the most used pretreatments before fermentation without light both in microorganisms and in substrates is the change in pH, with acidic and basic substances, which are added to generate a low and high pH, respectively, in microorganisms or substrates, with this the bacteria that do not produce spores are eliminated, while the bacteria with spores manage to survive without being affected (Deublein and Steinhauser 2011). Different conditions have been used for the pH pretreatment, in which acids are used to lower the pH, such as hydrochloric acid, sulfuric acid, nitric acid, and perchloric acid, concentrations between 0.1 and 6.0 M have been used with a pH ranging from 2.0 to 4.0 (Chaganti et al. 2012). For pretreatment with high pH, the most common agent is sodium hydroxide with concentrations ranging from 1 to 8 M with a pH of 10 to 12. The retention time most used in pH pretreatment is 24 h (Kan 2013).
- (d) *Biological*: The biological pretreatment of LCB materials consists of the use of organisms capable of partially altering lignin and hemicellulose, commonly known as wood rot, for these fungi are used, which are classified according to the type of rot they produce, some types are brown rot, rot soft, and white rot. (Sun and Cheng 2002). The fungi in addition also help in the alteration of the cell wall. Subsequently to these effects, the cellulose molecules are released in solution, and this improves enzymatic hydrolysis, therefore a higher yield in the production of biohydrogen is possible (Appels et al. 2008). The fungi involved in soft and white decomposition degrade both lignin and cellulose, while brown decompose fungi mainly degrade cellulose (Sun and Cheng 2002). On the other hand, several works report that another biological pretreatment option to increase biohydrogen production is using enzymes (Bundhoo et al. 2015). Technologies based on biological methods consume little energy and do not require chemical agents, therefore they are considered cheap compared to other technologies used for pretreatment, however, they need the investment of time in the care required for the growth of the microorganisms involved herein, besides, the rate of hydrolysis must be taken care of, for this reason, biological pretreatments are difficult to scale at an industrial level (Chandra et al. 2007).
3. *Microorganisms*: The production of biohydrogen by dark fermentation can be carried out both with pure cultures, with a great variety of strains of microorganisms, and with mixed cultures that generally come from waste products. Both

types of cultures serve as inoculums for fermentation (Lee et al. 2011). In the production of hydrogen, the most studied bacteria are of the genus *Clostridium* that carry out butyric fermentation, and the enterobacteria that carry out acid-mixed fermentation (Ishikawa et al. 2006). Both ingest glucose, therefore, they begin their degradation with glycolysis. Clostridia, for example, *Clostridium buytricum*, *C. thermolacticum*, *C. pasteurianum*, *C. paraputrificum*, and *C. bifermentans* are Gram-positive, anaerobic, spore-forming bacteria, and obligate fermenters since they lack the machinery to effect respiration (Liu and Shen 2004). The products of sugar fermentation are  $H_2$  and  $CO_2$ , acetic and butyric acids, if the pH of the medium is neutral. As the pH becomes acidic by the same action of clostridia, the products change to acetone and butanol (Madigan et al. 2002). These two phases are called hydrogen/acid production, in the exponential growth stage of the crop, and solvent production, in the stationary stage, respectively (Kapdan and Kargi 2006). Enterobacteriaceae, which are resistant to the presence of oxygen and therefore have the advantage of consuming oxygen quickly in bioreactors, guaranteeing anaerobiosis, are *Enterobacter*, *E. coli*, and *Citrobacter* (Bisaillon et al. 2006; Ishikawa et al. 2006). They are Gram-negative, non-sporulating, facultative anaerobes, and capable of fermenting glucose. They generally have lower yields than *Clostridium*, as other by-products of hydrogen metabolism such as lactate are produced, although mutants have been developed to block these unwanted metabolites and improve production (Reith et al. 2003). With *E. coli*, the production of hydrogen from formate in anaerobiosis has been demonstrated due to its formate-hydrogen-lyase activity and with *Citrobacter* produces hydrogen by gas exchange reaction (Reith et al. 2003).

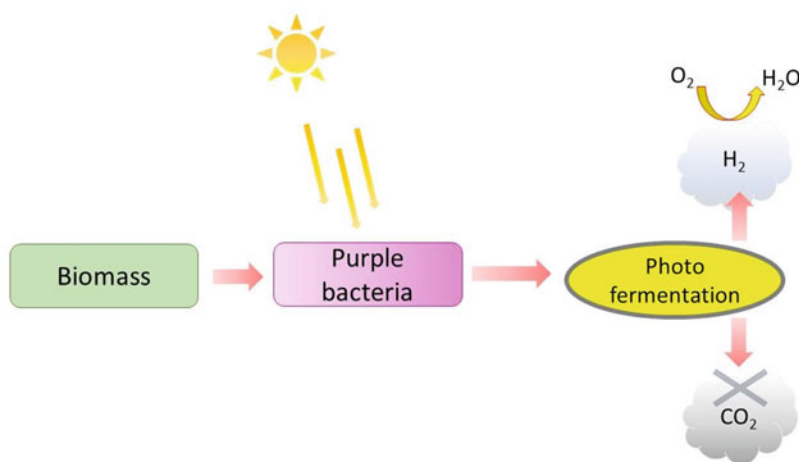
4. **Operating conditions:** The conditions of the medium are fundamental to produce  $H_2$  by dark fermentation. Mainly, the environment must be anaerobic. pH influences many important parameters, such as yield, production rate, and type of fermentation products (Chaganti et al. 2012). The optimal pH reported reaching the highest  $H_2$  yield and the best production rate is between 7.0 and 8.0 (Liu and Shen 2004), it has been reported that when the pH is less than 5, fermentation can occur in ethanol type in which the main soluble products are ethanol and acetic acid (Rafieenia et al. 2018). Furthermore, as bacteria ferment producing acids, the pH decreases and the hydrogenase enzyme is negatively affected and inhibited (Dabrock et al. 1992). Therefore, controlling the pH of the medium is very important because the acidic products of the same fermentation continually acidify it. These products, in addition to  $H_2$  and  $CO_2$  gases, are mainly composed of volatile organic acids acetic, butyric, and propionic acid and bacteria release them into the environment (Rafieenia et al. 2018). In dark fermentation, the reaction temperature is a critical factor, it can vary from 25 to 40 °C, 40 to 65 °C, and above 80 °C for mesophilic, thermophilic, and hyperthermophilic conditions, respectively (Argun and Kargi 2011).

### 3.2.2 Photo-Fermentation

The production of biohydrogen through photo-fermentation using photosynthetic bacteria was first carried out in 1949 by Gest and Kaman (Zhu et al. 2011), since then, this technology has demonstrated an effective production of high-purity hydrogen without the generation of oxygen (Das and Veziroğlu 2001), such as shown in Fig.5. The biohydrogen produced by photo-fermentation uses bacteria capable of producing energy through photosynthesis, this type is known as purple bacteria (PNSB) (Adessi et al. 2012). These microorganisms can use different organic compounds as raw material to produce  $H_2$ , they can even be used to clean waste or in the treatment of wastewater, which is rich in organic matter (Boboescu et al. 2014). Various reports mention that photo-fermentation has a better efficiency in the production of biohydrogen compared to dark fermentation, and also uses solar radiation as an energy source, which, being an inexhaustible source, provides a great advantage to this process (Ghosh et al. 2017).

PNSBs carry out anaerobic photosynthesis to produce ATP molecules driven by light under hypoxic conditions. On the other hand, PNSB with the help of ATP molecules and  $N_2$  presence can catalyze the reduction of  $N_2$  to  $NH_3$  through the enzyme nitrogenase capable of fixing  $N_2$ . This enzyme is the main catalyst present in PNSB to produce biohydrogen. Thus, for each molecule of nitrogen fixed in ammonia, one molecule of  $H_2$  is produced (Ghosh et al. 2017).

The development of the PNSB depends largely on the available conditions of the environment, for example, they can change from one form to another as photoheterotrophs, photoautotrophs, or chemoheterotrophs, depending on the amount of oxygen, carbon, and of course, the amount of light supplied. On the other hand, these bacteria sometimes use sulfur as an electron donor when it is in low



**Fig. 5** Photo-fermentation process

concentrations and they feed on sunlight, hence the term, sulfur-free (Basak et al. 2014).

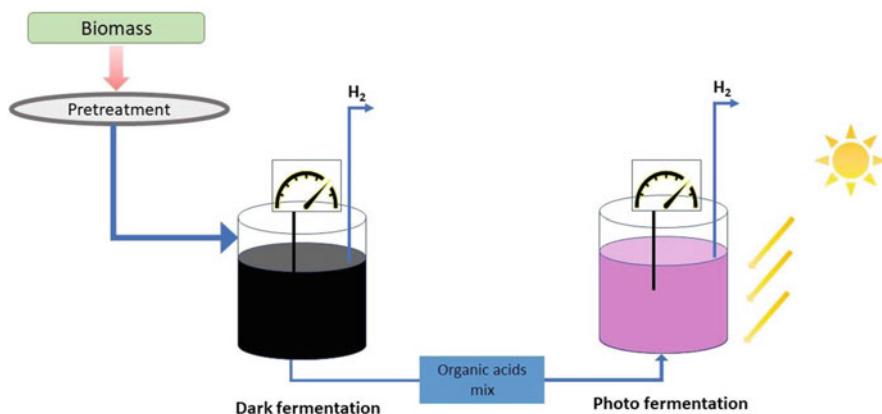
Among the PNSBs the most studied, in relation to  $H_2$  production, are *Rhodobacter sphaeroides*, *Rhodopseudomonas capsulatus*, *R. pallustris*, or *Rhodospirillum rubrum* (McKinlay and Harwood 2010). They are called “purple” because they are phototrophic and capture the energy of light by means of bacteriochlorophylls and carotenoid compounds that give them that color. These phototrophic bacteria do not capture  $CO_2$  from the air like green plants, but instead use a great diversity of organic compounds as a carbon source, for example, fatty acids, sugars, amino acids, alcohols, and even aromatic compounds such as benzoate (Madigan et al. 2002).

### 3.3 Hydrogen Production by Hybrid System

Of all the biological methods to produce hydrogen described so far, there is not one that is highly efficient in practice, because each process has different types and amounts of by-products depending on the reactions, raw materials, and conditions involved in each process. However, to make the biological processes more efficient for obtaining hydrogen, various combinations of biological processes have been used, such as processes that are made up of two fermentations known with and without light, another is made up of 3 components that include the two fermentations and biophotolysis.

#### 3.3.1 Two-Component Systems of Dark Fermentation and Photo-Fermentation

It has been widely reported that the joint use of the two types of fermentation both in light and in the dark, either in one stage, that is, in the same bioreactor, or in two stages, as can be seen in Fig. 6, it can significantly improve the yield of biohydrogen production and increase the conversion efficiency of a substrate (Hay et al. 2013). This is because the theoretical yield of biohydrogen obtained from glucose by dark fermentation is estimated to be around 4 ml  $H_2$  with 1 mole of glucose, as mentioned in detail in the previous section (Levin et al. 2004). However, past performance is still low from a financial perspective. It has been previously reported that if residues from the food industry are used, the yield of biohydrogen production can be significantly increased, because this material contains simple sugars and during its biohydrogen production process some by-products are also obtained, such as fatty acids which favor the production of  $H_2$  and reduce  $CO_2$  (Łukajtis et al. 2018). According to Hay et al. With the photo-fermentation process, it is possible to obtain 8 moles of  $H_2$  with 1 mole of acetate, therefore it is theoretically estimated that by combining dark fermentation with photo-fermentation, 12 moles of  $H_2$  could be obtained with 1 mole of glucose (Hay et al. 2013). Works on biohydrogen

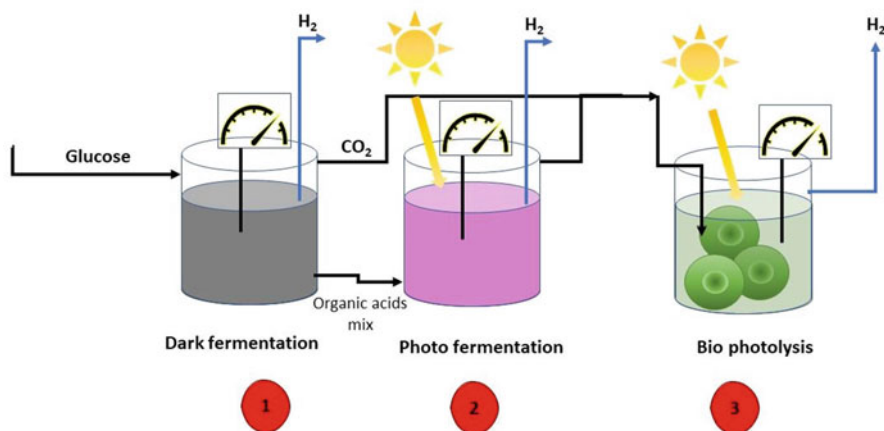


**Fig. 6** Two-component system of dark fermentation and photo-fermentation in biohydrogen obtention

production have confirmed the theoretical yield proposed by Hay et al., where they have managed to improve the yield of biohydrogen production by combining photo-fermentation and dark fermentation in a two-step process (Łukajtis et al. 2018).

### 3.3.2 Three-Component System of Dark Fermentation, Photo-Fermentation, and Bio-Photolysis

The combination of the three most efficient biological processes in obtaining hydrogen was proposed for the first time in 2006 (Melis and Melnicki 2006). This combination can be exemplified in Fig. 7. Since the products of *Clostridium* bacteria in dark fermentation are acids, alcohols, and other short-chain organic molecules, these same compounds are ingested by non-sulfur purple bacteria as a carbon source. Therefore, it was proposed that the effluent obtained from dark fermentation be used as part of the medium, more precisely the carbon source, for photo-fermentation. Furthermore, biomass accumulated by photosynthesis in reactors with photosynthetic organisms could be employed as C and energy fount in dark fermentation. Likewise, the  $\text{CO}_2$  produced by anaerobic bacteria could be separated from  $\text{H}_2$  and bubbled up as a C fount for algae in biophotolysis (Ghirardi et al. 2000; Melis and Melnicki 2006). In this integrated way, the  $\text{H}_2$  yield is higher, and the light can be harnessed in a broader spectrum since the algae use visible light and the purple non-sulfur bacteria absorb the near-infrared light the algae use visible light and the purple non-sulfur bacteria absorb the near-infrared light.



**Fig. 7** Three-component system of dark fermentation, photo-fermentation, and bio-photolysis in biohydrogen obtention

## 4 Conclusions

The high demand for hydrogen currently makes hydrogen production from fossil fuel resources dominant today. Because a highly efficient biological process has yet to be found to meet the world's demand for hydrogen. This is where the development of biotechnology has an extremely important role, to improve the performance of biohydrogen. One of the most efficient methods to produce biohydrogen is photo-fermentation, however, the efficiency of this process is limited by the amount of radiation of the visible spectrum that is absorbed in the system, this is known as photoconversion efficiency. On the other hand, fermentation in the dark to obtain biohydrogen, despite its low yield, is the most studied and used process, since it can use a great diversity of raw materials, including food, industrial waste, of agriculture, and everything that is considered as biomass, generates a high interest in this process since its raw material is inexhaustible and economical. Bio-photolysis is a process in development, since its performance is not yet sufficient for current demand, however, great efforts have been invested in it, since through genetic engineering they have managed to obtain genetically modified algae capable of increasing biohydrogen production. In this way, the conjunction of two or more biological processes, known as hybrid processes, are a profitable option soon to increase the yield of biohydrogen and at some point it will be sufficient to satisfy world demand and thus displace hydrogen from fossil fuel resources.

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# Global Conclusions and Future Perspectives



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*The use of plant oil as fuel may seem insignificant today. But  
such products can in time become just as important as  
kerosene and these coal-tar-products of today.*

*Rudolf Diesel (March 1858–September 1913)*

**Abstract** This chapter will summarize the most relevant ideas from all chapters to have a basis that justifies firstly, the implementation of biofuels by different pathways, the potential benefit related to the use of biotechnology in the production of biofuels (third and fourth generations) in the future given the fact that thermochemical and physical approaches are the most used so far and this includes first- and second-generation biofuels. A wider outlook on strategies for the implementation of biofuels will be discussed. Some current and complementary examples added to the ones already presented throughout the book related to biofuel applications will be mentioned to continue to support their presence in the energy sector. Collaborations among countries and fuel companies in the energy sector will be added too, to give a

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more real sustaining and framing on the application of biofuels as efforts mainly from the private sector.

**Keywords** SynBio · Renewable energies · Biofuels industry · LCAs

## 1 A General and Final Overview on Biofuels

Throughout this book these are the biofuels that were covered: methane, bioethanol, biodiesel, bio-oil, vegetable oil, biogas, and to some extent although not as a chapter topic, the sustainable aviation fuel (SAF). It has been remarked that the methods to generate or obtain them are mostly based on physical and thermochemical procedures and to a much lesser extent on a biotechnological ground (at research laboratories by means of genetic engineering), but despite this, efforts in this area are being carried out to overcome this and lead to more sustainable production paths. Also, the fact that the sources for these biofuels are crops, waste, and microorganisms such as algae and the like represents a good start to begin refining processes to a more biotechnology and green-oriented production as it has been discussed throughout the book. And as time passes by, there is hope for waste and algae to be the biomass that at a certain moment covers the most demand to avoid monopolizing crops due to the already mentioned food production/supply vs biofuels production.

If the mentioned biofuels were categorized in their order of use and importance, we would have the following order: bioethanol, biodiesel, biogas, hydrogen or biohydrogen, bio-oil, and vegetable oil. Nowadays, awareness of biofuels is being raised in distinct and varied ways, no matter how elementary or more elaborate they are. Figure 1 displays a scheme of examples of different initiatives and efforts. To start with, there is the World Biofuel Day celebrated since 2015 on August 10th, because in this date but in the year 1893, Rudolf Diesel successfully ran a Diesel engine on peanut oil and this event represents a turning point when it comes to biofuels early stages (Punia et al. 2021). The proposal for this date was initiated in India by the Ministry of Petroleum and Natural Gas and the Ministry of Environment, Forest and Climate Change (Ministry of External Affairs 2022). There are also educational programs aimed at the general public which are focused on providing information about biofuels as well as evidence to support their incorporation in substitution of fossil fuels. Life cycle assessments (LCAs) were mentioned in chapter “Introduction” and have become a source of evidence for decision-making on the environmental risks related to biofuels although their data is considered to be limited by far in most cases.

Policy making is a crucial step in biofuels implementation whose aim is the reduction of greenhouse gas (GHG) emissions and amelioration of energy security given the fact that it regulates details like biomass handling for biofuels production which also includes in some cases, what crops and in what amount they can be used; determine what biofuels and in what quantities they can be manufactured, if they can be used already directly or in blends with other fuels in a certain region, regulates the percentage of use in transport, authorizes incentives and supports investors through



**Fig. 1** Biofuels implementation strategies

the creation of programs, sets import tariffs, issues standards for the different biofuels, provides of subsidies just to mention some of the most important (Sorda et al. 2010). Policy making relies on the expertise of people with different profiles among which environmental scientists and economists can be found. Some examples of policies from the United States, China, France, Brazil, Australia, the United Kingdom, Canada, Germany, and Russia are included in Table 1.

Russia represents a particular case since there seems to be no interest whatsoever in transitioning to biofuels in replacement of fossil-based ones. This might be attributed to two main reasons: Russia is a big natural gas exporter and the second biggest producer and exporter of crude oil. This is reflected concerning policies, to the lack of clear statements or mandates which include specific aspects on the inclusion, development, and production of biofuels (Karel Janda 2017).

In addition to this, biofuels are being used in real settings with certain limitations that have already been discussed in the book and they have a presence in the news, too, with respect to their gradual introduction by companies that belong to the energy, automotive, aircraft, and transportation sectors and are investing in biofuels production. This, at the same time, is also on its way to the creation of an emerging economy that demands feedstock/biomass suppliers, researchers, engineers,

**Table 1** Renewable energy policies list on the biofuels topic

Country	Policies	Regulations	References
United States	<ul style="list-style-type: none"> <li>– Energy Policy Act of 2005, -Energy Independence and Security Act of 2007</li> <li>– 2002 Farm Bill and the Biomass Research and Development Act of 2000</li> </ul>	<ul style="list-style-type: none"> <li>– Quantification of targets for renewable fuels.</li> <li>– Stipulation of volumes of gallons of 9 billion gallons of renewable fuels (2008) and an expected increase to 36 billion gallons by 2022.</li> <li>– Development of bio-refineries, incentives for feedstock producers, education programs (outreach) for farmers, local authorities the general public to promote the benefits of biofuels</li> </ul>	FAO, The State of Food and Agriculture, Biofuels: Prospects, Risks and Opportunities (2008) Chapter “Biotechnological Formation of Biogas ”, pp. 30–31
India	National Policy on Biofuels 2018	<ul style="list-style-type: none"> <li>– Permits the production of ethanol using flawed food grains like wheat, broken rice, and others, not intended for human consumption</li> <li>– Allows for the transformation of excess and unused amounts of food grains to ethanol</li> </ul>	Government of India
Brazil	RenovaBio (2017)	<ul style="list-style-type: none"> <li>– Introduction of marketing strategies to detect the capacity of biofuels individually to decrease emissions per production unit</li> <li>– Biofuels manufacturing certification, carbon-reduction credits (CBIO) as well as annual obligatory GHG emission reduction goals</li> </ul>	(Grangeia et al. 2022)
China	E10 ethanol-blend policy (2017)	<ul style="list-style-type: none"> <li>– Decrease of China’s large corn stockpiles</li> <li>– Restriction of air pollution and Green House Gas (GHG) emissions are generated by 194 million cars</li> <li>– Decrement of smog and an intent to attain its commitments to the Paris Climate Agreement</li> </ul>	(Zhang et al. 2017) (Si et al. 2017)
France	<ul style="list-style-type: none"> <li>– The Energy Transition for Green</li> </ul>	<ul style="list-style-type: none"> <li>– The energy sector must commit and aid in Reach the</li> </ul>	(Pelkmans et al. 2021)

(continued)



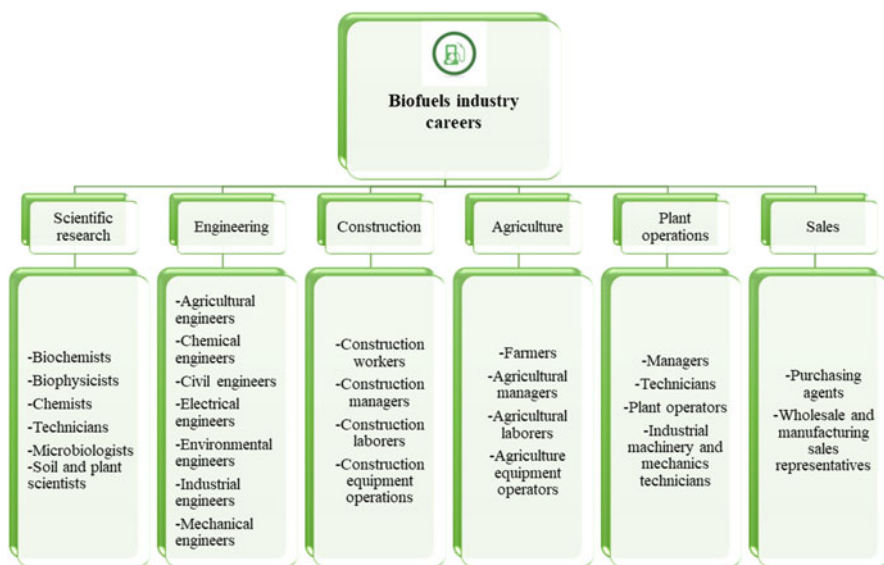
**Table 1** (continued)

Country	Policies	Regulations	References
	Growth Act of 2015 – Energy and Climate Law of 2019 – Energy Transition for Green Growth Act (LTECV, 2015)	reduction GHG emissions by 40% and to develop renewable energies to attain 33% in the French energy mix by 2030	
Australia	Queensland sustainable biobased petrol and biobased diesel mandates (2017)	– Assistance in the assurance to the current ethanol and biodiesel producers and encourage the investment in Queensland – Taking advantage of second-generation biofuels technologies from diverse feedstocks – Participating and enhancing regional growth and jobs creation	(Resources <a href="#">2019</a> )
United Kingdom	Renewable Transport Fuel Obligation (RTFO, 2018)	– Setting up Europe's first waste based jet biofuel plant that will lead to new jobs and economic growth – Obliging owners of transport fuel who supply at most 450,000 liters or more per to ensure fuel blend is at least 12.4% biofuel by 2032	UK Government
Canada	Renewable Fuels Regulations (SOR/2010-189)	– Increment in the prices of agricultural commodities to support farms. – Federal regulations have requested a minimum renewable content in gasoline and diesel since 2010	Canada Government (Rajagopal <a href="#">2011</a> )
Germany	Biofuel Sustainability Regulation (Biokraft-NachV)	– Establishment of warranties regarding the environmental compatibility of biofuels – Biofuels production by sustainable pathways whenever they save at most 35% on greenhouse gases in comparison to their fossil counterparts – Exclusively raw materials coming from sustainable cultivation are permitted	German Government

(continued)

**Table 1** (continued)

Country	Policies	Regulations	References
Russia	Russian Energy Strategy till 2035 (2015)	<ul style="list-style-type: none"> <li>– More focus on electricity generated from renewables</li> <li>– More cutting-edge biofuels as a part of energy balance is not contemplated</li> <li>– It is not actually obligatory to use a blend of a gasoline and bioethanol or biodiesel</li> </ul>	(Karel Janda 2017)

**Fig. 2** Career paths in the biofuels industry

analysts, CEOs, and other kinds of workers, enhancing the opportunities for professionals as shown in Fig. 2. In the figure, the different career paths necessary for the biofuels industry have been broken down to visualize in more detail what individuals interested in the field could work on (Richards 2022). It is worth mentioning that scientific staff from research centers, national laboratories, and universities are working and making contributions to the field, too. Thus, a large number of people are increasingly getting engaged in the biofuels industry. Lastly, collaboration and work among different countries will be also necessary to reach this sustainability challenge related to new fuels because not only one country can attain total independence when it comes to fuel production and consumption.

Bioethanol and biodiesel are the most promising and used biofuels. They both come from biomass and for bioethanol, the main sources are maize (64%), sugarcane

(26%), molasses (3%), and wheat (3%). On the other hand, biodiesel sources are vegetable oils from rapeseed (37%), soybean, and palm oil (9%) or used cooking oils (23%). Concerning the technologies that treat cellulose feedstocks, they do not actually contribute to the total shares of biofuel production in the world yet.

Bioethanol is usually used as motor fuel or as an additive in gasoline and is considered a convenient choice as a “renewable” energy element. The future perspective for ethanol looks quite encouraging because it is an effective, environment-friendly, and a strong substitute for gasoline. There are currently engines that can run on 100% pure ethanol and improvements might help generalize this trend of pure biofuels and not only blends (Bajpai 2021). GS E&C is South Korea’s representative global EPC contractor and on August 29th, 2022, along with Finnish company ST1 Renewable Energy joined forces on a technological cooperation that will be focused on cassava-based bioethanol production (The Korea Herald 2022).

As for biodiesel, in 2019, in the European Union its production reached 15.7 billion L. In the United States, it was 8.4 billion L and, for other countries: Indonesia, 7.2 billion; Brazil, 5.9 billion L and in Argentina it reached 2.4 billion L (IEA (2020), Renewables 2020, IEA, Paris <https://www.iea.org/reports/renewables-2020>). It is important to mention that the cost of biodiesel is influenced in a great manner by the kind of feedstock, production volume, production process, government incentives, food prices, as well as research and development prices. Since comestible oils consist of more than 80% of the world’s biodiesel feedstock, biodiesel prices jointly pursue vegetable oil prices. Furthermore, with regard to environmental benefits, biodiesel favorably reduces particulate matter generation by approximately 88% relative to conventional diesel fuel. The use of 100% biodiesel in heavy-duty highway engines generates almost 70% less hydrocarbons, 50% less particulates and carbon monoxide, and 10% more NO<sub>x</sub> emissions. Biodiesel bears nonsignificant sulfur oxide emissions and half the ozone-forming potential of conventional diesel (Rouhany and Montgomery 2019). In August 2022, it was reported that Restaurant Technologies (Minneapolis, MN, USA), a supplier of cooking oil management and back-of-house hood and exhaust cleaning solutions, has sold collected cooking oil to the Renewable Energy Group (REG, recently acquired by Chevron in June 2022) to produce biodiesel or renewable diesel for 10 years and that now REG is known as Chevron Renewable Energy Group is already providing biofuels to power Restaurant Technologies’ fleet located in Des Moines, Iowa and expecting to expand to more locations for biofuel commercialization and distribution (Chevron 2022).

The biofuels global market is headed by first- and second-generation bioethanol and biodiesel. In contrast, global biogas provision was 27% of the global biofuel market or nearly 0.25% of the global energy market back in 2011 (Koonaphapdeelert et al. 2020). In 2020, The International Renewable Energy Agency (IRENA) reported that 88,378 GWh of biogas is produced worldwide and the five countries where it is produced in the greatest quantity are: Germany, the United States of America (USA), Italy, the United Kingdom (UK), and China with 33,416, 13,259, 8300, 5701, and 3204 GWh, respectively (IRENA 2020). Biogas can also be targeted for electricity production in co-generation or by mixing heat and power. Moreover, its future would depend mainly on feedstock, guidelines to refine biogas

and hence, attain pipeline quality. In Sweden, trains and buses are run on biogas with refueling stations on target spots in the city (Kadam and Panwar 2017).

Biohydrogen, bio-oil, and vegetable oils are well covered in the scientific literature when it comes to the methodologies to obtain them as well as some potential applications. However, statistics, future perspectives, and similar topics are not easily found. This might be because their contribution to the transition to clean energies is not so clear. Even though the demand for hydrogen, has grown threefold since 1975 up to 2018 and continues to rise, its uses are limited to petroleum refining, metal treatment, and fertilizer production, whereas transportation and utilities are emerging and potential possibilities that need to be worked on further. Hydrogen biofuel has been studied for its use with fuel cells to generate water, electricity, and heat which later on could be extended to energy supply for use in miscellaneous applications such as combined heat and power; backup power; systems for storing and enabling renewable energy; portable power; supplementary power for trucks, aircraft, rail, and ships; professional vehicles such as forklifts; passenger as well as freight vehicles including automobiles, trucks, and buses (Sunitia Satyapal 2017). With respect to the aircraft industry, efforts have been made by Fabrum (Christchurch, New Zealand) a leader company in zero-emissions transitioning, the consultancy Filton Systems Engineering (Bristol, UK), and GKN Aerospace (Redditch, UK) to work on shifting aviation to hydrogen flights with edge-to-edge hydrogen fuel system technologies (Fabrum Team 2022).

In the case of bio-oil it has the advantage of having a heating value of 16–19 MJ/kg. Still, there are some drawbacks to overcome, namely, low-grade properties such as high water content, high viscosity, low pH, and lack of stability that retard bio-oil application as a fuel (Panwar and Paul 2020). On the other hand, there are efforts focused on improving the methodologies to produce bio-oil and hence, representing an area of opportunity to propel its usage as a biofuel. One of these efforts is the hydrofaction technology intended to be used in a biorefinery. This technology involves the transformation of low-energy-density feedstocks to high-energy density and valuable bio-oil known as hydrofaction Oil. As opposed to pyrolysis and gasification, hydrofaction offers the possibility of the treatment of wet biomass with moisture contents as high as 80%. This means moisture is desirable in hydrofaction and is the reaction medium for the conversion process. The usage of supercritical water, inexpensive catalysts, and anticoking additives, and the fact that in this process biomass is liquefied and deoxygenated biomass without the need for additional inputs such as hydrogen makes the process an alternative to hydrolysis and hydrothermal liquefaction (Toms et al. 2013).

Finally, as for vegetable oils (VO), studies show that they are particularly investigated to be used as the basis for biodiesel production, this, because VOs have problems like when they are used in diesel engines because of carbonization surplus of the combustion chamber; contamination and degradation of the lubricant causing precipitated wear of moving parts. Notwithstanding, pure VOs or their mixture with diesel have proved the reduction of gas emissions compared to diesel. Furthermore, research reports evaluating the emissions of engine gases fueled with preheated palm oil at 85 °C, for example, have reported that in order to be promising

and adequate to some extent, for design adjustments (compression ratio, injection point, fuel demand) it will be necessary to improve engines functioning (Delalibera et al. 2017). The recently created Irish fuel startup Certa will provide hydrotreated vegetable oil (HVO) to building sites, namely, machinery owned by John Sisk and Son, a construction company based in Cork, Ireland. This project has the aim of achieving carbon neutrality by 2030 and approximately €two million has been invested in this effort so far (John Sisk and Sons 2022).

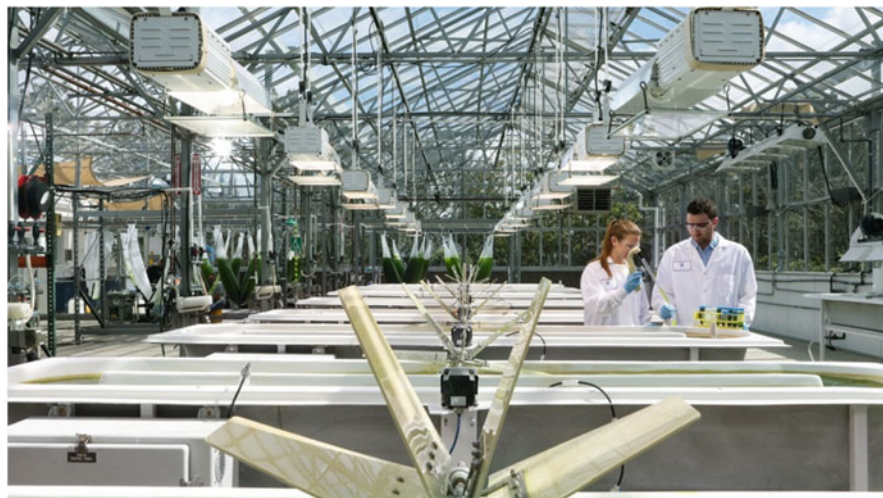
The previously mentioned biofuel-producing partnerships work on first- and second-generation biofuels. Although biofuels from it are not applied properly speaking but are in the development stage as it will be noted in Sect. 2.

## **2 Biotechnology Specific Contribution to the Production of Biofuels**

The role of biotechnology, although limited in the production of biofuels, is still in the works with favorable outcomes and it is related to third- and fourth-generation biofuels. The necessity of incorporating biotechnology into biofuels production arises because there are different tasks that can get complicated, like biomass conversion, specifically, the significant cost and difficulty to manipulate chemically the structure of cell walls to convert them to sugars. Another one is the efficient conversion of the obtained sugars into biofuels (like in the case of cellulosic biomass).

There is an important concept to introduce and avoid just referring to biotechnology and such a concept is synthetic biology, which has to do with concepts, approaches, and tools in the realm of biotechnology that allow for either the alteration or creation of biological organisms. Synthetic biology (SynBio) can be applied to biofuel production by investigating and synthesizing more efficient enzymes to decompose solid biomass or engineered robust microbes that produce biofuels in a straightforward manner and more efficiently. Microbes are going under genetic engineering with synthetic DNA to produce novel enzymes special proteins that speed up a chemical reaction, that in turn, can increase the proportion at which biomass is disintegrated. Microorganisms can also be altered to produce renewable hydrocarbon fuels that are the same as petroleum-based gasoline, diesel, or jet fuel. These technological advances can give rise to improved biomass conversion efficiency and lower production costs (Mackenzie 2013).

Amidst the many types of biomass, microalgae are quite attractive to be used as feedstock considering their high carbohydrate and lipid content, fast growth ratio, and resistance to variable environmental conditions. Biotechnological engineering tools among which genome sequencing, genetic transformation, gene targeting, different promoters, selection markers can be found, and molecular biology techniques such as clustered regularly interspaced short palindromic repeats (CRISPR) (Yang et al. 2020), transcription activator-like effectors (TALEs) (Mandal and



**Fig. 3** Viridos laboratory bioengineering site. Image Credit: ExxonMobil Climate Solutions

Chaurasia 2018) and zinc-finger nucleases (ZFN) (Dai et al. 2018) are targeted at improving biofuels production (Jagadevan et al. 2018). Regarding other microorganisms, microbial strains' development and improvement by means of genetic engineering as well as the upgrading of fermentation specifications have increased the production of biofuels. Despite the fact that one of the main disadvantages is the method for ameliorating various processes for maximal yield, the incorporation of process engineering, fermentation technology, enzyme engineering, and metabolic engineering. The arrival and advance of metabolic engineering and the increase in the number of whole-genome sequenced organisms have been devoted to the enhancement of microbial metabolic handling options and the assembly of numerous important chemicals to produce biofuels (Adegboye et al. 2021).

Third- and fourth-generation biofuels which are more biotechnological-oriented in their production are not applied yet properly speaking but are in the development stage. Better explained by an example, ExxonMobil, the American giant of oil and gas companies partnered with Viridos, a private biotechnology company known before as Synthetic Genomics to work on the conversion of CO<sub>2</sub> into renewable diesel and sustainable aviation fuels (SAFs) to reach the decarbonization in the heavy duty transportation industry by means of bioengineering tools like genomics. ExxonMobil is also investing in biofuel research programs on non-food-based biomass feedstocks. Figure 3 shows Viridos facilities where investigation related to their bioengineering work on biofuels takes place (Vijay Swarop 2022).

### 3 Conclusions on the Future of Biofuels

A large number of the existing fossil fuel alternatives bear lower costs of production than biofuels. But, as it has been mentioned throughout this book, such resources are depleting at high rates as years pass by and they represent a source of pollution. And as it occurs with new technologies, they tend to be expensive but with adequate research, technology improvement, and policies, this could be surmounted remarkably.

Biofuels and gasoline release CO<sub>2</sub> when they are burned. Biofuels are, in theory, carbon neutral, letting out CO<sub>2</sub> recently absorbed from the atmosphere by the crops used to produce them. Gasoline and other nonrenewable fuels add to the CO<sub>2</sub> supply in the atmosphere by discharging CO<sub>2</sub> absorbed and captured in plants many years ago. The main asset of biofuels is not so clear in a “life-cycle” analysis that screens, not just combustion, but also the generation and processing of the feedstock into fuel. Several investigations point to the fact that the net energy balance of biofuels is positive (e.g., the energy output is greater than the corresponding input), but estimations vary among authors. Net balances are limited for corn ethanol and more relevant for biodiesel from soybeans and ethanol from sugarcane and cellulose. The biofuel with the highest net energy balance cuts down greenhouse gases generation (GHG) the most when in comparison with that for gasoline (Coyle 2008).

Another issue is related to the competition of the food supply/production chain vs biofuels generation, hence, a compromise between both must be established and with a rapidly increasing population, it is more feasible to use human food feedstock byproducts, known as second-generation feedstock to manufacture second-generation biofuels (Dahman et al. 2019).

Finally, the forthcoming scenario of biofuels at a global level will stand on their profitability, which at the same time leans on several interrelated factors like crops as feedstocks prices. Additionally, even though biotechnology potentially serves as a possible choice to boost biofuel production, it is still under research and although slow, its advancements by means of Syn Bio, it portrays hope for the future of renewable energies. Nowadays, countries around the world have devoted money, time, and efforts to generate policies that permit the gradual insertion of renewable energies in any of their forms and thus, replace or at least act together with fossil fuels. A good start has been achieved with first- and second-generation biofuels and the third and fourth ones are on the way to be implemented step-by-step via biotechnology approaches (mostly in research domains) which also entail a green path to avoid environmental damage.

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