Chapter 12
New Trends and Commercial Aspects of Enzymatic Saccharification of Lignocellulosic Biomass

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Abstract Currently, society is looking for new alternative energy sources, cleaner and less harmful to the environment, and an example of this is the depletion of fossil fuels and the search for biofuels from various renewable materials, which can be classified as first and second generation. The most common in the industry is the first-generation biofuel obtained through edible oils or vegetable sugars, mainly corn and sugar calla, and the second-generation biofuel obtained from the exploitation of residual raw material residues from food industries, forest residues among others. On the other hand, the third-generation biofuels are obtained from non-food species by using molecular biology techniques in which microalgae currently stand out, and finally, in a similar way, the fourth-generation biofuels are manufactured from non-arable land. However, unlike third-generation biofuels, it does not require the destruction of biomass. The relationship between the different types of biofuels is the search for the saccharification process, which is a process in which a polysaccharide is transformed into fermentable sugar. Enzymatic hydrolysis is a type of saccharification in which the process is catalyzed by a group of enzymes generically called cellulases, which are a mixture of different enzymatic activities whose combined action degrades cellulose. During enzymatic hydrolysis, cellulose is degraded by cellulases to reducing sugars that can be fermented by yeast or bacteria to ethanol. This chapter will address the recent developments in the enzymatic saccharification

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(ES) technologies for biofuel production in their current state, challenges, products in the market, and prospects.

Keywords Saccharification · Enzyme · Biofuel · Cellulase · Lignocellulose

12.1 Biofuels

Mainly, the energy demand has been covered by fossil fuels; however, the increase in the population has generated the development of different energy supplies to improve the quality of life (Rodionova et al. 2016). The use of fossil fuels presents some disadvantages such as the generation of pollutants and the emission of greenhouse gases (Beig et al. 2021). Recently, other energy generating alternatives have been investigated, for example, biofuels, which are energy-rich chemicals generated through biological processes or derived from the biomass of living organisms such as microalgae, plants, or bacteria (Rodionova et al. 2016). The production of biofuels has been studied in some species of bacteria such as *Escherichia coli* and *Bacillus subtilis* (Hasunuma et al. 2013). *Saccharomyces cerevisiae* is an another microorganism that has been utilized for the efficient production of ethanol by a fermentative process (Rodionova et al. 2016). It has been found that some species of algae can produce biofuels; *Botryococcus braunii* and *Chlorella protothecoides* contain high amounts of terpenoid hydrocarbons and glyceryl lipid, which can be transformed into shorter hydrocarbons to produce bioethanol, triterpenic hydrocarbons, isobutyraldehyde, and isobutanol (Rodionova et al. 2016).

Biofuels can be used in a wide variety of ways – liquid fuels (long-chain alcohols, bioethanol, biodiesel, and biobutanol) and gaseous products (methane and hydrogen) (Beig et al. 2021). They are composed of ethanol, 1-butanol, isobutanol, isobutene, isoprene, and farnesene. To obtain, it requires the fermentation of sugars derived from biomass such as corn, sugar cane, and vegetable oil (Choi et al. 2020). Biofuels have been classified into three different generations, the first generation which is derived from food biomass (Immethun et al. 2016); the second generation which is derived from non-edible biomasses, or lignocellulosic biomasses (Saladini et al. 2016), and the third generation which is derived from photosynthetic microorganisms as microalgae (Alaswad et al. 2015).

12.1.1 Conventional Biofuel Production

The application of the biomass resource that is produced in vicinity of the site of production of the biofuel has some advantages in its procurement and less cost in transportation (Grisolia et al. 2020). Biofuel production requires some characteristics, for example, easy availability, technical and environmental feasibility, and

economic competitivity (Grisolia et al. 2020). Commonly, biofuel production is realized by the biomass from organisms and plants, such as firewood, wood chips, pellets, animal waste, forest and crop residues, and landfill gas. These materials are composed of ethanol, alcohols, triglycerides, fatty acids, lipids, carbohydrates, and cellulose, which are considered as the major biofuels sources (Rodionova et al. 2016). Bioethanol and biomethane are produced through the fermentation of starch or sugars; biodiesel is obtained by the transesterification of oil crops and the hydrogen from microalgae and microbes (Dragone et al. 2010). Lignocellulosic biomass is an important source of sugars for the production of bioethanol. Currently, lignocellulosic biomass from rice straw or cane has been used for the biofuels production; also, some plants with a high content of starch such as maize has been applied. Bioethanol is produced by distillation, hydrolysis, and subsequent fermentation (Dias et al. 2009). Lipids are commonly accumulated in cell biomass which can be converted into multiple products (Dong et al. 2016). Biofuels are also produced by the oleaginous microorganisms; obtention of biological lipids is favoured because direct lipid extraction from the wet cell biomass eliminates the need for costly dehydration (Dong et al. 2016).

12.1.2 Use of Enzymes in the Production of Biofuels

In the production of second-generation biofuels, lignocellulosic materials are employed. These materials come from a wide variety of sources and their composition can vary. In general, lignocellulosic materials are composed of cellulose, hemicellulose, and lignin. The process involves several steps: fermentation, pretreatment, and enzymatic saccharification (ES) (Binod et al. 2019).

The pretreatment is considered to be a very crucial initial step as it needs to be well chosen to ensure the hemicellulose and lignin removal from the biomass to improve the enzyme contact with the matrix (Guo et al. 2018). Different kinds of pretreatments can be applied to the lignocellulosic biomass such as chemical, physical, and biological and their severity can deteriorate the biomass to release polymeric sugars (Guo et al. 2018; Nargotra et al. 2018; Rattanaporn et al. 2018). When choosing the pretreatment method, it should be considered that some pretreatments can release inhibitors for fermentation (Guo et al. 2018).

Oxidation pretreatments have also been reported to be successful for lignocellulosic materials. A recent study (Xiao et al. 2017) evaluated two oxidation pretreatments (Fenton reagent and peroxyacetic acid) for biofuel production employing sugarcane bagasse, *Eichhornia crassipes*, and *Metasequoia glyptostroboides*. Peroxyacetic acid resulted in the improved lignin removal for sugar cane bagasse (reaching carbohydrate content up to 90.63%) and *Metasequoia glyptostroboides* (up to 93.73% of carbohydrates). On the other hand, the Fenton reagent displayed better performance on *Eichhornia crassipes*. Also, the results showed higher porosity and improved surface area for the action of enzymes. This highlights the influence of different pretreatments methods on the different types of materials.

To perform the enzymatic saccharification, the enzymes to be used need to be properly chosen due to the complexity of the biomass. One single enzyme is not sufficient to perform the enzymatic hydrolysis; so a pool of enzymes is often selected to carry out the process. These enzymes are grouped as cellulases, xylanases, peroxidases, and laccases (Binod et al. 2019; Guo et al. 2018; Siqueira et al. 2020).

The cellulases are composed of endoglucanases EC 3.2.1.4 (which randomly hydrolyze internal β-1,4-glucosidic bonds), cellobiohydrolases EC 3.2.1.91 (which can produce cellobiose by hydrolyzing β-1,4-glycosidic linkages at the reducing and non-reducing ends), and β-glucosidases EC 3.2.1.21 (which may act on cellobiose degrading it into glucose) (Binod et al. 2019; Guo et al. 2018; Gupta et al. 2016; Siqueira et al. 2020). Lignocellulosic enzymes are also composed of xylan which can be degraded by xylanases. These englobe endo-1,4-β-xylanase (EC 3.2.1.8), β-xylosidase (EC 3.2.1.37), and α-arabinofuranosidases (EC 3.3.1.55) (these enzymes release xylooligosaccharides that are further degraded to xylobiose and sequentially to xylose) (Biely et al. 2016; Binod et al. 2019; Cui and Zhao 2012; Thomas et al. 2013). Peroxidases such as lignin peroxidase enzyme (LiP, EC 1.11.1.7) and laccases (EC 1.10.3.2) help cellulases in hydrolyzing lignocellulose (Gupta et al. 2016).

12.1.3 Enzymatic Saccharification and its Use for the Production of Biofuels

For efficient saccharification, the process parameters need to be well defined. These parameters involve pH, optimal enzyme concentration, temperature, and time (Bala and Singh 2019; Faizal et al. 2020; Rattanaporn et al. 2018).

A recent study (Narra et al. 2020) employed response surface methodology to optimize the culture conditions for four hydrolytic enzymes from the fungi *Aspergillus tubingensis* M7. Among those, the optimized parameters were incubation time, inoculum size, moisture content, and substrate (g%). The results showed a high saccharification efficiency up to 86.02%.

An optimized saccharification process for the bioethanol production was reported by (Faizal et al. 2020) utilizing four species of duckweeds, *Lemnaaequinoctialis*, *Landoltia punctata*, *Spirodelapolyrrhiza*, and *Wolffia arrhiza*. Best starch conversion to sugar was achieved after 24 h at 50 °C with a 2: 1 (v/v) of α-amylase and amyloglucosidase. Sugar conversion was further carried out obtaining 0.16–0.19 g of ethanol/g of dry biomass.

Enhancement in the enzymatic saccharification yield is highly influenced by the pretreatment; several authors focus on pretreatment to reach more efficiency by their enzymatic methods. (Nargotra et al. 2018) reports an improved enzymatic digestibility (163.42 mg sugars/g biomass) followed by an alkali (NaOH) and ionic liquid 1-butyl-3-methyl imidazolium chloride pretreatment. (Rattanaporn et al. 2018) reports a chemical pretreatment composed of organic acids (acetic acid, oxalic

acid, and citric acid) in which enhancement of enzymatic saccharification was observed to be around 2.3 times higher sugar yield compared to the untreated biomass.

12.2 Challenges for Research and Development

Biofuel production from lignocellulosic residues is one of the most common alternatives (Beig et al. 2021). However, there are many challenges in obtaining biofuels from lignocellulosic biomass. Biomass is a highly oxygenated and highly functionalized material, so it is necessary to increase energy density and reduce reactivity when generating a biofuel (Alonso et al. 2012). The conversion of lignocellulosic materials into fermentable sugars as fuel precursors such as ethanol (Lin et al. 2019) has several stages that start with a pretreatment step (Yang et al. 2020; Wang and Lü 2021), followed by enzymatic hydrolysis, and ends with the fermentation of the obtained sugars (Yang et al. 2020; Wang and Lü 2021). The pretreatment applied to biomass represents 20–30% of the total costs associated with biofuel production (Axelsson et al. 2012; Beig et al. 2021). Hydrolysis of hemicellulose and cellulose into reducing sugars is a critical point in the conversion process (Alonso et al. 2012). Thus far, biofuel production from lignocellulosic material has been limited (Lin et al. 2019). This is mainly attributed to the hydrolysis stage, where some barriers are present, which affect the viability of the process. Some of them are the cost associated with the enzymes in charge of hydrolyzing the biomass (Lin et al. 2019), the low hydrolysis efficiency, and the high production costs (Wang et al. 2020). On the other hand, there is a need for low-cost feedstocks that can be effectively digested by hydrolytic enzymes (Lin et al. 2019) with low processing costs(Sandesh and Ujwal 2021).

Hydrolyzing the lignocellulose into monosaccharides remains a technical challenge due to the indigestibility of the cellulose structure (Khaire et al. 2021). Pretreatment of the lignocellulosic biomass is paramount to improve the cellulose accessibility for the enzymes to release fermentable sugars at the hydrolysis stage and reduce the enzyme usage (Marulanda et al. 2019). Several studies have focused on determining the process conditions that allow better yield at a lesser cost.

Pretreatments applied to lignocellulosic biomass before enzymatic hydrolysis that require high energy demands (Beig et al. 2021) can be biological, physical, chemical, and physicochemical (Shafiei et al. 2015; Lin et al. 2019; Houfani et al. 2020). These are applied independently or in combination to improve the enzyme efficiency during saccharification (Jamaldheen et al. 2018). The combination of methods has greater advantages than a single pretreatment method as it favors the monosaccharide production, reduces the inhibitor formation at high concentration, and reduces the effects of extreme pretreatment conditions. The combination of pretreatments results in higher productivity. The most effective pretreatments for lignin and hemicellulose removal are the combination of dilute acid with a steam explosion,

alkaline pretreatments, and microwave-assisted alkaline pretreatments (Lu et al. 2009).

Accordingly, the current challenge lies in the development of an efficient pretreatment process that meets the following requirements:

1. To be energy efficient without compromising production.
2. Minimize the loss of compounds and products, particularly sugars.
3. Avoid the application of products that may act as inhibitors to the reactions. Avoid washing or neutralization steps that increase the cost of operation.
4. Synchronize subsequent operations to increase the overall efficiency of the process.
5. Include a subsequent sugar preconcentration step to improve the efficiency of the process.
6. Optimize the fermentation time between 3 and 4 days (Houghton 2006; Axelsson et al. 2012; Beig et al. 2021).

Other challenges for the practice of large-scale enzymatic saccharification are the low enzymatic activity and the high costs (Chen and Fu 2016; Guo et al. 2018).

12.3 Marketing and Products in the Market

The development of biofuels from the renewable sources is an essential issue for the conservation of the planet's fossil resources. Different raw materials have been reported as substrates to produce biofuels. Biomasses such as food crop, non-food lignocellulosic biomass, microalgae, forest, agricultural residues, and agri-food residues (Raud et al. 2019) have gained global importance due to their environmental impact on the ecosystem (Torres-Valenzuela et al. 2020). An important case is that of agro-industrial waste, which could generate up to five billion tons of waste per year globally (Naidu et al. 2018). The use of this wastes in the production of biofuels requires an initial stage of enzymatic hydrolysis or saccharification which is decisive for the viability of the process with a contribution of 25% of the operational costs (Valdivia et al. 2016). The saccharifying enzymes are used as the complex carbohydrate degraders in biofuel production and play an important role in the optimization of the process conditions.

As analyzed by the Business Communication Company (BCC), in 2023, total world-wide industrial enzyme market should reach $7.0 billion and the estimated compound annual growth rate is 4.9% from 2018 to 2023. By 2021, market studies predict an increase in the production of technical enzymes including those used to produce biofuels which is directly related to the creation of new production processes.

The countries with the highest demand for enzymes are North America, Western Europe, Japan, and Canada. By 2021, it is estimated that the global enzyme market could increase by 6.8–7.9% for the North American and Asia-Pacific regions. Other markets such as Eastern Europe, Middle East, and Africa have also been highlighted.

In 2016, the global technical enzymes market was dominated by Europe, the Middle East, and Africa, accounting for approximately 35%; however, the market may grow further in North America and the Asia-Pacific region in the coming years (Dewan 2014). For the biofuel enzyme market, sales of more than $300 were predicted in the Europe and North America regions by 2020 (BBC Research 2015).

New enzymatic technologies have made it possible to overcome the problems of converting recalcitrant biomasses or lignocellulosic materials. Currently, saccharolytic enzymes are produced in the market by making blends of enzymes (Lange 2017; Lange et al. 2021). Many enzymes are available on the market mainly from Novozymes (Denmark), Danisco/Dupont (US), BASF (Germany), DSM (Netherlands), and Abengoa representing an important segment of total enzyme production (Dewan 2014). Other important companies are Denykem (UK), Megazyme (Ireland), Advanced Enzymes Technologies (India), and MetGen (Finland). Novozymes released an annual report in 2019, estimating that it comprises approximately 48% of the global enzyme market; it also reported that sales during the same year increased moderately with predominant growth in India and a weakening of the market in China and emerging markets. Currently, Novozymes has launched the product Fortiva®, composed of alpha-amylase, to increase ethanol production yields by 1%. It should also be noted that this company has focused its efforts in the production of yeasts to produce the first-generation biofuels, under the name Innova® yeast technology. According to its annual report, sales in the bioenergy sector are expected to grow 1–5%.

Commercially available enzymes for biofuel production from different feedstocks can be grouped into cellulases, amylases, β-glucosidases, xylanases, proteases, lipases, keratinases, laccases, lignin peroxidase, and manganese peroxidase (de Pereira Scarpa et al. 2019). The applications of these enzymes can vary according to the type of fermentation such as solid-state (SSF) or submerged (SmF) fermentation (de Castro and de Castro 2012). Among the saccharifying enzymes that dominate the market are cellulases that are popular due to the wide range of industrial applications; other enzymes such as lipases, catalase, and xylanase are being investigated based on catalytic activity (Chapman et al. 2018). In the market, these enzymes can be prepared as cocktails which contain different enzymes with specific properties and other substances such as secondary metabolites produced by microbial strains (Álvarez et al. 2016). The main option in the biofuel enzymes market is Spirizyme®, portfolio launched by Novozymes (Table 12.1), which contains eight gluco-amylases for saccharification. The most outstanding enzyme is trehalase which has allowed to increase the starch ethanol production yields with reducing fermentation times (Novozyme 2021).

Market studies have shown that the price of the enzyme should stabilize at $0.4/gallon; however, this cost may increase in a commercial presentation. By 2020, the total cost of enzymes for biofuel has been estimated at $1.0 billion (BBC Research 2015; Lopes et al. 2018)). Previous studies report that the enzyme costs higher than 30% in the bioethanol production (Solarte-Toro et al. 2019). Stabilization of the enzyme cocktail costs requires increasing demand and competition. The design of

Table 12.1 Commercially available enzymes in the biofuel market

Trade names	Companies	Enzyme type	Reference
Spirizyme®	Novozymes	Glucoamylases	Novozyme (2021)
Celluclast®, Cellic CTec2 Cellic CTec3	Novozymes	Cellulase	Khare et al. (2015); Scott et al. (2016); Brar et al. (2019)
HTec3®	Novozymes	Cellulase	Sharma et al. (2016)
Termamyl1®	Novozymes	Amylase	Fasim et al. (2021)
AMG1®	Novozymes		Fasim et al. (2021)
Viscozyme L®	Novozymes	Multienzyme	Gama et al. (2015)
Novozyme 188®	Novozymes	Glucosidase	Khare et al. (2015)
BrewZymeLP®	Danisco	β-Glucanase	Sharma et al. (2016)
Boli GA-150®	Boli bioproducts	Glucoamylase	Sharma et al. (2016)
Spezyme®	Genencor	Cellulase	Khare et al. (2015)
Accelarase 1500®	Genencor	Cellulase	Khare et al. (2015)
Optimax L-1000®	Genencor	Pullulanase	Sharma et al. (2016)

new enzymes or preparations from lignocellulosic biomass is necessary to ensure the stability of this market segment (Valdivia et al. 2016).

Genetic engineering has played a crucial role in the design of new enzymes or enzyme preparations. Currently, there is an increasing interest in improving the thermal stability of the enzyme, the temperature being a determining factor in the viability of the plant material. The enzyme production yield and its catalytic efficiency, as well as the reduction of protein production costs, and inhibition of the final product are some of the issues under study (Elleuche et al. 2014; Valdivia et al. 2016).

The production of technical enzymes is affected by the Research & Development (R&D) activities and the environmental policies and legislation in each country. The Paris Climate Agreement encourages the production of fuels from renewable sources to reduce greenhouse gases, a situation that has favoured the demand for saccharifying enzymes (Dewan 2014). However, the cost of technical enzymes remains an important factor in the growth of the market.

12.4 Success and Failure Stories and New Trends in Enzymatic Saccharification Technologies

12.4.1 Success and Failure Stories

The enzymatic saccharification is an efficient and environment friendly process to enhance the reducing sugars from polymeric sugars in the lignocellulosic biomass (Tan et al. 2016; Manisha 2017).

The lignocellulosic material was decomposed to monosaccharides using acid-catalyzed or alkali-catalyzed hydrolysis. Acid hydrolysis can be performed using dilute acid or concentrated acid. Alkaline hydrolysis results in efficient lignin removal and low inhibitor formation, but this technique is expensive and results in alteration of the lignin structure (Saeed and Saleem 2018). However, it causes corrosion of the gas equipment and produces by-products that inhibits further fermentation.

The enzymatic hydrolysis is of great interest because it could overcome the disadvantages of acid and alkali catalyzed hydrolysis. However, there are still some downsides such as slow reaction rate and limited enzymatic accessibility to polysaccharides. Pretreatment is necessary to open the biomass cell wall structure which would increase the enzymatic accessibility during enzymatic hydrolysis (Saeed and Saleem 2018).

The obstacles for carrying out enzymatic saccharification on a larger scale are increased costs and little profitability (Chen and Fu 2016). Genetic engineering has been one of the solution tools for enzyme technology. However, it suffers from various drawbacks such as posttranslational modifications, inclusion bodies, costs, tediouness, time-consumption, and expertise requirement. Immobilization has been the foremost enzyme technology being used due to its simplicity, decreased labor, and cost-efficacy. It leads to physical confinement or localization of enzymes in a specifically defined region of space with retention of their catalytic activities and less sensitivity towards their environment with insistent usability (Dwevedi and Kayastha 2011).

Since several obstacles are encountered in the process, it was first necessary to use extensive pretreatment processes. Since the objective is to fractionate the cellulose, hemicellulose and lignin of the biomass, to later hydrolyze it using selected enzymes at reduced doses. (Zhang et al. 2016). Pretreatment with an organic solvent has been studied. (Li et al. 2016). However, the selection of the solvent should meet several requirements such as low risk to health, production of cellulose for the subsequent phases, and low cost.

The reducing sugars obtained in the saccharification stage will be fermented in the next phase and will be able to produce some biofuel. Separate saccharification and fermentation (SHF) is a standard practice; however, its optimal conditions are generally different (Guo et al. 2018). Therefore, there is strong research interest to seek combined processes to increase general enzymatic saccharification and subsequent fermentation of yeast that include simultaneous saccharification and fermentation (SSF), presaccharification and simultaneous saccharification and fermentation (PSSF), and consolidated bioprocesses (CBP) (Loaces et al. 2017; Hilares et al. 2017).

12.4.2 New Trends in Enzymatic Saccharification Technologies

As mentioned above, the enzymatic saccharification processes have been accompanied by the genetic recombination processes of microorganisms to obtain higher enzyme titers and enzyme recovery for reuse in search of an increase in the production yields (Guo et al. 2018). Nowadays, research on the production of biofuels continues to make use of the enzymatic saccharification processes. The main difficulties derive from the crystallinity and degree of polymerization of cellulose, the accessibility to the substrate surface, and mainly from the presence of lignin. The last one prevents the swelling of fibers and produces non-productive adsorption of cellulases (Sheng et al. 2021). Novel pretreatments accompany the enzymatic saccharification processes to contribute the delignification of the lignocellulosic biomass.

The hybrid pretreatment of ultrasound and organic solvents consists of solvents synergistic action with free radicals production. These radicals are produced through the sonochemical effect to exert an attack on the biomass components and reduce the cellulose crystallinity by rearrangement of molecules through mechanoacoustic development (Lee et al. 2020). Research has been carried out on the use of lytic polysaccharide monooxygenases (LPMOs) in conjunction with cellulase enzymes to improve saccharification yields through the oxidation of substrates surface, facilitating the access of hydrolytic enzymes (Velasco et al. 2021).

In recent years, the use of deep eutectic solvents (DES) as a pretreatment in the enzymatic saccharification has been reported. DES has properties similar to the ionic liquids, although they stand out for being simple to synthesize, biodegradable, and have a low cost (Ling et al. 2020). As lignin is an essential component of lignocellulosic materials, its revaluation in saccharification processes could contribute to biofuel production's profitability (Huang et al. 2021). Research has recently been conducted using DES with lignin derivatives. In 2020, the first report of DES prepared with *p*-hydroxybenzoic acid (derived from lignin) and choline chloride for the pretreatment of woody biomass improved the percentage of delignification enzymatic hydrolysis, also achieving a sustainable process by recycling the DES used (Wang et al. 2020). Similarly, Huang et al. (2021) reported that using a DES pretreatment consisting of choline chloride/guaicol (derived from lignin) with traces of $AlCl_3$ contributed significantly to the degradation of hemicellulose and lignin, resulting in complete enzymatic hydrolysis from wheat straw.

Use of the alkaline hydrogen peroxide has been reported to increase the enzymatic digestibility of corn stubble leading to the breaking of the hydrogen bonds of cellulose and hemicellulose and the elimination of lignin, reducing the non-productive adsorption of cellulases to the biomass (Yang et al. 2021). The use of a novel hybrid pretreatment has recently been reported by Tang et al. (2021). They combined an organic surfactant (humic acid) with dilute sulfuric acid, achieving an increase in the percentage of lignin and hemicellulose removal and the ES of wheat straw, reaching a saccharification percentage of 92.9% (Tang et al. 2021). Similarly,

a pretreatment effect based on ozonolysis with a subsequent washing with sulfuric acid under mild conditions before enzymatic saccharification has been evaluated with good results in cane bagasse (Perrone et al. 2021).

There is currently a trend toward the use of enzymatic saccharification using the macroalgal biomass. The use of macroalgal biomass is because, in contrast to the terrestrial lignocellulosic biomass sources, they do not requireland for agriculture or fertilizers, water, or pesticides. In some cases, they respond better to the thermal pretreatments, increasing the percentage of saccharification compared to that achieved with some terrestrial biomass (Thygesen et al. 2020).

12.5 Conclusions

In this chapter, after reviewing, it was concluded that the need for replacement and overexploitation of fossil fuels is imminent due to the consequences and risks they represent, for which it is very important to continue research and studies of new sources of renewable energies such as biofuels. It is essential to invest time and efforts to improve their production and yields and investigate new technologies that offer greater benefits. Enzymatic saccharification is an option, as we observed in this chapter, is quite interesting and attractive for its use and that has already been used with active products in the market and with very interesting success stories; however, there is still a long way to go, starting with research focused on this issue since there are still complications that have not been studied and it is important to consider better production processes at the industrial level.

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