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Second-generation ethanol: concept, production and challenges

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ABSTRACT: The use of biological agents for the large-scale production of biofuels has stood out as successful processes for the advancement of science in the world. The growing exploitation of biomass in the agricultural sector and the emergence of new energy sources generated from food industry waste have become attractive and viable due to the potential and variety of possibilities for using different sources of biomass. The present review was carried out through careful bibliographical research in the literature and in scientific journals for the current discussion of concepts, production methodologies and challenges for the energy sector considering second-generation ethanol (2G ethanol). Several 2G ethanol production methodologies have been implemented as a potential low-cost alternative energy production that follows the principles of Green Chemistry.

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1. Introduction

Sugarcane originated in the Southeast Asian region, near India. This desired product that passed through lands such as Genoa, Venice and Sicily had contact with Brazilian lands through Portuguese colonization, being brought by settlers from Madeira Island, famous for being the largest producer of sugarcane in the 15th century. During the Portuguese colonization, the province of Pernambuco, in a short period of time, became one of the most important and profitable lands in Portuguese possession. In addition to its coastal location, Pernambuco was ideal for planting sugarcane, with geographical (intense solar radiation) and natural (soil and climate) conditions favoring its exploitation and use in mills throughout the region. The mills produced not only sugar, but also one of the biggest by-products of sugarcane, ethanol, which was essential for the Portuguese rise in the world.

Nowadays, six centuries later, occupying the second largest producer and consumer of ethanol in the world, only behind the USA (UNEM, 2021), Brazil is responsible for producing about 31.6 billion liters of ethanol in 2019, according to the website of Agency of Brazil and Conab (National Supply Company of Brazil) (Agência Brasil, 2019). Hydrated ethanol sold at gas stations reaches 18.9 billion liters and anhydrous ethanol, used in the mixture with gasoline sold at gas stations, reaches 10.5 billion liters. In addition, the Midwest region is the one that most uses cereals for the production of ethanol in the national territory, about 1.27 billion liters in 2019.

The production of ethanol in abundance has an equivalent generation of residues that presents difficult decomposition, causing damage to the environment due to the accumulation of these materials (Herrera-Ruales and Arias-Zabala, 2014); therefore, techniques have been developed that use residues that are usually discarded from the main processes in the production of ethanol, but which are rich in cellulose, hemicellulose and lignin that, after undergoing pretreatments, steps and chemical processes, are converted into biofuels.

2. Second-generation (2G) ethanol

The USA occupies the position of one of the largest emitters of carbon dioxide on the planet; 2019 data record about 6,558 million tons of emission (US EPA, 2021). On the other hand, we celebrate the encouraging return of the USA, in 2020, to the Paris Climate Agreement proposed by COP21-2015 (21st Conference of Parties to the United Nations Framework Convention on Climate Change), which aimed to bring positive climate change, in order to reduce the environmental impacts resulting from the drastic increase in the temperature of the planet.

The matter is urgent for our planet, as it triggers other problems for all of us, such as the low yield of food production, the constant threat to biodiversity, compromised water quality in rivers and seas, and the possibility of extreme natural events as well.

Solutions that can minimize the effects mentioned above are frequently studied and developed around the world. The idea that has drawn the attention of many researchers is the use of discarded matter in agriculture, known as biomass or lignocellulosic biomass and confirmed as the most abundant renewable resource in nature (Yu *et al.*, 2018). One of the ideas that has been gaining strength is the use of biomass from sugarcane bagasse to produce 2G ethanol.

Raízen, a joint venture between Cosan and Shell, announced in 2022 the construction of four industrial plants to produce 2G ethanol using biomass from sugar cane, becoming the only one in the world to operate with a greater number of cellulosic ethanol plants on an industrial scale (Boechat *et al.*, 2022).

Most of the raw biomass, after pretreatment, are composed of hemicellulose (~28%), cellulose (~40%), lignin (~33%), extracts (~2%) and ash (< 1%), as shown in Fig. 1. The extracts are low molar mass chemical compounds. They consist mainly of terpenes, fats, waxes, and phenolics, and their content and composition vary among species, location and season (Jönsson and Martín, 2016). These components are associated with different types of cellular organization and biochemical processes of biomass, which makes their composition relative from one species to another. The use of lignocellulosic biomass discarded in biorefineries has been surprisingly positive. Recent study showed the possibility of obtaining monomers for polymers, high-value fuels and pharmaceutical intermediates that have a wide variety of structural complexity (Bender *et al.*, 2018).

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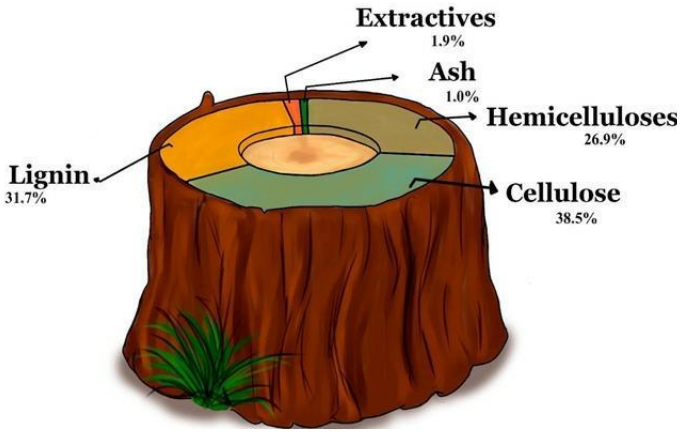

Figure 1. Representation of lignin, hemicellulose, cellulose, extractives and ash sources from plant biomass.
Source: Adapted from Jönsson and Martín (2016).

Regarding the issue mentioned above, the alternative of using 2G ethanol fits as a sustainable option for reducing greenhouse gases, in addition to increasing ethanol production per area of land. This alternative envisages being able to reduce the exploitation of other resources that are sources of high emission of gases, such as fossil fuels.

However, the production of 2G ethanol is not consolidated as it presents economic and technological obstacles (Elias *et al.*, 2021). The process of obtaining 2G ethanol needs to be detailed, because every type of raw material needs a pretreatment to successfully release the sugars contained in cellulose fibers that are incorporated into the bases of plant cell walls (Agbor *et al.*, 2011).

The main steps listed for the manufacture of 2G ethanol consist of pretreatment, hydrolysis and fermentation, those of which require greater care for each type of biomass exploited. The general model for representing the transformation of biomass is shown in Fig. 2.

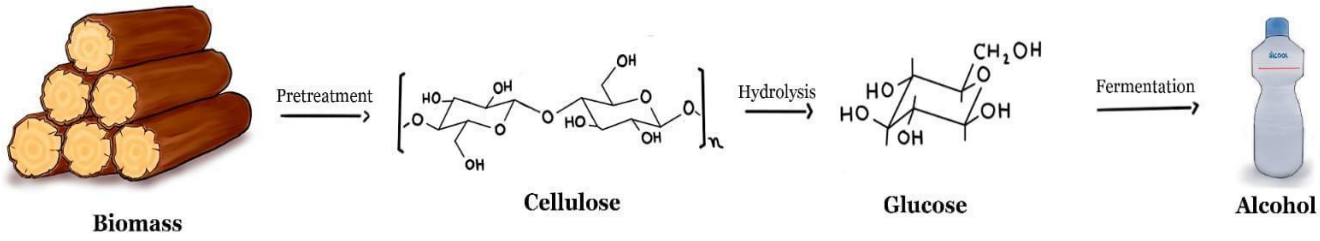

Figure 2. Representation of 2G ethanol production from lignocellulosic material.
Source: Adapted from Santos *et al.* (2012).

2.1 Pretreatment

Jönsson and Martín (2016) stated that pretreatment is an important step in the process that aims to eliminate the physical and chemical barriers that make native biomass recalcitrant and cellulose accessible for enzymatic hydrolysis, which is a fundamental step in the biochemical processing of lignocellulose based on the sugar platform concept. The efficiency of the process comes from increasing the surface of accessible cellulose through the solubilization of lignocellulosic residues such as lignin or/and hemicelluloses that cover the biomass.

Usually, pretreatment is carried out with the objective of reducing the degree of crystallinity of the cellulose, increasing the contact surface of the material leaving them porous, thus facilitating the conversion of sugars, eliminating hemicelluloses and lignins, which in contact with the hydrolysis step form monomeric sugars not fermentable by yeast (Beig *et al.*, 2021). The breakage of the lignocellulosic wall is schematized in Fig. 3.

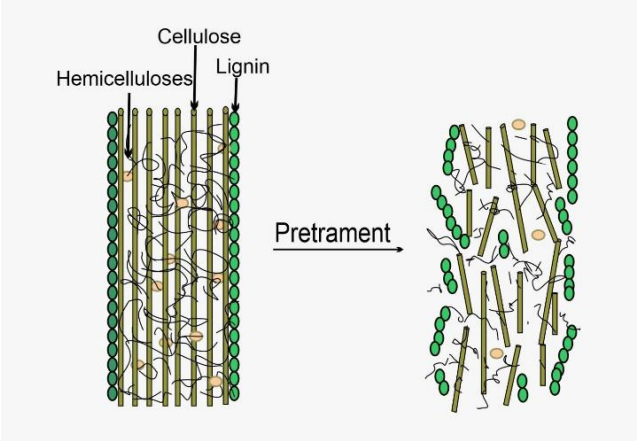

Figure 3. Biomass submitted to pretreatment, with the elimination of hemicellulose and lignins.
Source: Adapted from Santos *et al.* (2012).

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The concept of lignin-carbohydrate complex is well accepted in biomass chemistry, which is a cross-linked structure formed by the interconnection of polysaccharides (cellulose and hemicellulose) (Cui *et al.*, 2022). Lignin is a highly branched aromatic macromolecule composed of guaiacyl propane (methoxy-3-hydroxy-4-phenylpropane), syringyl-propane (dimethoxy-3-5-hydroxy-4-phenyl-propane) and hydroxyphenyl propane units that bind cellulose and hemicellulose together (Yu *et al.*, 2018).

Due to the complex matrix of lignocellulosic biomass and the peculiarity of recalcitrance of the material, we are faced with the obstacle of water-insoluble matter and the impossibility of being directly hydrolyzed to produce sugars. Therefore, the pretreatment step is mandatory.

Often, the reactions involved in pretreatment result in products derived from lignocellulosic materials that are inhibitors to the biochemical process, mainly because the lignin dissolved in the solution can be attached to the biomass surface, leading to decreased access of enzymes to cellulose and lignin, which can also cause inhibition of cellulase activity due to nonproductive adsorption through hydrophobic, electrostatic and hydrogen bonding interactions (Huang *et al.*, 2022), which become significant in large quantities of products. Hitherto, pretreatment records cannot be quantified, as we have numerous different sources of biomass, each with its own particularities and cellular organizations. However, some techniques show good results with different types of biomass and are therefore more commonly used and are routine procedures.

Some studies are investigating the interaction between lignin and cellulase, which is aimed to understand how lignin inhibits the enzymatic hydrolysis efficiency of cellulose (Cui *et al.*, 2022; Lu *et al.*, 2016; Zhao *et al.*, 2022). They also provide new evidence for the structural information between cellulose and lignin in poplar using ^{13}C NMR, ^{1}H NMR and 2D NMR analyses.

Pretreatment methods can be divided into categories including, physical (milling, microwave, ultrasound, and pyrolysis), chemical (acid, alkali, ozonolysis, and organic solvent, ionic liquids), physicochemical (hot water, steam explosion, ammonia based, wet oxidation, and carbon dioxide, CO_2) and biological (microbial and enzymatic). For each material, there may be more than one pretreatment considering that the most efficient methods may not be according to availability, there are adaptations of pretreatments or substitutions to arrive at the desired material (Meenakshisundaram *et al.*, 2021; Silva *et al.*, 2022; Zanivan *et al.*, 2022).

2.1.1 Acid-based methods

Acid hydrolysis is one of the most promising pretreatment methods regarding industrial implementation, due to the low methodological complexity. Acid-based methods are divided into weak acid and strong hydrolysis (Karatzos *et al.*, 2012).

The dilute acid treatment (e.g., maleic and fumaric acids) is one of the most effective pretreatment methods for lignocellulosic biomass. In general, there are two types of weak acid hydrolysis: High temperature ($T > 160$ °C) and continuous flow process for low-solids loading (5–10 wt% substrate concentration) and low temperature ($T < 160$ °C) and batch process for high-solids loading (10–40% substrate concentration) (Harmsen *et al.*, 2010).

Concentrated strong acids, such as H_2SO_4 and HCl, have been widely used for treating lignocellulosic materials because they are powerful agents for cellulose hydrolysis, but organic acids and sulfur dioxide are also used to a lesser extent (Assumpção *et al.*, 2016; Harmsen *et al.*, 2010).

This type of pretreatment results in high recovery of hemicellulosic sugars in the pretreatment liquid and a solid cellulose fraction with enzymatic convertibility. Acid pretreatment also has some disadvantages, such as high cost of materials used for reactor construction, gypsum formation during neutralization after sulfuric acid treatment, and formation of inhibitory by products (Jönsson and Martín, 2016). In addition, acid pretreatment can also lead to increased toxicity of the waste generated during the process.

2.1.2 Chemical pulping processes

Sulfite pretreatments are well-known and use acids, alkalis or neutral sulfite that show high recovery of hemicellulose sugars and enhance the susceptibility of the cellulose to enzymatic hydrolysis efficiency (Huang *et al.*, 2022). Despite being a technique that delivers satisfactory results, its execution is expensive due to the materials used in the construction of the reactors and can result in the production of inhibitory by-products after their neutralization (Jönsson and Martín, 2016).

2.1.3 Alkaline methods

Alkali pretreatment is based on saponification of intermolecular ester bonds crosslinking xylan hemicelluloses and other components such as lignin. This method removes acetyl and the various uronic acid substitutions on hemicellulose that lower the

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accessibility of the enzyme to the hemicellulose and cellulose surface (Harmsen *et al.*, 2010).

Alkaline pretreatment with heating results in lignin dehydration processes, promoting the formation of reactive compounds such as furfural and hydroxy-methyl-furfural, which are inhibitors of the metabolism of ethanol producing microorganisms. For this reason, it is important to control the temperature so that these reactions are not promoted. On the other hand, cleavage of acetyl groups may contribute to the formation of more reactive compounds such as furfural. Thus, alkaline pretreatments are highly efficient for the degradation of lignin and hemicellulose, which can increase bioethanol. However, it is important to control the temperature to avoid the formation of undesirable compounds (Carrillo *et al.*, 2005).

2.1.4 Oxidative methods

The crystallinity index can be reduced in pretreatment by adding oxidants to the biomass using alkali metal peroxide, wet oxidation and ozonolysis (Jönsson and Martín, 2016). The feasibility of the method is due to hemicelluloses solubilized and recovered as oligosaccharides in wet oxidation, which are of great interest to the pharmaceutical industry and the food sector since they have prebiotic agent and body agent properties (Maugeri Filho *et al.*, 2019). The combination of wet oxidation with alkaline compounds reduces the formation of phenolic aldehydes and furans.

2.1.5 Chemical pulping processes

Chemical pulping pretreatment is a method that targets lignin and to some extent hemicelluloses. This method can be applied to both soft and hard biomass. The major technologies used are Kraft (based on NaOH and Na_2S) and sulfite pulping. In sulfite pulping, which is based on an aqueous mixture of bisulfite (HSO_3^-) and sulfite (SO_3^{2-}), the hemicelluloses are hydrolyzed and removed to the spent sulfite liquor, while the cellulose is maintained almost intact (Mboowa, 2021).

Sulfite pretreatments are well-known and use acids, alkalis or neutral sulfite that show high recovery of hemicellulose sugars and enhance the susceptibility of the cellulose to enzymatic hydrolysis efficiency (Huang *et al.*, 2022). Despite being a technique that delivers satisfactory results, its execution is expensive due to the materials used in the construction of the reactors and can result in the production of inhibitory by products after their neutralization (Jönsson and Martín, 2016).

2.1.6 Hydrothermal processing

One alternative procedure is the hydrothermal processing, which consists of water in the vapor phase or liquid phase used in the biomass, being positive because it does not require the use of a catalyst and does not present significant corrosion problems. The key to the method is to control the pH around neutral values, which minimizes the formation of fermentation inhibitors, since in the process the water penetrates the biomass, hydrating the cellulose and removing part of the hemicelluloses and a small portion of the lignin (Jönsson and Martín, 2016).

Hydrothermal pretreatment was developed for cellulose recovery processes for conversion into ethanol. It is an energy-efficient, economical and eco-friendly technique because it employs water as a solvent in high temperature and pressure ranges. The conditions of the reaction medium can be divided based on the specific critical point of water (374 °C and 22.1 MPa), which separates into subcritical or supercritical hydrothermal pretreatment (HTP) (Ilanidis *et al.*, 2021; Saritpongteeraka *et al.*, 2020).

In addition, the systemic organization of the hemicellulosic matrix and the strong association of hemicellulose with the other components of the lignocellulosic complex confer resilience to the biomass cell wall. HTP stimulates the direct dissolution of hemicellulose into its sugars (arabinose, xylose, galactose, glucose, mannose), resulting in the synthesis of industrially attractive by-products, such as xylooligosaccharides, furfural and 5-hydroxymethylfurfural, and levulinic, acetic and formic acids (Ilanidis *et al.*, 2021).

The expansion and strengthening of multiproduct biorefineries have highlighted HTP, and it has been adapted for biomass fractionation through hemicellulose solubilization and lignin redistribution. The significant diffusion of HTP is a result of numerous advantages, such as competitive cost, limited use of catalysts, and sustainable and environmentally friendly properties (Scapini *et al.*, 2021).

Despite all the benefits of hydrothermal pretreatment, there are still some disadvantages of the process, such as high costs, the need for equipment that can withstand high pressures and temperatures, and the degradation of some compounds. In addition, the complexity of the reaction and the need for strict monitoring of the operating parameters also represent challenges for the commercial application of the process.

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2.1.7 Ionic liquid

A variety of ionic liquids (ILs), such as ammonium, pyridinium, imidazolium, and phosphonium-based cations, attached to alkyl or allyl side chains coupled to various anions, such as chloride, acetate, and phosphonate, has been used in the pretreatment of lignocellulosic biomass and presents an attractive method for presenting promising results; however, the final product presents potentially toxic by-products for fermentation microorganisms (Karatzos *et al.*, 2012).

ILs present high thermal stabilities and negligible vapor pressures; they do not release toxic or explosive gas when used, giving environmentally friendly characteristics (Karatzos *et al.*, 2012).

There are reports lignin removal from biomass, 29% total lignin removal from triticale straw using 1-ethyl-3-methylimidazolium acetate ([Emim]Ac) as a pretreated solvent at 150 °C for 1.5 h (Fu and Mazza, 2011). 1-Butyl-3-methylimidazolium chloride ([Bmim]Cl) was investigated as an IL solvent for pretreatment of legume straw at 150 °C for 2 h and observed 30% lignin removal (Wei *et al.*, 2012). Currently, studies (Asakawa *et al.*, 2015; Li *et al.*, 2007) have been conducted regarding lignin yield, as lignin is a potential renewable source for valuable products. Pretreatment of corn stalks with [Emim]Ac at 125 °C for 1 h resulted in a lignin yield of 44% (Li *et al.*, 2007). Bagasse fractionation was performed at 110 °C for 16 h with choline acetate, and 20% of the lignin was fractionated as lignin-rich material (Asakawa *et al.*, 2015).

ILs such as [Emim]Cl, [Bmim]Cl and [Emim]Ac have been widely advertised for the pretreatment of lignocelluloses; however, high pretreatment temperatures and long processing times are always required (Wang *et al.*, 2017).

Although the pretreatments appear separate, there is a way to use them in collaboration with other methods to obtain and investigate better results, as done by Yu *et al.* (2018), who used ultrasound pretreatment with ionic liquid at frequencies of 20, 28, 35, 40 and 50 kHz with 100 W power.

2.2 Hydrolysis

Hydrolysis is the step in which the cellulose and hemicellulose present in the biomass matrix, partially free from the lignin envelope, can be converted into pentoses and hexoses to be fermented, as schematically shown in Fig. 4 (Harmsen *et al.*, 2010; Grasel *et al.*, 2017). Despite expressing the acid hydrolysis process, the idea can be extended to the general concept of cellulose hydrolysis, since there are two varieties of possibilities widely used for this step: enzymatic hydrolysis or acid hydrolysis.

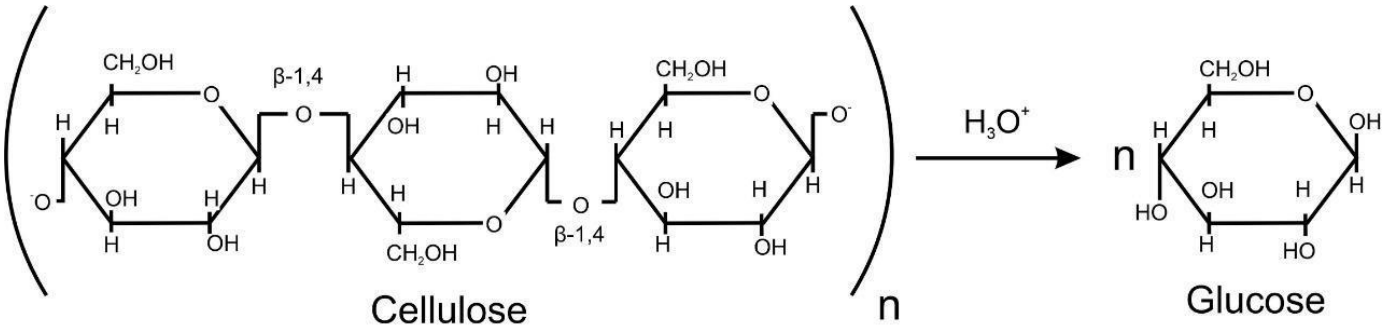

Figure 4. Cleavage of the β-1,4 glycosidic bonds of cellulose in an acidic medium and obtaining glucose.
Source: Adapted from Grasel *et al.* (2017).

2.2.1 Enzymatic hydrolysis

Enzymatic hydrolysis is carried out mainly by using the enzyme cellulase and hemicellulose, which breaks down cellulose and some other polysaccharides while keeping the lignin intact. Several factors can affect the yield and rate of enzymatic hydrolysis as well as limit the duration of the process depending on the substrate and enzyme. Substrate-related factors are cellulose crystallinity, degree of polymerization, available surface area, porosity, lignin barrier, hemicellulose content, particle size, cell wall thickness and capacity access to glucan, while enzyme-related factors are cellulase activity, reducing the cost and composition of the cocktail (Martins *et al.*, 2015).

Hemicellulose can also be attacked at intermediate positions along its skeleton, releasing oligomers made of many sugar molecules; these oligomers can be successively broken down into even smaller oligomers before a single sugar molecule can be formed. However, the breakdown of the released sugars can be moderate

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enough to recover about 80–90% of the maximum possible sugars (Ogeda and Petri, 2010).

The enzymatic hydrolysis of cellulose to produce 2G ethanol is a good alternative as it has temperature conditions of 40–59 °C at atmospheric pressure. However, as it is a process that takes 48 to 72 h, including catalytic deactivation by inhibiting enzymatic activity, as well as the use of enzymes such as cellulase, which have less environmental impact compared to acid hydrolysis (Cunha *et al.*, 2012), the process has a high cost to be accomplished. The residual lignin has an affinity for enzymatic adsorption, making it difficult for the enzymes to hydrolyze lignocellulosic matter, drastically increasing the amount of enzymes necessary for the glucose conversion to cellulose, increasing the cost of procedure (Florencio *et al.*, 2016).

The costs can be solved with methodologies that aim not only to reduce the number of enzymes used in the process, but also to improve the yield of saccharification and fermentation reactions, which consequently have a greater number of sugars to be fermented. A method that can reduce the total cost of enzymatic hydrolysis is the addition of lignin blocking agents at this stage, these blockers indicate increases in the alcoholic conversion rate, as the study by Kristensen *et al.* (2007) in the evaluation of the use of Tween 80 (Polysorbate 80) in Abies, which showed a 58% increase in the rate of conversion of biomass into available glucose. It is worth noting that the effect of the increase varies according to the type of biomass and pretreatment used.

2.2.2 Acid hydrolysis

Acid hydrolysis has inorganic acids, mostly sulfuric acid under high pressure conditions, for the cleavage or separation of the cellulose glycosidic bonds to obtain glucose (Harmsen *et al.*, 2010).

Although the two options are the most widespread in the sphere of the study on biomass, there are procedures used together that can meet the demand for breaking down cellulose, such as the one used by Tsubaki *et al.* (2017), who investigated the use of polyoxometalates with activated carbon support to the acceleration of hydrolysis under microwave irradiation.

Following these fundamental steps, sugars produced in the hydrolysis will be the direct source to produce ethanol; monosaccharides, which are simple carbohydrates with 4 to 6 carbons in their structure (Allinger *et al.*, 1976). These sugars are found in different proportions after hydrolysis of cellulose and hemicellulose; although it is not possible to predict the exact amounts of the sugars obtained, it is possible to have a projection of what they will be based on the type of material chosen.

Some examples: Pentoses (xylose and arabinose) are major monosaccharides in the hydrolysis of biomass from hardwoods (cane sugar, ipê tree and andiroba) and annual plants (corn and soybean). Hexose (glucose, mannose, galactose rhamnose) are major monosaccharides in the hydrolysis of softwood biomass (cane sugar and Paraná pine) (Agbor *et al.*, 2011; Jönsson and Martín, 2016; Rabemanolontsoa and Saka, 2013). It is important to point out that these statements are of major monosaccharides in these species, it does not mean that a species is devoid of one or another sugar. Such sugars will serve as an energy source for fermentation.

2.3 Fermentation

Fermentation is the step in which a microorganism (fungus or bacteria) transforms carbohydrates and produces ethanol. The options depend on what is worked on, considering that in many cases different rates of yield are obtained for different raw materials. This fact is given by the different types of sugars obtained, and the limitations of microorganisms (Tse *et al.*, 2021).

Due to their ease of acquisition and handling, the fungus *Saccharomyces cerevisiae* and the bacterium *Zymomonas mobilis* are the most used microorganisms in biomass fermentations, as they have high yields of ethanol rates. Values around 70–80% and higher at converting hexoses can be reached, despite their inactivity with xylose—the main pentose of hemicellulose (Hasner *et al.*, 2015). There are other types of microorganisms popular for being able to ferment hexoses and pentoses, such as *Scheffersomyces stipitis* and *Candida shehatae*; however, with limitations such as low consumption of sugars (Mengesha *et al.*, 2022).

The knowledge of species that ferment pentoses is still limited. Among the main species that ferment xylose, it can be mentioned *Pachysolen tannophilus, S. stipitis, Scheffersomyces shehatae, Kluyveromyces marxianus, Candida guilliermondii, Candida tenuis, Brettanomyces naardenensis, Scheffersomyces segobiensis* have been studied in the fermentation processes of pentoses. There are still challenges to controlling the fermentation of pentoses to ethanol, such as the low tolerance to ethanol by pentose fermenting yeasts. Also, the presence of glucose in hemicellulosic hydrolysates may act as a repressor of genes responsible for xylose utilization (Tse *et al.*, 2021).

Fermentations can occur using only one type of yeast (monoculture) or the combination of two or more yeasts (coculture). Regarding this topic, cocultures with

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genetically modified microorganisms have drawn attention in more recent studies, as their exception can supply the needs that isolated cultures have. Fermentation is a delicate step, as it requires the stability of factors such as temperature, reaction time, pH, bacterial contamination and organic and inorganic nutrients, which vary in quantity and type according to the material and microorganism chosen (Mengesha *et al.*, 2022).

3. Cellulose sources for ethanol production

The agricultural environment faces adversities due to demands that are not expected and supported by cycles of nature. In view of this, the negative impacts on the environment are almost inevitable, and if they are not minimized, they may be irreversible, as reinforced by Marques *et al.* (2007). These researchers stated that, given the great demand required by a socioeconomic system of society, the self-cleaning capacity of the aquatic cycle is compromised. Therefore, the importance of methodologies that have the ability to decelerate or reduce environmental degradation is no longer a small attraction to become a major priority.

It is important to mention that Brazil, as one of the largest holders of biological diversity, whether fauna or flora, has a variety of resources from unexplored sources for the conversion of sources of lignocellulosic materials. Moreover, we are faced with the use of various parts of a plant. As mentioned below, the cases range from studies involving parts of the same *Musa cavendishii* plant to the use of waste, such as the fiber present in the green coconut husk. The summarized list of the cases presented below are shown in Table 1.

Table 1. Different methodologies used in recent studies of 2G ethanol production through the purchase of biomass source, pretreatment, microorganism and yield results of pulp fermentation.

Cellulosic source	Pretreatment	Microorganism	Yield results
Cavendish banana (*Musa cavendishii*)	acid hydrolysis	*Saccharomyces cerevisiae*	34%
Orange albedo	acid hydrolysis	N.F*	49.7%
Elephant grass (*Pennisetum purpureum*)	acid hydrolysis	*Saccharomyces cerevisiae*	79%
Citrus pulp bran and orange pomace	enzymatic hydrolysis	*E.C* (Xanthomonas axonopodis, Saccharomyces cerevisiae)*	Monoculture: 50-99%
		Candida parapsilosis (IFM 48375 and NRRL Y-12969)	Coculture: 74-100%
Avocado seed (*Persea americana* Mill.)	enzymatic hydrolysis	*Yeast*	33.8%
Banana pseudostem	acid hydrolysis	*Saccharomyces* and *Zymomonas mobilis*	60%
		Scheffersomyces stipitis and *Pachysolen tannophilus*	
Green coconut husk fibers	Alkaline pretreatment and enzymatic hydrolysis	*Saccharomyces cerevisiae*	30.5%

*N.F. (not fermented), E.C (enzyme cocktail).
Source: Elaborated by the authors using data from Souza *et al.* (2012), Montagnoli *et al.* (2018), Antunes *et al.* (2015), Grasel *et al.* (2017), Cypriano *et al.* (2017), Cabral *et al.* (2016), Kowalski *et al.* (2017).

The growing agricultural production contributes to the increase of agricultural waste. Thus, this provides a diverse and encouraging variety of unexplored lignocellulosic materials to be selected and used.

Having this potential in mind, Souza *et al.* (2012) conducted a study that used banana pulp to produce 2G ethanol. Thus, the authors analyzed the potential of using the bark and pulp of *Musa cavendishii*, known in Brazil as banana in nature, previously hydrolyzed by enzymes and acid as a substrate for alcoholic fermentation. Two series of tests were carried out containing 18 pretreatments for each case of residue evaluated, using the isolated yeast *S. cerevisiae* for fermentation. The values of total ethanol yield (Qp) obtained using banana pulp and peel were, respectively, 3.04 and 1.32 g L^{-1} h^{-1}.

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The results showed superior yield when compared to sources such as wheat bran, corn flour and wood chips.

Proving again to be an excellent source of biomass, the banana and its plant was also approached by Montagnoli *et al.* (2018). They used the banana pseudostem to produce 2G ethanol, using different types of microorganisms for the fermentation of pentoses and hexoses: *S. cerevisiae* and *Z. mobilis*, *S. stipitis* and *P. tannophilus*. Despite the routine used in the steps, there was an application of the use of active charcoal for the detoxification of the banana pseudostem juice, which showed an increase of up to 60% in yield.

Considering that potential, Brazil is a country with high worldwide production and consumption of citrus fruits, with oranges being the one with the highest expressive numbers for the ranking. Accordingly, Antunes *et al.* (2015) dedicated to studies of better results from fruit byproducts. Then, they aimed to study the pretreatment of orange albedo with sulfuric, nitric, hydrochloric and phosphoric acids, with concentrations of 0.5% and 1%, for the determination of reducing sugars and total reducing sugars (TRS). The acid that generated the highest values of sugars was sulfuric acid, even at a concentration of 1%. However, the quantity of sugars obtained in the form of TRS were higher only when 0.5% acid was used, regardless of the acid chosen, indicating that higher concentrations of acid can degrade the sugars generated in secondary products. Consequently, an inhibitory effect on other steps in the production of 2G ethanol, such as fermentation and hydrolysis, may occur.

Another study developed by Grasel *et al.* (2017) used the biomass from elephant grass (*Pennisetum purpureum*). Unlike other biomasses, such as runny banana, corn and sugarcane, which are seasonal crops, elephant grass can yield up to four crops in a 1-year period. In addition to the advantage of being a crop that has a short production period, elephant grass does not need specific climates or soils for cultivation. For production, the material was previously treated to separate the cellulose from other components of the plant biomass, then acid hydrolysis was used to produce glucose and, finally, the alcoholic fermentation was carried out with the unisolated *S. cerevisiae* yeast. The result of sugarcane was 96 g of ethanol 100 g^{-1} of dry biomass compared to 79 g of ethanol 100 g^{-1} of dry biomass of elephant grass.

Using a cocktail of enzymes isolated from Gram-negative, *Xanthomonas axonopodis* and *S. cerevisiae* yeasts, and two strains of the *Candida* genus (*Candida parapsilosis* IFM 48375 and NRRL Y-12969), the conversion of biomass from citrus pulp bran was evaluated: 74.8 to 100% of 1 g of industrial orange pomace was converted into 2G ethanol in fermentations, while in monocultures the conversion was from 50 to 99.0%. The production of 2G ethanol corresponded to 51.1% of fermentable sugars. Based on the data, the study developed by Cypriano *et al.* (2017) stated that 6.69 to 130.7 thousand tons of 2G ethanol could be obtained annually. In addition to the production of 2G ethanol, the author was successful in the extraction of nanocellulose—managing the combination of chemical, enzymatic, defibrillation or partial hydrolysis processes, from the orange biomass. Nanocellulose has advantages over synthetic nanofibers such as improved thermal, biodegradability and mechanical properties in addition to its renewable character (Machado *et al.*, 2014).

Another fact that has caused concern is the inadequate disposal of green coconut husk. This situation presents a set of problems to the environment, such as the difficult degradation of coconut husk, which can take from 8 to 12 years and the production of methane gas, when it is inadequately disposed of in sanitary landfills. Taking this into account, Cabral *et al.* (2016) investigated the use of green coconut husk fiber to produce reducing sugars and conversion into ethanol. Despite a significant 17.9% loss in cellulose content, enzymatic hydrolysis was successful in converting about 87% of the sugars and the fermentation consumed 81% of the hydrolyzed matter, resulting in 22.34 g TRS per 100 g of coconut fiber.

Kowalski *et al.* (2017) studied enzymatic hydrolysis processes in avocado seeds to produce second generation ethanol. The ethanol obtained was characterized and the results showed that the raw material used is excellent for biofuel production, with 1.0579 g/L of starch producing 44 L of ethanol per ton of seed, 33.8% of yield ethanol.

The constant investigation of agricultural residues has proved to be extremely advantageous and ecological, due to the global concerns as a result of population growth, global warming and the rising of oil prices (Rosa and Garcia, 2009). The number of supplies to satisfy the energy needs of the society considering the constant exponential population growth will require an increase outside the natural capacity of cropland used as sources of raw material for consumable goods, increases that compromise food production, as they require significant growth in arable land.

4. Final considerations

Evidencing the potential of different sources of biomass to produce 2G ethanol, the exploitation of these resources showed multiple possibilities of use such as obtaining biogas and the value of cellulosic nanofibers for being biodegradable, expressing properties of higher performance and superiors to synthetic nanofibers. In addition, the exploitation of 2G ethanol not only

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combines with the current appeal of green chemistry, but also brings low-cost materials available in abundance in the nature of photosynthesis (Cypriano *et al.*, 2017).

Even in some cases of biomass, pretreatments require more expensive methodologies, the use of biomass becomes not only an excellent alternative for a cleaner future, but also a set of affordable solutions. Although the Brazilian Company Raízen is one of the few that includes 2G ethanol in its production sphere, the 50% increase in production after the implementation of the use of biomass may encourage others to join the method. These reservations and initiatives show the positive impact that can be caused by the global implementation of 2G ethanol.

The 2G ethanol production methodologies are diverse and have difficulties in implementing projects that are sufficiently attractive for large corporations, however, there are expansion projects for Asia, India and Thailand as they are sugarcane producers. Even with the possibility of expanding to new 2G ethanol producing countries, Brazil will still be the largest producer of this alternative energy due to its enormous agricultural potential and available biomass.

Authors' contribution

Conceptualization: Medeiros, P. V. C.; Theophilo, P. H. M.; Ribeiro, L. P. D.; Lopes, G. S.
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Data availability statement

All data sets were generated or analyzed in the current study.

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