Comprehensive Review on Potential Contamination in Fuel Ethanol Production with Proposed Specific Guideline Criteria

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Abstract: Ethanol is a promising biofuel that can replace fossil fuel, mitigate greenhouse gas (GHG) emissions, and represent a renewable building block for biochemical production. Ethanol can be produced from various feedstocks. First-generation ethanol is mainly produced from sugar- and starch-containing feedstocks. For second-generation ethanol, lignocellulosic biomass is used as a feedstock. Typically, ethanol production contains four major steps, including the conversion of feedstock, fermentation, ethanol recovery, and ethanol storage. Each feedstock requires different procedures for its conversion to fermentable sugar. Lignocellulosic biomass requires extra pretreatment compared to sugar and starch feedstocks to disrupt the structure and improve enzymatic hydrolysis efficiency. Many pretreatment methods are available such as physical, chemical, physicochemical, and biological methods. However, the greatest concern regarding the pretreatment process is inhibitor formation, which might retard enzymatic hydrolysis and fermentation. The main inhibitors are furan derivatives, aromatic compounds, and organic acids. Actions to minimize the effects of inhibitors, detoxification, changing fermentation strategies, and metabolic engineering can subsequently be conducted. In addition to the inhibitors from pretreatment, chemicals used during the pretreatment and fermentation of byproducts may remain in the final product if they are not removed by ethanol distillation and dehydration. Maintaining the quality of ethanol during storage is another concerning issue. Initial impurities of ethanol being stored and its nature, including hygroscopic, high oxygen and carbon dioxide solubility, influence chemical reactions during the storage period and change ethanol's characteristics (e.g., water content, ethanol content, acidity, pH, and electrical conductivity). During ethanol storage periods, nitrogen blanketing and corrosion inhibitors can be applied to reduce the quality degradation rate, the selection of which depends on several factors, such as cost and storage duration. This review article sheds light on the techniques of control used in ethanol fuel production, and also includes specific guidelines to control ethanol quality during production and the storage period in order to preserve ethanol production from first-generation to second-generation feedstock. Finally, the understanding of impurity/inhibitor formation and controlled strategies is crucial. These need to be considered when driving higher ethanol blending mandates in the short term, utilizing ethanol as a renewable building block for chemicals, or adopting ethanol as a hydrogen carrier for the long-term future, as has been recommended.

Contents

1. Introduction

Industrial ethanol is mostly produced for use as fuel. Ethanol is also used in many applications such as solvents, alcoholic beverages, and feedstocks for synthesizing various organic substances in the chemical industry, such as ethylene, polyethylene, 1,3-butadien, and ethyl acetate [1]. The trend of renewable energy and alleviating greenhouse gas emissions from fossil fuels has promoted greater ethanol fuel demand.

The contamination of ethanol can increase fuel corrosivity, which causes the swelling of some elastomer engine parts [2]. Thus, ethanol for gasoline blending must meet the anhydrous ethanol specification to ensure sufficient quality when it is used in vehicles, to ensure it is environmentally friendly and not harmful [3–5]. Certain impurities influence ethanol characteristics, such as acidity, pHe, water content, and electrical conductivity. Table 1 compares the anhydrous and hydrated ethanol specifications of some countries, including the United States, Brazil, Thailand, and those in the European Union. It can be noticed that fuel ethanol specifications used to control ethanol quality are different due to different markets, climatic conditions, and raw materials used in ethanol production [5]. The differences in water content specification between different countries rely on ethanol–gasoline blending ratios and the methods of gasoline transportation. Only the EU has a phosphorus specification based on ethanol producers. Brazilian and Thai ethanol standards provide criteria for electrical conductivity, since conductivity can simply and quickly detect impurities in ethanol [6]. In Thailand, anhydrous ethanol specification can be categorized into three major applications: denatured ethanol for gasohol production (TIS 2324), ethanol for pharmaceutical use (TIS 640-1), and ethanol for industrial use (TIS 640-2). When compared to the EU, USA, and Brazil, Thailand does not include sulfate limitation in anhydrous ethanol for blending with gasoline. The maximum quantities of permitted sulfate in the USA, Brazil, and EU specifications are 4, 4, and 3 ppm, respectively. For the USA, 4 ppm is the sulfate limitation for E10 fuel, which is agreement with the refining, automotive, and ethanol industries. Thus, this limitation may be updated in the future due to the increasing ethanol concentration in ethanol-blended gasoline [7,8]. Hence, Thailand should include sulfate specification in the future when ethanol demand increases.

Recently, there has been more attention given to second-generation ethanol, owing to the conflict between food and fuel. However, it contains higher amounts of impurities than first-generation ethanol. Some scientific confirmation is needed to prove which impurities in lignocellulosic ethanol can cause an adverse effect on vehicle engine performance. This finding could lead to the adoption of new specifications or the revision of existing ones to make them more compatible with second-generation ethanol. According to the literature review, phosphorus should be limited in fuel ethanol to protect automotive catalyst systems from deactivation if ethanol is produced from non-traditional feedstocks. The phosphorus content in ethanol is affected by feedstock composition, the fertilizers used in the cultivation stage, and nutrients used in the fermentation process [6,9]. Acetic acid in ethanol has the greatest impact on ethanol acidity, causing corrosion to automobile engines. Since the acetic content of lignocellulosic ethanol is more than that of first-generation ethanol [10], it is challenging for ethanol producers to meet the required standards. Furthermore, lignocellulosic ethanol contains a significant amount of furanic substances. The remaining furanic compounds in ethanol–gasoline blended fuel can lead to lower oxidative stability and the possibility of the formation of dangerous organic peroxides [11].

For anhydrous ethanol for pharmaceutical purposes, the limitations of non-volatile materials, benzene, acetaldehyde, acetal, and any other volatile impurities are included in the specification. If lignocellulosic ethanol is going to be used for pharmaceutical purposes, the separation technique should be improved to remove these impurities, especially acetaldehyde and acetal [12].

Habe et al. [10] reported impurities in 17 different types of bioethanol samples. They concluded that lignocellulosic-derived ethanol contains more impurities than sugar- and starch-derived ethanol because lignocellulosic feedstock requires a pretreatment to modify the lignocellulose structure and improve the accessibility of enzymes and chemicals. Lig-

nocellulosic ethanol has high concentrations of acetic acid, acetaldehyde, methanol, and furan. On the other hand, these contaminants are lower in sugar- or starch-derived ethanol. Considering sulfur-containing compounds, dimethyl disulfide and thiazole are only found in lignocellulosic-derived ethanol. In contrast, dimethyl sulfide and dimethyl sulfoxide are sulfur-containing compounds in sugar- and starch-derived ethanol.

In addition to the type of feedstock and production process, storage procedure also has an influence on ethanol quality. Naegeli et al. [13] concluded that decreasing fuel ethanol pH over storage periods correlates with ethyl sulfate formation, which also increases ethanol conductivity. During ethanol distillation, sulfite, a fermentation byproduct, is carried over with the ethanol vapor. Then, sulfite is oxidized to sulfate during the storage period. Recently, this sulfate contamination issue has gained interest due to its effect on vehicle engines. Many studies have reported that the contamination of sulfate causes deposit formation on inlet valves in combustion chambers and on injector tips [7,8,13,14].

Although the investigation of the impurities in final fuel products has received much attention [15–17], there are a few studies focusing on impurities occurring throughout the production process, and only some previously published works attempting to set guidelines to control blended gasoline quality during storage periods [18,19]. The lack of collective information regarding the quality control of anhydrous ethanol from the up-stream to downstream process is a current knowledge gap, which brings about the first aim of this review—to create an understanding of the causes of impurity formation throughout the whole production process (starting from feedstock acquisition), and identify the effects on the subsequent processes (fermentation, ethanol recovery, and storage) and on the final ethanol properties. Finally, specific guidelines to control ethanol quality, from anhydrous ethanol production until the storage period, can be proposed. The strategies and methods for reducing contamination are integrated from current knowledge. Additionally, recommendations and future perspectives also provided in the last part of the review.

Table 1. Comparison of anhydrous and hydrated ethanol specification [20–23].

Specification	Unit		European Union	USA	Brazil	Thailand		
			prEN 15376	ASTM D-4806-16a	ANP Resolution n° 19	TIS 2324	TIS 640-1	TIS 640-2
Ethanol type	-	-	Anhydrous	Denatured anhydrous	Anhydrous	Denatured anhydrous	Anhydrous	Anhydrous
Ethanol	% by volume	Min.	-	-	98	-	-	-
Ethanol and higher saturated alcohols	% by volume, (% by mass)	Min.	(98.7)	92.1	(99.3)	99	99.5	99.5
Higher saturated mono-alcohols-C3-C5	% by volume, (% by mass)	Max.	(2)	-	3	2	-	-
Methanol	% by volume, (% by mass)	Max.	(1)	0.5	0.5	0.5	0.02	0.05
Water content	% by volume, (% by mass)	Max.	(0.3)	1	(0.7)	0.3	-	-
Density at 20 °C	kg/m^3	Max.	-	-	791.5	-	790–793	-
Total acidity (as acetic acid)	mg/L, (% by mass)	Max.	(0.007)	56 (0.007)	30	30	30	(0.005)
Electrical conductivity	μS/m	Max.	-	-	300	500	-	-
pHe	-		-	6.5~9.0	-	6.5~9.0	-	-
Copper	mg/kg, (mg/L)	Max.	0.1	0.1	0.07	0.07	-	-
Inorganic chloride	mg/kg, (mg/L)	Max.	1.5	6.7 (5)	1	(20)	-	-
Solvent-washed gum	mg/100 mL	Max.	-	5	-	5	-	-
Sulfur	mg/kg, (ppm)	Max.	10	(30)	Report	-	-	-
Total sulfate	mg/kg	Max.	3	4	4	-	-	-
Phosphorus content	mg/L	Max.	0.15	-	-	-	-	-
Non-volatile material	mg/100 mL, (% by mass)	Max.	10	-	5	-	2.5	(0.005)

Table 1. *Cont.*

Specification	Unit		European Union	USA	Brazil	Thailand		
			prEN 15376	ASTM D-4806-16a	ANP Resolution n° 19	TIS 2324	TIS 640-1	TIS 640-2
Denaturant content	vol. %	Max.	-	1.96~2.5	-	-	-	-
Iron	mg/kg	Max.	-	-	5	-	-	-
Benzene	mL/kL	Max.	-	-	-	-	2	-
Acetaldehyde and acetal (as acetaldehyde)	% by volume, (% by mass)	Max.	-	-	-	-	0.001	(0.10)
Any other volatile impurity (as 4-methylpentan-2-ol)	mL/kL	Max.	-	-	-	-	300	-
Absorbance - Lower than 240 nm - 250 to 260 nm - 270 to 340 nm		Max.	-	-	-	-	0.4 0.3 0.1	-
Sodium	% by mass	Max.	-	-	0.0002	-	-	-
Permanganate time	Minute	Min.	-	-	-	-	-	15
Aspect	-		Clear and colorless	Clear and colorless	Clear and no impurities	Clear, colorless and no visible suspended solids	Clear and colorless	Corresponding to ISO 2211

2. Ethanol Production from Different Types of Feedstock

Ethanol can be produced from different feedstocks. There are two main types of ethanol production feedstock in first-generation technology: sugar-containing feedstock and starch-containing feedstock. An increase in fuel demand and concern regarding the potential negative risks of using food feedstock led to the utilization of lignocellulosic feedstock for fuel ethanol production in second-generation technology. Ethanol production processes from any feedstocks can be divided into three main steps: (1) converting feedstock into fermentable sugar; (2) the fermentation process to convert fermentable sugar to ethanol; and (3) the ethanol recovery and storage process. Although the production feedstocks are different, the fermentation and downstream processes are significantly similar. Hence, when considering different feedstocks, the difference in contamination is mainly affected by the feedstock stage involving the conversion to fermentable sugar [24].

3. Impact of Different Feedstocks on Impurities in Fuel Ethanol

As mentioned previously, the ethanol production process from each type of feedstock includes three major steps: conversion of feedstock, fermentation, and ethanol recovery. This section describes the conversion of each separate feedstock. The key to this process is to release sugar molecules from the feedstock structure. The difficulties in releasing sugar molecules depend on feedstock type, which involve different required steps to convert feedstock, and consequently result in various contamination profiles in the ethanol product.

3.1. Conversion of Sugar-Containing Feedstock

In many countries, such as Thailand, Brazil, India, and Colombia, sugarcane is cultivated for sugar production [25,26]. The valuable byproduct from sugar production is molasses, which is used in ethanol production. Besides, sugarcane juice is also utilized to produce ethanol in some countries such as Thailand [25,27,28]. Therefore, the sugar production process needs to be considered, as it determines the quality and impurities of the feedstock during ethanol production.

Attached and autonomous distilleries are two types of sugarcane-derived ethanol production plants, classified by ethanol feedstocks. The overall production process and chemical additions in each step for these two categorized sugarcane-derived ethanol production plants are shown in Figure 1. In the case of autonomous distilleries, the process section in the dashed–blue box can be excluded.
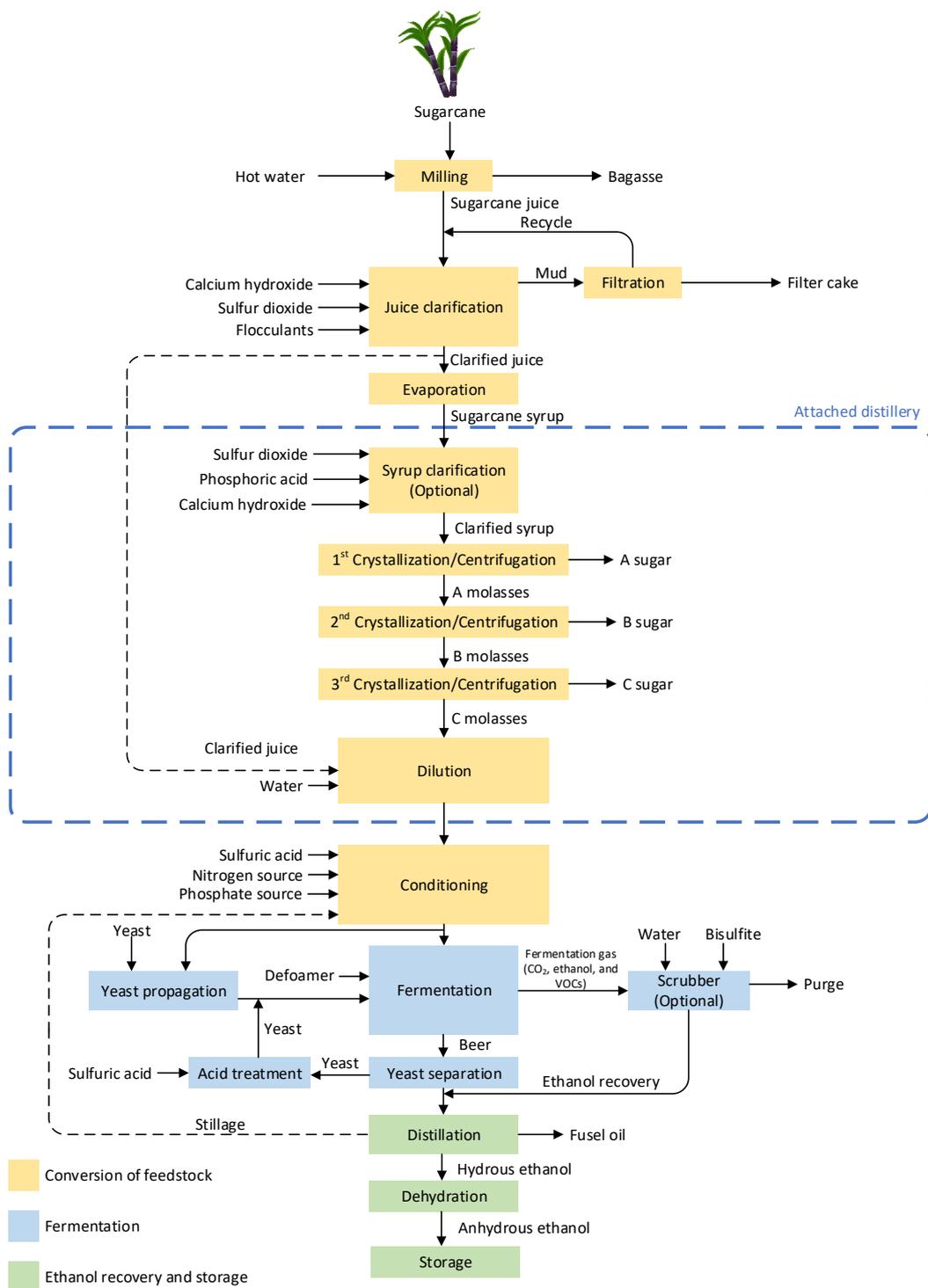
Figure 1. Type of sugarcane-derived ethanol production plant.

3.1.1. Attached Distillery

The attached distillery mainly produces sugar from sugarcane juice, while molasses appears as a byproduct. In the case of attached distilleries, molasses can be considered as the primary feedstock for ethanol production. However, sugarcane juice can be allocated between sugar and ethanol production, depending on the product demand [25,29,30]. The production process of the attached distillery is illustrated in a schematic diagram shown in Figure 1.

1.	Sugarcane plantation and harvesting

Sugar production from sugarcane begins with plantation. Gilbert et al. [31] have reported that the main climatic factors that have influence on cane crops are rainfall, temperature, and sunlight. Moreover, Cardona, Sanchez and Gutierrez [26] also described that the composition of sugarcane depends on the cultivated condition. Most variations in sugarcane composition are based on the difference in moisture content, sugar, and ash.

The harvesting of sugarcane can be performed by two methods, including manual harvesting and mechanical harvesting. Thai and Doherty [32] found that the sugarcane harvesting method has an influence on the chemical composition of the cane juice. Almost all manually cultivated sugarcane fields are burnt before harvesting. The composition of the burnt cane differs significantly from the non-burnt cane. Non-burnt cane juice contains a higher proportion of soluble inorganic ions and ionizable organic acids than burnt cane juice. In addition to the harvesting method, harvesting age is another factor affecting the juice extraction method, which will be discussed in the further sections.

After harvesting, sugarcane must be processed into ethanol production quickly because sucrose loss has been reported relating to invertase activity and the proliferation of microbial which produce acid, ethanol, or dextran. Besides, biodeterioration can occur due to delays between harvesting and milling. Biodeterioration also relates to other factors such as ambient temperature, humidity, cane variety, storage period, invertases activities, and maturity status [33].

As shown in Figure 2, the average composition of sugarcane can be simply classified into 86.7% broth and 13.3% fiber. Generally, most fibers are separated prior to the juice extraction process for electricity generation. The broth consists of 69.7% water and 17% soluble solids. Mostly, soluble solid contains 15.35% sugar and some non-sugar, which is removed in the juice clarification step. The sugar content comprises both non-fermentable sugar and the fermentable sugars necessary for fermentation, such as sucrose, glucose, and fructose [26,34].
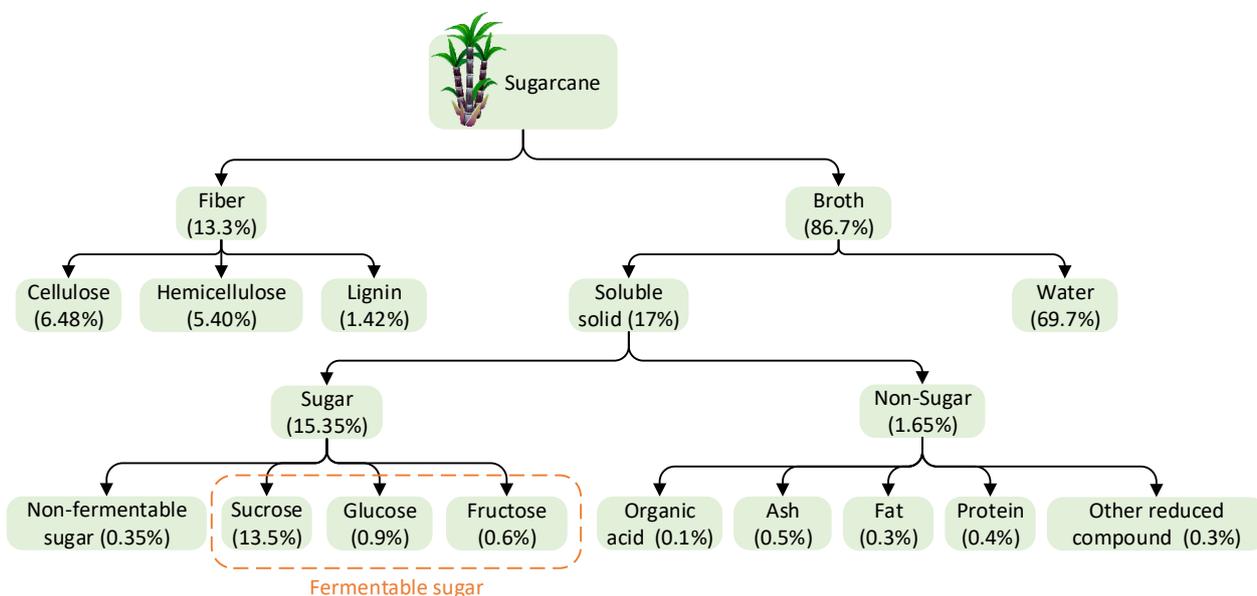
Figure 2. Average sugarcane composition, modified from: [26,34].

2. Juice clarification

Raw juice is obtained from the extraction. It contains various impurities such as minerals, salts, organic acids, dirt, and fiber particles [35]. In this step, raw juice is fed through the clarification process with the addition of sulfur dioxide to eliminate bacteria, consequently inhibiting reactions which enhance color appearance and coagulation of the suspended colloids. The clarification process includes three steps: coagulation, flocculation, and precipitation [36]. In the first step, coagulation, lime (calcium hydroxide) is added to

neutralize and alleviate the loss of sucrose content due to sucrose inversion. Then, lime juice is heated to coagulate the colloid particles, and proteins and polysaccharides are adsorbed into the colloidal particles. In the flocculation step, calcium from the lime reacts with the phosphate in the sugarcane and the sulfur dioxide in the calcium phosphate [37] and calcium sulfite [26], respectively. Calcium phosphate and calcium sulfite particles are involved in the formation of flocs which are responsible for the removal of impurities. In the precipitation step, flocs are precipitated in the clarifier tank as mud [38]. Mud is separated from clarified juice as a filter cake by vacuum rotary filters. The sucrose concentration in clarified juice is approximately 10–15% [39].

3. Evaporation

The primary purpose of evaporation is to remove water from clarified juice. However, there are some differences between the case of autonomous distillery and attached distillery. In the autonomous distillery, the evaporation step is carried out before the fermentation process to adjust juice concentration to achieve 60–70 °Bx, approximately [40,41].

In the attached distillery, evaporation is performed before the crystallization and centrifugation steps. The achieved steam or condensate from this step can be reapplied in other process steps. After the water has been removed, around 60 °Bx of sugarcane syrup is obtained.

4. Crystallization and centrifugation (For attached distillery only)

During the crystallization step, excess water in the sugarcane syrup is removed by the vacuum pan. Seeding with sucrose crystal is necessary to form sugar crystals in the mother liquor. The mixture of sugar crystals and mother liquor is called Massecuite [26,42]. Then, sugar crystals are separated from the mother liquor by centrifugation. After crystallization and centrifugation, the raw sugar and C-molasses (final molasses) are yielded as feedstock for ethanol production.

5. Dilution (for attached distilleries only)

In attached distilleries, molasses needs to be diluted before fermentation. It is not appropriate for direct use as the fermentation medium because of the high osmotic pressure on the yeast cells. In attached distilleries, molasses should be diluted by clarified juice or water below 25 °Bx because high osmotic pressure can affect yeast metabolism, or decrease yeast viability [26,43,44]. The adjustment of pH and the elimination of bacteria by sulfuric acid are also needed [26,42,45]. The obtained molasses from sugar production is a dark-brown viscous liquid. Considering the composition of molasses, it is composed of up to 50% soluble carbohydrates, such as sucrose, D-glucose, and D-fructose. Its major components, excluding carbohydrates, are calcium, potassium, and magnesium salts, such as magnesium chloride and magnesium sulfate. Its minor constituents include cuticle wax, sugarcane fats and sterols, plant phenolics, polysaccharides, aconitic, plant pigments, amino acids and proteins, inorganic ions (such as sodium-ion, iron, aluminum), silicon compounds, and trace metals [42].

- Water used in the dilution step

For ethanol production, water quality is a crucial factor in the production process since water is the main component of fermentation media for yeast [46]. So, the dissolved constituents in added water can significantly affect the ethanol production process and ethanol quality.

Dissolved constituents, usually found in surface water and groundwater, can be divided into major, minor and trace constituents. The major constituents with concentrations higher than 1.0–1000 mg/L are Ca, Mg, Na, Cl, Si, SO_4^{2-}, H_2CO_3, and HCO_3^-, while other minor constituents with a concentration between 0.01 and 10 mg/L are B, K, F, Sr, Fe, CO_3^{2-}, and NO_3^-. Al, As, Ba, Br, Cd, Co, Cu, Pb, Mn, Ni, Se, Ag, Zn, and others are dissolved with a trace amount lower than 0.1 mg/L [47,48].

Iowa State is the highest ethanol production state in the United States. Research has found that ethanol production relies on the water quality in the municipal wells

pumped from Cambrian–Ordovician groundwater sources. Water samples from this area contain high amounts of chloride and sulfate: at concentrations of 160–230 mg/L and 560–720 mg/L, respectively. Besides chloride and sulfate, the water also contains other dissolved constituents (such as Ca, Na, K, HCO_3^-, CO_3^{2-}, Cl, SO_4^{2-}, F, SiO_2, and Fe) [49].

A high concentration of dissolved constituents can cause osmotic stress, which negatively affects the function of yeast cells in the production process. Variation in water quality can have a significant impact on yeast's growth rate, and consequently on conversion efficiency. To avoid this problem, the water quality utilized in the fermentation process must be carefully monitored. The common parameters for testing of water are pH, nitrate, nitrite, and trace elements. The indication of polluted water by sewage or animal waste can be determined from the concentration of nitrate and nitrite salts. When these are higher than 50 ppm, it would be advisable to avoid using this water in the fermentation process.

The properties of the fermentation medium after the dilution with water are important for yeast growth. For example, many types of yeast can grow in a pH range of 4 to 6.5. The minimum and marginal concentrations dissolved in fermentation medium will be summarized and discussed in Section 4.1.

6. Conditioning

Sucrose-containing feedstocks, such as sugarcane juice and molasses, can contain substances which can inhibit microorganisms for converting sugar to alcohol. However, there can be difficulty in predicting the composition of sucrose-containing feedstocks because of several related factors, including cultivation techniques, sunlight, weather conditions, fertilizers, water availability, and harvesting methods [26]. The concentration of inhibitors in feedstock is difficult to control. To improve the fermentability of feedstocks, inhibitors in feedstock should be removed or diluted before fermentation.

- Synthetic zeolites

Synthetic zeolites are conventionally applied for eliminating inhibitory substances [50] by their ionic exchange and adsorption properties. When zeolites are added to the fermentation system, Na^+ is mostly found in the fermentation medium as an inhibitor that can be removed through ion exchange resin by replacing K^+-containing zeolite [50,51]. Potassium salt was found to be less inhibitory than sodium salt [52]. Moreover, zeolites also serve as pH regulators during fermentation, and maintain cellular viability and metabolic activities [53].

- Antiscalant

Sucrose-containing feedstocks can contain ash. In particular, sugarcane molasses feedstock consists of 10–16% ash [54]. Cardona, Sanchez and Gutierrez [26] claim that more than 10% ash content can cause scale problems in pipelines and distillation towers. Antiscalant, or scale inhibitor, is a chelating compound. It can be applied to water or molasses beer to reduce scale formation in heat exchangers or distillation columns by preventing calcium sulfate formation [26,43].

- Nitrogen source

Nitrogen source plays a vital role in fermentation; inadequate nitrogen can slow down sugar utilization because nitrogen functions in protein synthesis and sugar transport [55]. Thus, starting feedstocks for ethanol production should contain not only sufficient carbon sources but also other nutrients, such as free amino nitrogen (FAN), mineral, vitamin, and other growth factors [56], which are essential components for yeast health and efficiency.

High nitrogenous materials may be present in the fermentation medium, but they occur in a complex form that yeast cannot consume unless being hydrolyzed into amino acids, dipeptides, or tripeptides. Nitrogen that can be used as a nutrient source for yeast during the fermentation process is called free amino nitrogen (FAN) [57–60]. Depending on the feedstock, fermentation media sometimes contains a small amount of FAN, although this is insufficient and additional amino nitrogen sources need to be provided. An insufficiency

of FAN decreases yeast growth and reduces fermentation efficiency, leading to prolonged fermentation time [58,61] and the generation of hydrogen sulfide [62]. To release more FAN from soluble protein, protease is also added into the fermentation medium [26].

In addition to FAN, ammonium sulfate can be a nitrogen source for yeast [63]. Ammonium sulfate addition can also control the formation of fusel alcohols such as 1-propanol, 2-methyl-1-propanol, and 3-methyl-1-butanol [64,65]. However, the addition of ammonium sulfate may lead to sulfate salt precipitation in automotive fuel injectors.

Urea is a more preferable nitrogen source for ethanol fuel fermentation [7,14,26]. In terms of economics and yield, urea is the best option. Urea not only improves the ethanol yield and decreases the formation of byproducts, but it also increases the specific growth rate and capacity to tolerate ethanol [66]. In contrast, urea is unsuitable for alcohol fermentation in beverage production because of carcinogenic ethyl carbamate formation [56].

- Phosphate source

In the fermentation process, phosphate insufficiency leads to decreased cell growth rate. Typically, phosphate is necessary for nucleotide, phospholipid, and metabolite biosynthesis. The addition of di-ammonium phosphate as a phosphorous source could reduce the requirement of urea [26,67].

3.1.2. Autonomous Distillery

Autonomous distilleries usually use sugarcane to produce ethanol [25], which is different from attached distilleries, in that this plant is not used to produce sugar. Therefore, this type of distillery employs sugarcane juice as the primary feedstock. The feedstock conversion process for autonomous distilleries can be seen in Figure 1, with the exception of the dashed–blue box step.

3.1.3. Comparison of Contamination between an Attached Distillery and an Autonomous Distillery for Sugarcane-Based Feedstock

Brazil is the world's largest ethanol producer. Most of the ethanol production plants there are attached distilleries. This type of distillery allows the producer to take advantage of the synergy between sugar and ethanol [68]. In terms of production feedstocks in each type of ethanol distillery, autonomous distilleries use only sugarcane juice as the feedstock for ethanol production. In attached distilleries, molasses is used as the primary feedstock. Sugarcane juice is sometimes used in parallel with molasses.

Considering the impurities of these feedstocks, molasses has higher impurity levels than sugarcane juice (for example, inorganic salts, unfermentable sugars, sulfated ash, and pigment), as it is contaminated during the sugar production process [26,69]. Molasses composition depends on the sugarcane juice extraction process. Sulfur dioxide is usually added as a preservative when extracting cane juice from young sugarcane, and remains as sulfite in the ethanol product because of the difficulty in removing it in the distillation stage [70,71]. Due to the high impurities in molasses, Khoja et al. [72] have studied the effect of impurities in sugarcane molasses on fermentation. They reported that impurities in molasses may influence enzymatic activity. Ethanol yield can be improved by using some enzyme stabilizers or some agents/additives which can alleviate the effects of these impurities.

3.2. Conversion of Starch-Containing Feedstocks

Ethanol production from starch-containing feedstocks, such as corn kernels and cassava, can be classified into two processes: (1) the wet milling process and (2) the dry milling process, as presented in Figure 3. The major difference between these two methods is that the wet milling process has been developed to separate high-value products from the starchy feedstock, while the latter has not.
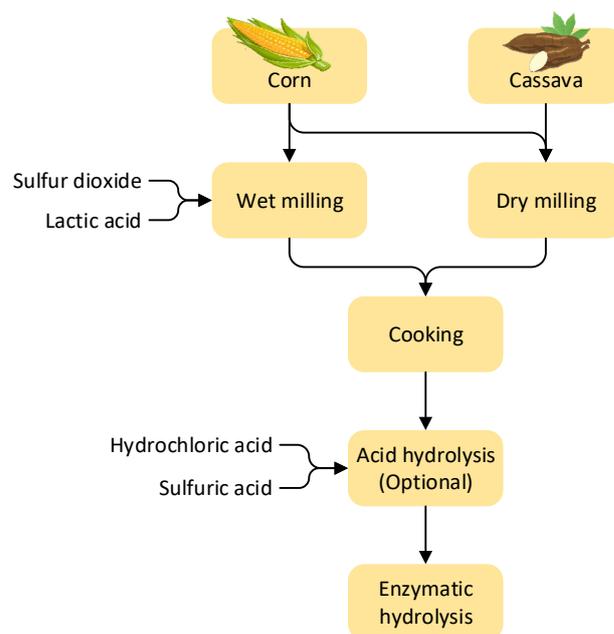
Figure 3. Conversion of starch-containing feedstock.

The wet milling process is applied for corn grain feedstock because it provides high-value products, such as corn gluten meal, corn gluten feed, and corn germ meal, which are usually applied as poultry feed. However, the drawbacks of the wet milling process include high capital cost, high energy consumption, and less ethanol yield. The dry milling process is often chosen as an alternative approach for corn grain feedstock.

The dry milling process is appropriate for cassava chip feedstock in ethanol production because cassava chips do not provide high-value components [3].

3.2.1. Wet Milling Distillery

In general, the wet milling process is applied for corn grain because it contains high-value components. Corn grain contains around 70–73% starch, 9–10% protein, 9–10% crude fiber, 4–5% fat, 1–2% ash, and 2% sugar [73].

As shown in Figure 4, the wet milling process begins with cleaning and soaking the corn kernels in a steeping solution consisting of sulfur dioxide and lactic acid [74]. The role of steeping is to soften the corn kernels, break down the protein coating the starch particles, and remove some soluble constituents. Then, soft corn kernels are milled with a corn degerminator, and corn germ is separated by the liquid cyclone. The degermed ground kernels are washed, ground, and screened to remove fiber. The centrifuge separates protein as a corn gluten meal (CGM) from the free fiber starch slurry. Steep liquor obtained from the evaporated steep water is mixed with corn fiber, or with condensed soluble, to achieve corn gluten feed (CGF) [75,76]. After completing the component fractionation, the starchy slurry is finally delivered to the cooking and enzyme hydrolysis processes [3].

3.2.2. Dry Milling Distillery

In Thailand and China, ethanol production from cassava usually operates through the dry milling process mainly carried out in the batch regime. This requires lower capital and energy costs because there is no need to fractionate the valuable products. The steps of this process are shown in Figure 5.
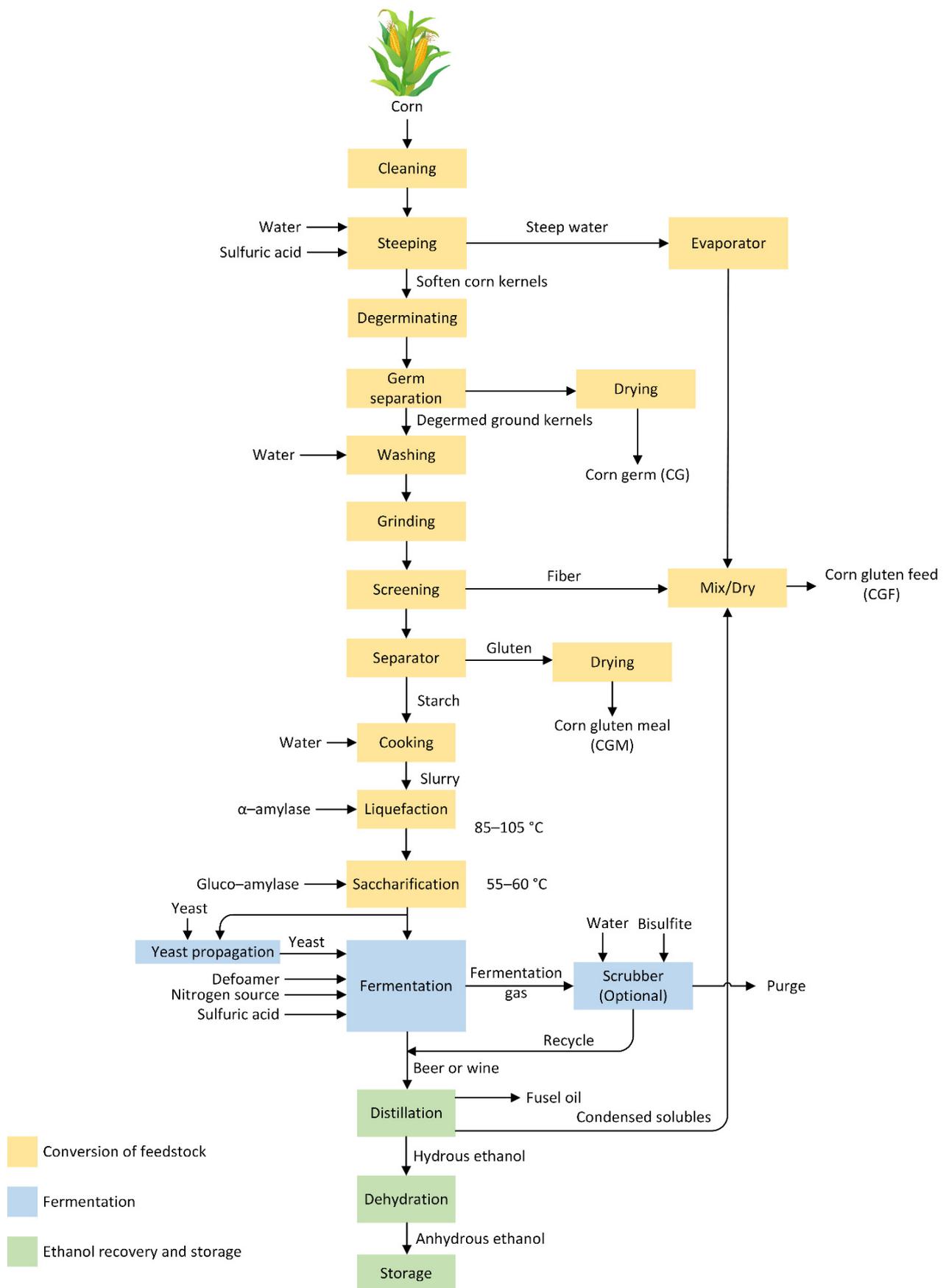
Figure 4. Schematic diagram for corn wet milling process.
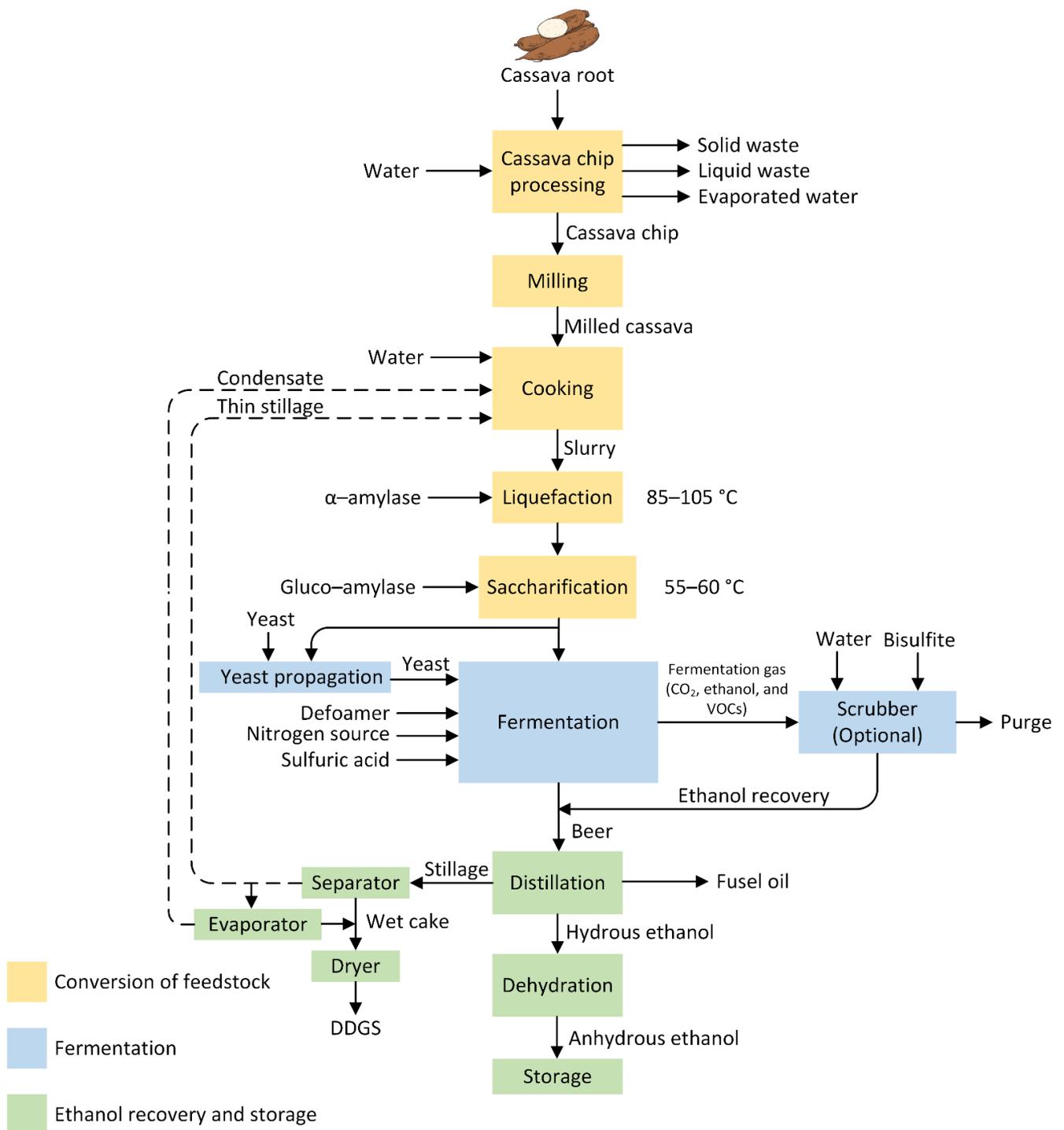
Figure 5. Conventional process for ethanol production from cassava.

1.   Cassava chip processing

       Various cassava forms, such as fresh root, cassava chip, and cassava starch, can be fed to ethanol production. The chemical composition of cassava contains sulfur compounds in amino acid forms such as cysteine and methionine. Then, this sulfur concentration increases by a factor of 2–3 times during the ethanol production process. However, nearly all of these sulfur-containing amino acids are removed from the ethanol product stream and remain in the distiller's dried grains with soluble (DDGS) fractions, as shown in Figure 5 [14,77].

Cassava fresh roots are used as raw materials after harvesting; they are cleaned, washed, peeled, and chopped into cassava chips [26,78]. Later, chips are distributed on a cement floor and exposed to sunlight for 2–3 days to reduce moisture content. For safe storage, moisture content should be less than ca. 14 wt.% [79].

2. Milling

In the dry milling step, cassava chips are sent to the hopper and metal detector, and then crushed and sieved to obtain a fine flour [3,26,80].

3. Cooking

Cassava starch is a polysaccharide that requires degradation to glucose. Initially, it is necessary to gelatinize starch via the cooking process in excess boiled water above the gelatinization temperature [3,81]. The α-amylase enzyme can be added for liquefaction at above 85 °C at the same time as gelatinization [81].

4. Starch hydrolysis process

During the hydrolysis process, water and enzymes breakdown the polymer chain into fermentable sugar. This can be carried out via two techniques: enzyme hydrolysis and acidic hydrolysis [82,83].

• Enzyme hydrolysis

Enzyme hydrolysis has two steps. It starts with liquefaction, followed by saccharification.

In the liquefaction step, an α-amylase enzyme is used for the hydrolysis of α-1,4 glycosidic linkage in the amylose and amylopectin of gelatinized starch into dextrin, maltose, and maltotriose [84]. After the liquefaction step, the temperature of the liquefied slurry is decreased before entering the saccharification process, ca. 60 °C in the case of cassava feedstock [85].

In the saccharification process, the glucoamylase enzyme is used for the hydrolysis α-1, 4 and α-1, 6 glycosidic linkages of dextrin into glucose [86,87].

• Acidic hydrolysis

Though the enzyme hydrolysis is typically employed for starch-containing feedstock, acidic hydrolysis can be performed to breakdown starch molecules into fermentable sugar [88]. One study, carried out by Candra et al. [89], conducted the hydrolysis of grated cassava by employing 0–1.0 M sulfuric acid and hydrochloric acid at 100 °C, at 1 bar for 30 min. The results showed that sulfuric acid offers higher hydrolysis efficiency than hydrochloric acid. The optimum concentration of sulfuric acid and hydrochloric acid resulted in a reducing sugar yield of 28.20 and 25.60%, respectively. However, the addition of hydrochloric acid during pretreatment could lead to high chloride levels remaining in fuel ethanol [90].

3.2.3. Comparison between Dry Milling and Wet Milling for Ethanol Production

The wet milling process is designed to fractionate starch and other high-value products from corn grain feedstock. After the grain is processed through cleaning, steeping, grinding, germ separation, fiber separation, and starch–protein separation, the starch slurry is further processed with cooking and enzyme hydrolysis for ethanol production.

In the dry milling process, cassava chips are fed into the hopper, and then metal and stone detectors. The chips are subsequently milled and sieved to obtain a fine powder which is slurried with water and later subjected to cooking and enzyme hydrolysis.

Even though ethanol has been produced by wet and dry milling processes for a long time, studies comparing the impurities in ethanol obtained from the different techniques are scarce. Sulfur dioxide addition in wet milling can cause sulfite and bisulfite. Their hydrogen cations are then transported into the ethanol fermentation with the starch, and can end up in the final beer product, eventually carrying over with the ethanol during distillation [7]. On the other hand, in dry milling, starch and sugars are not clearly removed from the other corn components, resulting in unconverted fractions remaining in the starch

slurry. Cyclic and heterocyclic compounds are generated from lignin in the corn hull. Some of these volatile byproducts remain in the distillate, causing unpleasant flavors and harmful ethanol [91]. Owing to the fractionation of starch and other high-value products from the feedstock, wet milling is generally suitable for food-grade ethanol production due to having lower impurities.

3.3. Conversion of Lignocellulosic Feedstock

First-generation ethanol production uses sugar and starch as feedstocks because they are easily converted into ethanol. However, the second generation allows the production of ethanol from lignocellulosic biomass. Its abundance and ability to grow in several areas makes lignocellulose a promising feedstock for ethanol production [92].

3.3.1. Lignocellulose Composition

Lignocellulosic biomass can be divided into many categories: agricultural residues, agro-industrial residues, hardwood, softwood, herbaceous biomass, cellulosic wastes, and municipal solid waste [26]. Lignocellulosic biomass comprises cellulose (40–60% of total dry weight), hemicellulose (20–40%), and lignin (10–25%) [24,93] with some acids, various minerals, and extractives [26,94]. Different types of lignocellulosic biomass have different chemical compositions affecting the yield and amount of substrate produced during the pretreatment stage, the size of the equipment, and the energy requirements [95].

1.　Cellulose

Cellulose has two regions based on different crystallinity orders: amorphous and crystalline. Amorphous cellulose nano-fibrils, arranging disorderly, comprise a linear polymer chain of beta glucose monomers connected by β(1,4) glycosidic linkage [26,93,94]. However, cellulose chains linked by hydrogen bonds between repeating chains or different chains lead to high crystallinity cellulose nano-fibrils regions, which are more difficult to hydrolyze. The hydrolysis of cellulose yields glucose sugar which can be further degraded into HMF [96].

2.　Hemicellulose

Hemicellulose is a branched-chain polymer that consists of 200 different types of sugar, mainly pentose and hexose. Pentose sugars include xylose and arabinose, whereas hexose sugars include galactose, glucose, and mannose. The rest are other carbohydrate-related compounds such as glucuronic, methyl glucuronic, and galacturonic acids [26,97]. Hemicellulose compositions are dependent upon the type of plant. In hardwood, hemicellulose compositions mainly contain xylans [98]. In softwood, hemicellulose compositions mainly consist of glucomannans, and their structures are mostly linear polymers, with a minor part being branched-chain [98,99]. Since hemicellulose is composed of various sugars, they can be degraded to yield several kinds of decomposition products. HMF is a degradation product of glucose, mannose, and galactose. In contrast, furfural is a degradation production of xylose and arabinose. Additionally, HMF can possibly degrade into levulinic and formic acid, while furfural can only degrade into formic acid [96]. Besides, acetic acid can be produced by the cleavage of acetyl groups [100].

3.　Lignin

Lignin is a complex phenolic polymer initiated by the polymerization reaction of monolignols, including coniferyl alcohol, sinapyl alcohol, and P-coumaryl alcohol [93]. These three acetyl alcohols are derived from units of guaicyl (G), syringyl (S), and p-hydroxyphenyl (H), respectively [26,101]. The differences in the proportions of guaicyl, syringyl, and p-hydroxyphenyl are based on plant types [93,102]. Since lignin is a phenolic polymer [103], it can further be degraded to phenolics and other aromatic compounds [96,104].

4.　Extractives

Extractives are natural compounds in biomass that can be extracted by polar or non-polar solvents (e.g., ethanol, water, acetone, benzene, toluene, dichloromethane, and

hexane). The major compositions of extractives are phenolics, fats, waxes, and terpenes. However, a minority comprise proteins, gums, resins, simple sugars, starches, essential oils, pectin, mucilage, glycosides and saponins, fatty acids, sterols, and flavonoids [104,105].

5.   Ash

Ash is usually considered as a residual after lignocellulosic biomass has been incinerated. Its content in biomass is dependent on the type of lignocellulosic biomass. Major elements with concentrations ranging between 1500 and 280,000 ppm are found in woody biomass ash and aligned in the following order: Ca > K > P > Mn > Fe > S. Minor elements with concentrations less than 400 ppm are aligned in the order: Zn > Cu > Ni > Cr > Pb > As [106].

3.3.2. Ethanol Production from Lignocellulosic Biomass

Figure 6 shows ethanol production from lignocellulosic biomass using steam explosion pretreatment. Steam explosion pretreatment comprises the majority of pretreatment used in commercial lignocellulosic ethanol production [107]. Sulfuric acid is widely used as a catalyst to improve the rate of hydrolysis and reduce sugar degradation [108]. Steam explosion solubilizes hemicellulose fractions into pentose sugar and inhibitors. The solid fraction contains mainly lignin and cellulose, and is called cellulignin. The separation of lignin can be performed in two different ways. First, lignin is removed after the fermentation process. Thus, the solid fraction is subjected to the enzymatic hydrolysis process containing cellulose and lignin, which can have a toxic effect on yeast [109]. In this case, enzymatic hydrolysis produces relatively low yields of sugar. For the second way to improve enzymatic hydrolysis efficiency, an alkaline delignification step is introduced to remove most of the lignin. It produces high purity cellulose hydrolysate that is more susceptible to enzymatic attack [110]. In some production processes, pentose liquor can be fermented to ethanol separately or simultaneously with hexose sugar.

3.3.3. Lignocellulosic Pretreatment

The cellulose part of lignocellulosic biomass is in the form of a microfibril structure surrounded by hemicellulose. In contrast, the lignin part is located in the void between the cell wall, cellulose, and hemicellulose [26,111,112]. Lignin in lignocellulosic biomass causes difficulties in bond-breaking and chemical/enzyme access. Therefore, pretreatment is essential to separate lignin and improve digestibility and suitability for dissolving cellulose and hemicellulose [112].

Although hemicellulose-encapsulating cellulose can be converted into sugar, sometimes approximately 50% of hemicellulose must be removed to increase cellulose digestibility [113]. However, hemicellulose can be degraded to undesired products, such as furfurals and hydroxymethyl furfurals [112].

Three purposes of the pretreatment stage are: (1) to break down cellulignin [112], (2) to increase amorphous regions of cellulose, making it to be easily hydrolyzed, and (3) to increase porosity which could enhance chemical and enzyme accessibility. Afterwards, cellulose is separated from hemicellulose and lignin [94,112]. Pretreatment can be classified as physical, chemical, physical–chemical, or biological in type [26]. The different pretreatment methods and concerning issues are shown in Table 2.
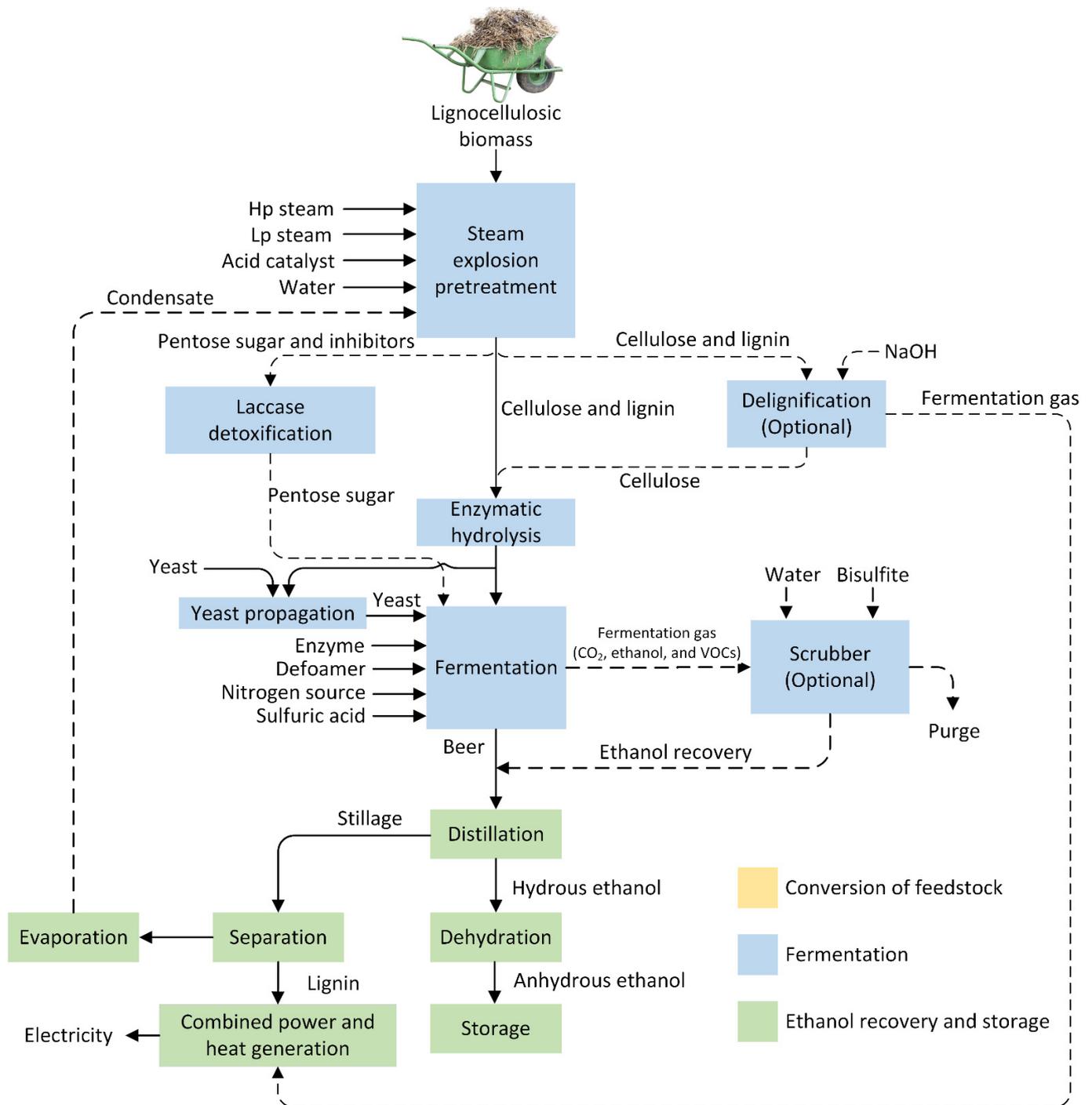
Figure 6. Ethanol production process from lignocellulosic biomass using steam explosion pretreatment.

Table 2. Concerning issues on different pretreatment methods.

Type of Pretreatments	Methods	Chemical/Enzyme Addition	Concerns
Physical	Mechanical	No additives	-
	Pyrolysis	No additives	• Possible to cause the formation of volatile products (aldehydes, phenol, benzene, furan, furfuryl derivatives, and other oxygenated compounds) and char residuals through mild dilute acidic hydrolysis [26,114–116].
Physical-chemical	Acid-catalyzed steam explosion	Sulfuric acid, sulfur dioxide, or carbon dioxide	• In severe conditions, cellulose can be depolymerized to form cello-oligomers or oligosaccharides [117,118]; • Possible to cause the formation of HMF from hexose dehydration (glucose) and furfural from pentose dehydration (xylose) [107,112,113,116,119]; • Incomplete destruction of lignin-carbohydrate complex [107]; • The use of H_2SO_4 increase sulfur components in ethanol [120].
	Uncatalyzed steam explosion	No additives	• Causes sugar decomposition [121]; • Inhibitor concentration depends on pretreatment condition severity [122]; • Hemicellulose degradation results in the generation of aliphatic acids (acetic acid and formic acid), as well as furans [122]; • Lignin is also partially degraded to phenolics [122].
	Liquid hot water (LHW)	Hot water	• Cellulose depolymerization can occur at a certain degree [26]; • In high temperatures, pentose can be degraded to form furfural. Acetyl groups in hemicellulosic polymers can be hydrolyzed to form acetic acid. Hexoses can be decomposed to form 5-hydroxymethyl furfural HMF [123]; • High energy and water consumption [107]; • Long residence times [107];
	Ammonium fiber explosion	Ammonia	• Low or no formation of inhibitors [124,125]; • Cellulose depolymerization can occur at a certain degree [26]; • Not suitable for high lignin content materials.
	Carbon dioxide explosion	Carbon dioxide	• Low or no formation of inhibitors [26,121].
Chemical	Ozonolysis	Ozone	• Low formation of inhibitors and xylitol, lactic, formic, and acetic acid were only found in hydrolysate [26,121,126]; • There is no formation of furan derivatives [126].
	Dilute acidic hydrolysis	Sulfuric acid, hydrochloric acid, nitric acid, phosphoric acid	• Generates inhibitors, such as furfural and phenolic components, and causes gypsum formation [26,123]; • Other inhibitors, such as chloric, phosphoric, or nitrous acids, are formed with the increasing temperature, depending on the hydrolyzing agent [123]; • It can increase material and equipment corrosion risk [107].
	Concentrated-acid hydrolysis	Sulfuric acid, peracetic acid	• Causes formation of inhibitors such as furfurals, 5-hydroxy methyl furfural, phenolic acids, and aldehydes [121,127].
	Alkaline hydrolysis	Sodium hydroxide, calcium hydroxide, hydrogen peroxide	• It results in low inhibitor formation [26,112]; • High cost of alkaline catalyst [107]; • Long residence times [107].
	Oxidative delignification	An oxidizing agent such as hydrogen peroxide, ozone, oxygen, or air	• Lignin polymer will be converted into carboxylic acids [128].
	Wet oxidation	Water, sodium carbonate, sulfuric acid	• Wet oxidation causes lignin degradation to CO_2, H_2O, and carboxylic acids [26,129]; • During the wet oxidation process phenolic compounds are degraded to carboxylic acids [125,130]; • Lower production of furfural and HMF compared to steam explosion or liquid hot water method [129,131].
	Organosolv process	Organic solvents (methanol, ethanol, acetone, ethylene glycol, triethylene glycol), sulfuric acid, hydrochloric acid, ethyl acetate	• Requires the removal of solvent [26,107,112,124,132]; • High inhibitor formation [121,132].
	Ionic liquid (ILs)	1-Ethyl-3-methylimidazolium acetate, 1-Butyl-3-methylimidazolium chloride	• The ionic liquid remaining in pretreated materials is toxic to the enzyme and fermentative microorganism [104]; • Ionic liquid may produce impurities, including water, halides, and other volatile substances [133,134]; • High solvent cost and requires solvent recovery [107].
Biological	Fungal	Cellulases, hemicellulase, ligninases, laccase, and quinone-reducing enzymes	• Low or no inhibitor formation [133,135,136]; • Long residence times [107].
	Bio-Organosolv	Ethanol	• Hemicellulose hydrolysis.

During pretreatment, inhibitors are generated depending on the pretreatment method and the fraction of lignocellulosic materials [137]. Acid pretreatment, commonly used at an industrial scale [138], breaks down complex structures such as hemicellulose, cellulose, and lignin into simple molecules such as pentoses and hexoses. However, this pretreatment method also brings about the formation of furanic compounds following lignin decomposition. Moreover, the decomposition of pentoses and hexoses yields furfural and HMF under acidic conditions. The possible inhibitors derived from the different fractions of lignocellulosic materials are visually summarized in Figure 7. Other details, including the reaction/pretreatment types that yield the inhibitors, and the effects of the inhibitors, are shown in Table 3. This table also presents detoxification methods for each type of inhibitor, which will be further discussed in Section 3.3.4.
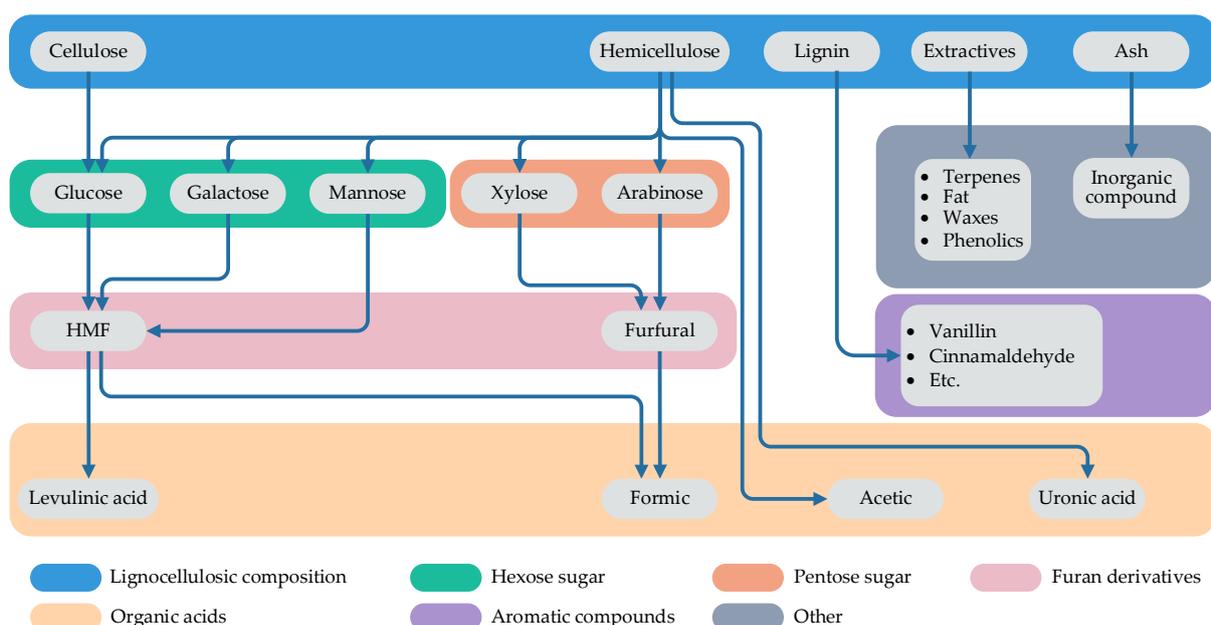
Figure 7. Inhibitors generated during lignocellulosic pretreatment.

In this review, the major inhibitors during lignocellulosic pretreatment are categorized into furan derivatives, organic acid, and aromatic compounds.

- Furan derivatives

The main furan derivatives in lignocellulosic hydrolysate are furfural and hydroxymethyl furfural (HMF). Part of hemicellulose can be hydrolyzed to pentose sugar. Furthermore, pentose can decompose to furfural. The hydrolysis of hemicellulose can be presented in Equation (1) [116]:

$$\text{Hemicellulose} \rightarrow \text{Xylan} \rightarrow \text{Xylose} \rightarrow \text{Furfural} \tag{1}$$
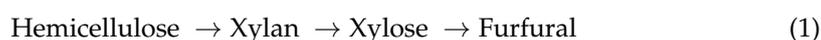
Table 3. Possible generated inhibitors during lignocellulosic pretreatment.

Compound Type	Compound	Reaction	Possible Methods Originated	Effects	Some Detoxification Methods
Furans derivatives	Hydroxymethyl furfural (HMF)	Degradation of hexose sugar [26,139]	Diluted acid [104,140,141], concentrated acid [104], steam explosion [142], liquid hot water [107], hydrothermal processing [104]	1. HMF reduces enzymatic and biological activities [143]; 2. HMF breaks down DNA and inhibits protein and RNA synthesis [143]; 3. Furfural and HMF synergistically suppress cell growth [143,144].	Adsorption with activated coal [145], pyrochar [146], PEI polymer [147], nanofiltration [148], anion exchange resin [149]
	Furfural	Degradation of pentose sugar [26,139]	Diluted acid [104,141], concentrated acid, steam explosion [142], liquid hot water [107], hydrothermal processing [104]	1. Furfural reduces enzymatic and biological activities [143]; 2. HMF breaks down DNA, inhibiting protein and RNA synthesis [143]; 3. Furfural and HMF synergistically suppress cell growth [143,144]; 4. At the same concentration of HMF and furfural, furfural causes higher inhibitory effects on cell growth than HMF [143].	Adsorption with activated coal [145], pyrochar [146], PEI polymer [147], nanofiltration [148], anion exchange resin [149], sodium borohydride [150]
Organic acids	Acetic	Hemicellulose hydrolysis [26,139]	Mild alkaline [104], diluted acid [141,151], concentrated acid, liquid hot water [107], hydrothermal processing [104], oxidative [104], steam explosion [104,142]	1. Acetic acid severely inhibits yeast cell growth [152]; 2. Acetic acid diffuses through yeast. Therefore, intracellular pH is reduced [96]; 3. It decreases ethanol yield [139].	Nanofiltration [148], adsorption with PEI polymer [147], anion exchange resin [149]
	Formic	Degradation of HMF and furfural [26,139]	Diluted acid [141], steam explosion [142], liquid hot water [107]	1. Formic acid diffuse through yeast cell leads to a decrease in the intracellular pH [96]; 2. It decreases ethanol yield [139].	Adsorption with PEI polymer [147], anion exchange resin [149]
	Levulinic acid	Degradation of HMF [26,139]	Acid, steam explosion [142], dilute acid [141]	1. The defusion of levulinic acid through yeast cells leads to a decrease in the intracellular pH [96]; 2. It decreases ethanol yield [139].	Adsorption with activated coal [145], anion exchange resin [149]
Aromatic compounds	Vanillin	Depolymerization of lignin [104,139]	Mild alkaline [104], steam explosion [127,142]	1. It may cause a negative impact on enzymatic saccharification [104,153]; 2. Phenolic compounds damage cell membrane and DNA repair mechanisms [139]; 3. Yeast growth rate and ethanol productivity are reduced [139].	Laccase enzyme [154], peroxidase enzyme [155], nanofiltration [148], anion exchange resin [149], adsorption with activated coal [156]
	Cinnamaldehyde	Depolymerization of lignin [104,139]	Mild alkaline [104], steam explosion [127]		Laccase enzyme [154], anion exchange resin [149]
	Benzoic acid	Degradation of lignin [157]	Acid [96,104], steam explosion [127]	Benzoic acid reduces growth rate and biomass yield [158].	Not available
	Cinnamic acid	Degradation of lignin [159]	Acid [96,104], steam explosion [127]	Cinnamic acid hindered yeast growth in ethanol fermentation [160].	Not available
	p-benzoquinone (BQ)	Oxidation of lignin and lignin-derived compounds [161]	Acid [96,104], steam explosion [127]	BQ at 20 to 200 ppm severely inhibited microorganism's cell growth and fermentability [162].	Sodium borohydride [150]
	2,6-Dimethoxy-1,4-benzoquinone (DMBQ)	Oxidation of lignin and syringyl-type compounds [161]	Acid [96,104], steam explosion [127]	DMBQ had a negative impact on balanced ethanol yield and productivity than on glucose consumption [161].	Sodium borohydride [150]

Hexose can be dehydrated into hydroxymethyl furfural (HMF) [163].

- Organic acids

Organic acids are derived from hemicellulose and lignin parts [104]. Acetic acid is a significant hydrolysis product of the acetyl group that can be found in lignin and hemicellulose [137,164]. The hydrolysis of the hemicellulose backbone also leads to uronic acid formation [104]. Under severe pretreatment conditions, formic and levulinic acid can be obtained as HMF degradation products [104,137].

- Aromatic compounds

Aromatic compounds are classified into three groups, including: (1) phenolic compounds, (2) non-phenolic compounds, and (3) benzoquinone. The aromatic compound is mainly caused by lignin degradation [137].

The first group of aromatic compounds, phenolic aromatic compounds, are formed mainly during lignocellulosic pretreatment via partial lignin degradation, depending on the pretreatment method. Alkaline wet oxidation pretreatment causes lignin and carbohydrate degradation to produce some phenolic compounds and furan aldehydes, which can be oxidized into carboxylic acids (acetic, propionic, formic, etc.) and non-carboxylic acids, i.e., furoic acid, respectively. The consequence of this oxidation leads to the formation of phenolic acids such as 4-hydroxy phenolic, vanillic and syringic acids [104,165,166]. Moreover, the quantities and types of phenolic compounds also depend on the type of lignocellulosic biomass. In wood acid pretreatment with hydrolysate, the phenolic compounds that are mostly found include 4-hydroxy benzoic acid, 4-hydroxy benzaldehyde, vanillin, dihydroconiferyl alcohol, coniferyl aldehyde, syringaldehyde, syringic acid, and Hibbert's ketones [104,167,168]. *p*-coumaric acid and ferulic acid are often found in the pretreated hydrolysate of annual plants, e.g., sugarcane bagasse, wheat straw, and switchgrass [166,169,170].

The second group of aromatic compounds, non-phenolic aromatic compounds, are the phenylic constituents of lignocellulosic hydrolysates, e.g., benzoic acid, benzyl alcohol, cinnamic acid, cinnamaldehyde, 3,4-dimethoxy-cinnamic acid, and para- and ortho-toluic acid [104,161].

The last group of aromatic compounds is benzoquinone, such as p-benzoquinone and 2,6-dimethoxybenzoquinone, which normally appear during lignin and lignin-derived compound oxidation [161,171,172].

3.3.4. Lignocellulosic Hydrolysate Detoxification

Since the main problem in lignocellulosic pretreatment is the formation of many inhibitors which hinder enzymatic hydrolysis and fermentation yeast, detoxification can be applied to improve the fermentability of lignocellulosic hydrolysates [150]. There are several categories of detoxification method, such as physical detoxification, chemical detoxification, and biological detoxification [173]. To choose the suitable method to detoxify each type of inhibitor, the key is to identify the potential inhibitors present in the hemicellulose hydrolysates, as provided in Table 3.

Physical detoxification, e.g., vacuum evaporation, can reduce the concentration of volatile compounds, including acetic acid, furfural, and vanillin [174]. However, this treatment has some drawbacks. Firstly, it can increase nonvolatile poisonous compounds such as extractives and lignin derivatives. Moreover, it is less effective in the removal of phenolic chemicals, and requires a large amount of energy [34,175]. Another example of physical detoxification is membrane filtration. In a recent work conducted by Pan et al. [176], membrane filtration (MF) was found to simultaneously improve sugar concentration and separate lignocellulosic hydrolysate inhibitors. Moreover, the organic acid removal efficiency of membrane can be enhanced by anionic polymer addition. In other previous works, membrane detoxification was also found to have high potential in eliminating various types of inhibitors such as carboxylic acids, acetic acid, furfural, formic acid, and HMF [177–179].

In chemical detoxification, overliming is an effective way of neutralizing and reducing the toxicity of the hydrolysates. This detoxification technique can be integrated with other techniques. Domínguez et al. [180] reported that hydrolysates could be firstly detoxified by overliming, and both HMF and furfural were mostly removed from hydrolysate by overliming detoxification. Additionally, a significant reduction in acetic acid and formic acid concentrations was then observed. Later, the activated charcoal was applied on treated hydrolysate which substantially minimized the furan and phenolic compounds. Lastly, the remaining acetic acid in hydrolysate was removed by ion exchange resin.

In biological detoxification, various strains of microorganisms can be utilized to remove inhibitors in hydrolysate. Fonseca et al. [181] demonstrated that the yeast *Issatchenkia occidentalis* showed a significant reduction in syringaldehyde, ferulic acid, furfural, and HMF. *Trichoderma reesei*, which is filamentous soft-rot fungus, can also remove acetic acid, furfural, and benzoic acid derivatives in willow hydrolysate [182].

3.3.5. Hydrolysis of Cellulose

Cellulose hydrolysis can be categorized as enzymatic hydrolysis and acidic hydrolysis.

1. Enzymatic hydrolysis

In enzymatic hydrolysis, cellulase and hemicellulase enzymes are used to depolymerize cellulose and hemicellulose into hexose and pentose sugar. This is preferable to acidic hydrolysis as there are greater yields and selectivity, no chemical additions, less energy consumption, mild reaction conditions, and non-toxic and less corrosive conditions. However, an expensive enzymatic cost and long retention time are still drawbacks. The high retention time of enzymatic hydrolysis is due to substrate structure and enzyme mechanism [112,183].

Cellulase enzymes can be categorized into endoglucanase, exoglucanase, and β-glucosidase. Due to hemicellulose complexity, many enzymes can be applied for hemicellulose hydrolysis, for instance, endo-1,4-β-xylanase, β-1,4-xylosidases, β-mannosidase, and α-glucuronidase [113,184]. The appearance of inhibitors during the pretreatment and hydrolysis stages, which are 5-HMF and phenolic compounds derived from lignin (i.e., trans-cinnamic acid, 4-hydroxybenzoic acid, syringaldehyde, and vanillin) could strongly affect enzymatic hydrolysis efficiency by inhibiting cellulase activity [113,132].

2. Acidic hydrolysis

Concentrated acids or diluted acids can hydrolyze lignocellulosic materials.

*   Diluted acid hydrolysis

In diluted acidic hydrolysis, sulfuric acid is often used at concentrations below 4% to generate monosaccharides by hydrolyzing glycosidic linkages. Diluted hydrolysis can be performed in one (single) or two stages [125,184].

The single-stage acidic hydrolysis can be conducted using 1.5% acid under 200–240 °C, in which the hydrolysis of crystalline cellulose region occurs. This hydrolysis step can generate inhibitors, such as hydroxymethyl furfural (HMF), from glucose degradation. In contrast, furfural and other derivative compounds form by xylose degradation [26]. These chemical compounds inhibit ethanol fermentation and reduce sugar yield [125,133,174].

The two-stage hydrolysis is another option of the single stage. There is less possibility to generate inhibitors or sugar degradation [125]. It is initially operated under mild conditions at a temperature of 190 °C with 0.7% acid for 3 min, where the amorphous region of hemicellulose can be degraded to the xylose monomer. Afterwards, the cellulose is degraded to glucose under harsh conditions at the temperature of 215 °C with 0.4% acid for 3 min, yielding 50% glucose [26,185].

*   Concentrated acidic hydrolysis

Concentrated acidic hydrolysis yields nearly 90% of glucose. There is economic concern that is the difficulty of acid recovery. There are several techniques to recover acid

from acid and sugar mixture solution. Ion exclusion chromatography, solvent extraction, and electrodialysis are the three most studied and best performing methods [186]. In concentrated acidic hydrolysis, 30 to 70% of sulfuric acid is applied to achieve 90% glucose. The process residence time is between 10 and 12 h. In this type of acidic hydrolysis, the high-cost reactor with acid resistance and high energy cost are critically concerned [26].

Concentrated acid hydrolysis can cause decomposition products: HMF ($C_6H_6O_3$), levulinic ($C_5H_8O_3$), formic acid (CH_2O_2), and levoglucosan ($C_6H_{10}O_5$). HMF can occur when three molecules of water dehydrate one molecule of glucose. Levulinic acid and formic acid are formed when HMF re-hydrates with two water molecules. Intense-severity acid treatment results in the dehydration of glucose to levoglucosan. Forming inhibitors including HMF, levulinic, formic acid, and levoglucosan should be considered, since these decomposition products can inhibit yeast activity in the fermentation process [187].

$$C_6H_{12}O_6 \rightarrow C_6H_6O_3 + 3H_2O \tag{2}$$

$$C_6H_6O_3 + 2H_2O \rightarrow C_5H_8O_3 + CH_2O_2 \tag{3}$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow C_6H_{10}O_5 + H_2O \tag{4}$$
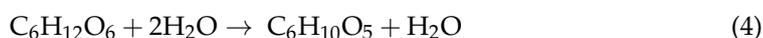
The distinctive advantage of biological detoxification is its mild operating conditions. Some microorganisms can effectively breakdown lignin while cellulose and hemicellulose remain in the substrate. Therefore, lignocellulosic substrate is easily hydrolyzed to fermentable sugars [34]. Currently, the biological method is gaining interest because of its simplicity, high effectivity, economics, and environmental friendliness [188]. However, prolonged incubation time and high costs of enzymes are still its drawbacks.

4. Fermentation

In general, sugar conversion to ethanol takes place in a fed-batch fermentation process with a cell recycling system, which recovers yeast cells from the previous batch into the next batch. After adding sugarcane juice into the fermenter, yeast converts fermentable sugar into ethanol and other fermented byproducts such as carbon dioxide, other alcohols, and organic acids. The yeast mostly employed to produce ethanol is saccharomyces cerevisiae [26]. Typically, the fermentation temperature is 30–37 °C [189].

4.1. Fermentation Media

Fermentation media contain a carbon source, water, nitrogen source, micronutrients, and salts [190]. The carbon source in ethanol production is sugar derived from the saccharification of different feedstock. Water is the major component of fermentation media [191]. In industrial ethanol production, urea or ammonium sulfate can be added as a nitrogen source. Yeasts require several micronutrients for optimum growth and fermentation performance at quantities typically between 0.1 to 100 mM depending on the yeast strain, fermentation conditions, and interactions with other components [192]. However, salts in the medium can cause osmotic stress to fermentation yeast. In Table 4, the impact of micronutrients and salts on ethanol production are provided along with their minimum concentration required and marginal concentration that possibly increase osmotic stress to yeast cells and induce other adverse effects.

Table 4. Micronutrients and salts in fermentation medium and their impacts on ethanol production.

Element	Impact on Ethanol Production		Concentration in the Fermentation Medium	
	Positive Effect	Negative Effect	Minimum Required	Marginal
Potassium (K^+)	• Potassium is a major cation involved in the yeast fermentation process [193]. Potassium plays a vital role in divalent cation transport and $H_2PO_4^-$ assimilation [43]; • Potassium is typically required at 160 ppm [192].	• At above 4–10 mM of potassium the fermentation rate could be decreased [43,192]; • Above 10 mM concentration it shows growth inhibition [43,192]; • Total inhibition was observed at about 2 M [43,193]; • Increases osmotic stress to yeast cells at high concentrations [194].	160 ppm	400 ppm
Magnesium (Mg^{2+})	• Magnesium is a major cation involved in the yeast fermentation process [193]. Magnesium regulates the metabolic enzyme of the fermentation pathway [195]; • Magnesium is necessary for the synthesis of DNA and ATP. It also stimulates essential fatty acids synthesizing [192]; • Magnesium concentrations of 300 ppm are required for good yeast activity [196]; • Magnesium concentrations of 500 ppm can increase yeast tolerance of temperature, ethanol, and osmotic pressure stress [192,197]; • Magnesium concentration in the fermentation medium should be controlled via adjusting the Mg:Ca ratio [198]. Increasing Mg to Ca ratio can increase fermentation performance in terms of the rate and yield of ethanol produced [198]. Anthony and Nwabueze [199] concluded that 2:1 Mg to Ca ratio with Zn supplemented results in maximum ethanol yield at 12.53% *v/v*.	• It can inhibit yeast growth at 1 M [43,192]; • Increases osmotic stress to yeast cells at high concentrations [194].	50 ppm	24,000 ppm
Zinc (Zn2+)	• Zinc ions positively affect the respiratory activity and the growth rate of yeast [200]; • Zinc is recognized as a major cation involved in yeast fermentation [193]. Zinc is an essential cofactor rapidly assimilated by yeast [43]; • At an appropriate concentration, it can increase yeast activity. De Nicola et al. [201] have reported the optimum Zn^{2+} concentration at 1.5–2.5 ppm, depending on yeast strain.	• Excess Zn^{2+} can inhibit yeast growth; • When Mn concentration is below 7 μM, growth inhibition occurs above ~30 μM [43]; • When Mn concentration is higher than 7 μM, Zn concentration can be as high as 1 mM before growth inhibition occurs [43].	0.3 ppm	2 or 60 ppm depending on Mn concentration
Calcium (Ca^{2+})	• Calcium may not be required, but some evidence may stimulate cell growth. It can also protect membrane structure and help maintain membrane permeability under adverse conditions [43,192]; • The concentration of Ca^{2+} of 4.5 mM is optimum for cell growth [43]; • Calcium concentration in the fermentation medium should be controlled by adjusting the Mg:Ca ratio. Increasing the Mg to Ca ratio can increase fermentation performance in terms of the rate and yield of ethanol produced [198]. Anthony and Nwabueze [199] concluded that 2:1 Mg to Ca ratio with Zn supplemented results in maximum ethanol yield at 12.53% *v/v*.	• Calcium inhibits the transphosphorylases enzyme of the glycolysis pathway, stimulated by magnesium [195]; • When Ca^{2+} concentration is over 25 mM, it can inhibit yeast growth depending on yeast strain [43]; • Calcium can react with carbonate to form calcium carbonate scale [43]; • Increases osmotic stress to yeast cells at high concentrations [194].	180 ppm	1000 ppm
Manganese (Mn^+)	• Manganese ions positively affect the respiratory activity and the growth rate of yeast [200]; • Yeast cells require manganese as an essential trace element at a concentration of 2–10 μM for optimal yeast growth [202].	• Mn^{2+} can inhibit cell growth at concentration more than 10 mM [192].	0.11 ppm	550 ppm
Iron (Fe^{2+})	• Iron is required as an essential nutrient for yeast, and is an enzyme cofactor [192]. Iron cations are involved in ribosome synthesis, protein translation, replication, and repair [203,204]; • Yeast typically requires 0.17 ppm of Fe^{2+}, which is usually abundant in mash [192].	• Iron concentrations higher than 10–15 mM can inhibit yeast growth [43]; • Excess Fe can decrease malate, pyruvate, and succinate dehydrogenase activity [43]. • At all levels up to 500 ppm iron is considered non-toxic [196].	0.2 ppm	500 ppm

Table 4. *Cont.*

Element	Impact on Ethanol Production		Concentration in the Fermentation Medium	
	Positive Effect	Negative Effect	Minimum Required	Marginal
Copper (Cu^{2+})	• Copper ions have a positive effect on the respiratory activity and the growth rate of yeast [200]. Trace amount of copper is an essential enzyme cofactor [43]; • The optimal concentrations of Cu^{2+} ions in the nutritive medium for the yeast growth and fermentation activity are in the range of 1–10 µM [202].	• Copper concentration higher than 1 ppm can inhibit yeast growth, and at 15,000 ppm cell growth completely ceases [192,196]; • Copper affects the changing yeast plasma membrane, leading to low molecular weight compounds' leakage and disturbing nutrient assimilation [43].	0.06 ppm	1 ppm
Sodium (Na$^+$)		• High sodium concentration reflects high osmotic stress on the yeast. The specific growth rate is reduced because yeast cell produces intracellular compatible solutes, such as glycerol and arabitol, against Na$^+$ diffusion into the cell [43]; • At acidic pH, sodium concentration of 5–100 mM can inhibit the enzymatic activity of yeast 38 to 44% [205]; • Sodium levels could increase floc formation during the clarification process [204].	-	115 ppm at acidic pH
Chloride (Cl$^-$)		• Some nutrients for fermentation yeast can be added in the form of chloride salts such as sodium chloride, potassium chloride, and ammonium chloride [196]. The addition of nutrients in the form of salt shows inhibitory effects on yeast depending on the type of cation (sodium, potassium, and ammonium) [52]; • Chloride is considered nondetrimental at all levels up to 500 ppm [196]; • Increases osmotic stress to yeast cells at high concentrations [194].	-	500 ppm
Sulfate (SO$_4^{2-}$)		• Some nutrients for fermentation yeast can be added in the form of sulfate salts such as magnesium sulfate, ammonium sulfate, zinc sulfate, calcium sulfate, and copper sulfate [196]. The addition of nutrients in the form of salt shows inhibitory effects and sugar consumption on yeast depending on the type cation (sodium, potassium, and ammonium). Compared to chloride salt, Casey, et al. [52] suggest that the addition of sulfate salt shows lower inhibitory effects than chloride salt; • Increases osmotic stress to yeast cells at high concentrations [194].	-	Depending on the cationic of sulfate
Fluoride (F$^-$)		• Fluoride concentrations higher than 160 ppm can inhibit yeast growth [196].	-	160 ppm
Nitrates (NO$_3^-$) and Nitrites (NO$_2^-$)		• When the concentration of these salts is higher than 50 ppm yeast is harmful in the fermentation process [196].	-	50 ppm
Tin (Sn^{2+})		• Tin concentrations higher than 360 ppm can inhibit yeast growth [196].	-	360 ppm
Tellurium (Te) and beryllium (Be)		• A higher concentration of Te and Be than 350 ppm can inhibit yeast growth [197].	-	350 ppm
Nickel (Ni)		• Nickel concentration higher than 185 ppm can inhibit yeast growth' [196].	-	185 ppm

4.2. Contamination during Fermentation

4.2.1. Bacterial Contamination

Bacteria can contaminate commercial ethanol during the fermentation process under poor sterile and pure-culture conditions through instruments, reactors, feed pipelines, chemicals/minerals, and yeast recycling systems [206,207]. This contamination brings about the formation of acetic acid and lactic acid. It reduces ethanol yield by inhibiting yeast from sugar and minerals, and reduces cell viability causing foam formation and yeast cell flocculation [40,208,209].

Most of the bacterial contamination in alcoholic fermentation is lactic acid bacteria. Lactic acid bacteria can be classified, according to glucose metabolism, into two types: homo-fermentative bacteria producing only lactic acid, and hetero-fermentative producing ethanol, lactic acid, acetic acid, and carbon dioxide [40,69,210,211].

Lactobacillus sp. are lactic acid bacteria usually found in ethanol fermentation because they can tolerate high ethanol concentrations. They can survive in low pH and low oxygen conditions. *Lactobacillus* sp. can produce both lactic acid and acetic acid. They also compete with other yeast cells for nutrients [209,212].

The source of bacterial contamination in sugarcane is soil [213]. Another source of bacterial contamination is borer. Sugarcane penetrated by borer leads to the accumulation of organic acid and phenolic compounds that can inhibit fermentation [69].

When bacterial contamination occurs during ethanol fermentation, antibacterial agents or antibiotics are required to reduce contamination. Sodium fluoride (NaF) or hydrogen peroxide (H_2O_2) can be used as antibacterial agents. Antibiotics, such as virginiamycin and penicillin, are usually employed [207,214]. However, these antibacterial agents cannot prevent long-term contamination because they can cause drug-resistant strains, reducing the effectiveness of antibiotics. Moreover, antibiotic utilization causes antibiotic residuals to be left over in byproducts [207,214].

The increase in metabolites (lactic acid and acetic acid) resulting from bacterial contamination leads to decreases in pH and increases in acidity during fermentation [208,212,215]. Additionally, produced metabolites inhibit ethanol production [209,214]. Lactic acid and acetic acid in undissociated form can diffuse through the cell membrane and dissociate to release hydrogen ions, according to (5) and (6). This mechanism can increase the acidity of the yeast cell's cytoplasm, resulting in the inhibition of ethanol production [212].

$$C_2H_4OHCOOH \leftrightarrow C_2H_4OHCOO^- + H^+ \tag{5}$$

$$CH_3COOH \leftrightarrow CH_3COO^- + H^+ \tag{6}$$

Yeast flocculation is usually found when contaminated by bacteria. The flocculation results in poor mass transfer, low cell viability, and a reduction in contact surface area between yeast and culture media, thus reducing the ethanol production yield [207,209]. In Brazil, yeast flocculation can be resolved by treating saccharomyces cerevisiae with sulfuric acid [216]. However, the use of sulfuric acid can cause contamination in co-products, which will be discussed further in Section 4.2.4.

4.2.2. Byproducts Generated by Yeast

In ethanol fermentation, glycerol, lactic acid, acetic acid, and succinic acid are major byproducts [217]. However, other byproducts can be generated. Campbell [218] summarized the main byproducts from the fermentation of sugars to alcohol into four groups: alcohols (ethanol, propanol, butanol, amyl alcohol, glycerol, phenethyl alcohol), acids (acetic, caproic, caprylic, lactic, pyruvic, succinic), esters (Ethyl acetate and any other combination of acids and alcohols), and others (CO_2, acetaldehyde, diacetyl, H_2S).

Fusel alcohols, such as 1-propanol, 2-methyl-1-propanol, and 3-methyl-1-butanol, are generated by yeast as byproducts from ethanol fermentation. In fuel ethanol, fusel alcohols must be controlled. Sanchez et al. [219] indicated that controlling fermentation conditions (pH, stirring rate, fermentation temperature, and hydrolysis condition) can

reduce fusel alcohol production. Among these parameters, fermentation temperature greatly affects fusel alcohol formation. Additionally, supplementing the growth medium with nutrients reduces the concentration of the contaminants in ethanol compared to non-supplementation [220].

Sulfite also can be produced by yeast metabolism via the sulfate assimilation pathway, in which yeast consumes sulfate from the fermentation medium to produce sulfur-containing amino acids that can also produce sulfite. The amount of produced sulfite depends on the yeast species, fermentation conditions, and sulfur-containing compounds in the fermentation feedstock.
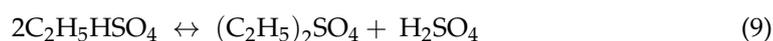
4.2.3. Sulfur Dioxide as an Antioxidant

In the ethanol fermentation process, sulfur dioxide is employed as a bactericide and antioxidant [13,221]. Sulfur dioxide is very reactive and inhibits ethanol fermentation [216]. Sulfur dioxide in dilute aqueous solution can occur in three forms: SO_2 (Molecular sulfur dioxide), HSO_3^- (Bisulfite ion), and SO_3^{2-} (Sulfite ion), depending on pH [7,222]. At low pH, sulfur dioxide is often found in molecular form, while bisulfite and sulfite are found at pH 5.0–9.0 [7,216,222]. The chemical equilibrium between molecular, bisulfite, and sulfite forms in an aqueous solution is shown in (7). Sulfite considerably affects ethanol pH in the form of SO_2 and HSO_3^- because it can react with carbonyl groups of aldehydes or organic acids to sulfonic acid [216,223].

$$SO_2 + H_2O \leftrightarrow H^+ + HSO_3^- \leftrightarrow 2H^+ + SO_3^{2-} \tag{7}$$
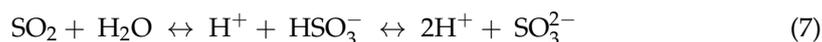
4.2.4. Sulfuric Acid as pH Regulator and Antimicrobial Agent

Sulfuric is used in different steps, especially as a pH regulator of fermentation. Moreover, it is also used after fermentation to remove bacteria from yeast cells before fermentation in the next batch [224]. Sulfuric acid utilization in these steps results in sulfate formation; it can react with ethanol to create ethyl sulfate and diethyl sulfate, as shown in Equations (8) and (9), respectively [13,225,226]. However, these sulfates from the sulfuric utilization could remain in the co-products. In cases where co-products are used for animal feed, these sulfates could be of concern in excessive levels [14,77,227].

$$C_2H_5OH + H_2SO_4 \leftrightarrow C_2H_5HSO_4 + H_2O \tag{8}$$

$$2C_2H_5HSO_4 \leftrightarrow (C_2H_5)_2SO_4 + H_2SO_4 \tag{9}$$

4.2.5. Addition of Defoamer

In ethanol production, foam formation normally occurs due to carbon dioxide production as a co-product of ethanol [228,229]. The foam reduces the fermentation tank's working capacity, resulting in higher production costs and lower productivity [230,231]. Therefore, employing a defoamer, such as a polypropylene glycol-based defoamer or a silicone polymer-based defoamer, is necessary. Different defoamers cause different effects on microbial physiology and cell growth rate [231].

However, the use of some defoamers can cause contamination. Silicone polymer-based defoamers can stimulate glycerol production during the fermentation process with *Saccharomyces cerevisiae* at low oxygen and excess glucose conditions [232].

4.3. Chemical Use for Fermentation Gas Removal

Fermentation gas is produced during the fermentation process. This fermentation gas, discharged through the vent stream, consists of carbon dioxide, vaporous ethanol, and other volatile organic compounds (VOCs) [233]. Presently, stringent pollutant emission regulations are in place in most countries. Typically, ethanol distilleries employ scrubbers connected to the fermentation tank to recover vaporous ethanol and control the emission of VOCs into the atmosphere [234]. Since ethanol is a good solvent for VOCs, the scrubber bottom contains water, ethanol, and VOCs [234]. Depending on the ethanol concentration

obtained from different scrubbing techniques, i.e., low ethanol concentration ca. 1–6 wt.%, the scrubber bottom can be recycled back to the cooking process to reduce water consumption. However, ethanol in a recycle stream will be consumed by bacteria in the cooking step [235,236]. Presently there are many techniques to recover ethanol in the vent stream. With a high concentration of ethanol, the scrubber bottom can be recycled directly to the distillation column [233,237,238].

VOCs can be divided into soluble and insoluble volatile organic compounds, as shown in Table 5 [239,240]. Sometimes, bisulfite may be used as an additive to increase the solubility of insoluble VOCs including acetaldehyde, ethyl acetate, acrolein, and acetone [234]. However, the use of bisulfite to control VOCs release may cause acid formation. Sodium bisulfite ($NaHSO_3$) can either react with acetaldehyde and convert to 1-hydroxy-ethane sulfonic acid salt (10), or with acrolein resulting in sulfonic acid salt (11) [7,14].

$$NaHSO_3 + CH_3CHO \rightarrow CH_3CH(OH)SO_3^- Na^+ \qquad (10)$$

$$NaHSO_3 + CH_2CHCHO \rightarrow CH_2CHCH(OH)SO_3^- Na^+ \qquad (11)$$

Table 5. Categories of volatile organic compounds generated during the ethanol fermentation.

Categories of Volatile Organic Compounds (VOCs)	
Soluble	**Insoluble**
Ethanol	
Formic acid	Acetone
Lactic acid	Acrolein
Acetic acid	Acetaldehyde
Amyl Alcohol	Ethyl Acetate
Formaldehyde	

Moreover, sodium bisulfite is an unstable substance that can decompose into sulfur dioxide. Therefore, acidity is increased, according to Equation (12) [241].

$$2NaHSO_3 \rightarrow Na_2SO_3 + SO_2 + H_2O \qquad (12)$$
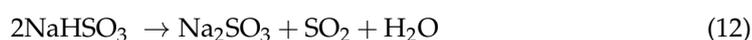
5. Ethanol Recovery

5.1. Distillation Process

In sugar and starch fermentation, other alcohols, aldehydes, ketones, fatty acids, and esters are produced as volatile byproducts, whereas cyclic and heterocyclic compounds are volatile byproducts in lignocellulosic ethanol fermentation [91]. After the fermentation process is finished, the centrifuged broth is obtained by separating the yeast from the fermented beer. The centrifuged broth containing ethanol at about 5–15 wt.% is passed to the distillation column to remove the water. The distillation column consists of two columns. The first one is called the distillation column, or the beer column. In this column, approximately 50 wt.% ethanol can be achieved. The second column is the rectifying column. Hydrous ethanol (about 93 wt.% ethanol) can be achieved in this column [30,35].

Distillation can remove some impurity from ethanol with increasing ethanol concentration. Furthermore, chemical molecules with low boiling points, or those similar to ethanol, show up in distillate because distillation is ineffective in removing them [17]. For example, volatile impurities (acetaldehyde, acetone, ester, methanol) still show up in distillate. These contaminants result in lower engine efficiency when ethanol is used as fuel [7,8,15,91,242].

5.2. Stillage Recycles

The remaining bottom liquid product after distillation of the ethanol from the beer column is called whole stillage. The whole stillage can contain ethanol up to 0.02 wt.%. Not only ethanol, but also solid particles, such as yeast cells, dissolved matter, and minerals, can be found [26,243]. After removing solid particles through a solid–liquid separation unit

(e.g., centrifuge or decanter), the obtained liquid product called thin stillage can be recycled back to different process steps, e.g., fermentation or saccharification, to minimize effluent treatment cost. However, thin stillage recycling can possibly cause some drawbacks, such as the accumulation of lactic acid, minerals, and unutilized substrates [26,243,244].

The difference in the type of feedstock affects the impurities in the stillage. When stillage is recycled, it causes different contaminations. In the case of cane molasses feedstocks, whole stillage (without yeast cell separation) can be recycled in the fermentation step [26]. In the case of starch-containing feedstock, 25–75% of the thin stillage can be recycled in the fermentation or saccharification processes [26]. Other feedstocks, such as corn, wheat, and triticale, can be recycled at 75%, 60%, and 60% of thin stillage, respectively [243,245].

In Thailand, produced stillage during ethanol production from molasses or cassava is often treated and converted into methane gas. Stillage can also be distributed to farmers because stillage provides minerals for plants [246,247].

5.3. The Fate of Electrolytes during Distillation

During ethanol distillation, sulfite as sulfur dioxide can be distilled into the final ethanol product. The presence of sulfite in distilled ethanol appears to be a common experience in the distilled spirits industry [7,248]. Zhang et al. [249] reported that the distillate of chardonnay contained 12% ethanol and 176 mg/L sulfite as SO_2. After two stages of distillation, the concentration of ethanol and sulfite as SO_2 were increased to 69 vol% and 654 ppm, respectively. This phenomenon can be explained with the vapor–liquid equilibria for dilute aqueous solutions of SO_2 as volatile weak electrolyte [250].

5.4. Dehydration Process

The distillation process produces 95 vol% ethanol, approximately, because of the azeotropic mixture of ethanol and water (95.6 wt.% at 78.15 degrees Celsius). Before mixing ethanol with gasoline, it is necessary to increase the ethanol concentration to 99.3 wt.%, to make anhydrous ethanol. Anhydrous ethanol can be obtained by several dehydration methods such as molecular sieves, azeotropic distillation, and pervaporation. The molecular sieve is most commonly used because it has lower investment costs than pervaporation and requires less steam than azeotropic distillation [30,35].

The most common dehydration methods in Brazil are heterogeneous azeotropic distillation, extractive distillation, and molecular sieve adsorption [35]. The heterogeneous azeotropic distillation method requires an entrainer to increase separation. Many entrainers, such as benzene, toluene, and cyclohexane can be used to separate ethanol from water [35,251]. However, using an entrainer can cause product contamination [252,253].

Extractive distillation, as an alternative method, requires the addition of a third component to change the relative volatility of ethanol and water. The third component acts as a separating agent, such as ethylene glycol, glycerol, 1,3 diamino pentane, diethylenetriamine, or hexachlorobutadiene. The separating agent and water mixture is obtained at the bottom of the column, which is fed to the second column to recover the separating agent. Anhydrous ethanol is obtained at the top of the extractive column. Compared to azeotropic distillation, this method provides less energy consumption and less ethanol contamination [35].

In the case of molecular sieve adsorption, there is no requirement to add solvent. Ethanol vapor is fed to zeolite beds. When hydrated ethanol contacts zeolite, water molecules are absorbed. When compared to azeotropic distillation and extractive distillation, molecular sieve adsorption offers lower energy consumption and no chemical contamination [35].

Pervaporation, a membrane dehydration method, is a relatively new alternative to the dehydration process. While adsorbents need regeneration, membrane separation offers continuous operation and energy saving. Industrial applications of zeolite membranes have been reported [254].

6. Ethanol Storage

Of course, ethanol derived from different biomass feedstock may have inconsistent compositions, which can cause storage stability issues. Besides, ethanol characteristics also change during storage due to its nature.

6.1. Oxidative Degradation

Normally, ethanol acidity increases along with storage periods due to oxidative degradation [255]. The oxidation reaction in ethanol relates to oxygen solubility in ethanol. Oxygen solubility in ethanol is approximately 44 cm^3/L at 25 °C, compared to 6.4 cm^3/L for distilled water [256,257].

Acetic acid is the main component affecting acidity [215]. During storage periods, acetic acid is produced from the oxidation reaction of acetaldehyde. Ethanol contains acetaldehyde as impurities from pyruvate decarboxylation in the fermentation stage [258]. Another source of acetaldehyde is the product of ethanol oxidation. Acetaldehyde can be oxidized to acetic acid during storage periods [91,256,259]. Additionally, ethyl acetate can form by the esterification reaction between acetic acid and ethanol [91,260].

$$(13)$$

$$(14)$$

$$(15)$$

$$(16)$$
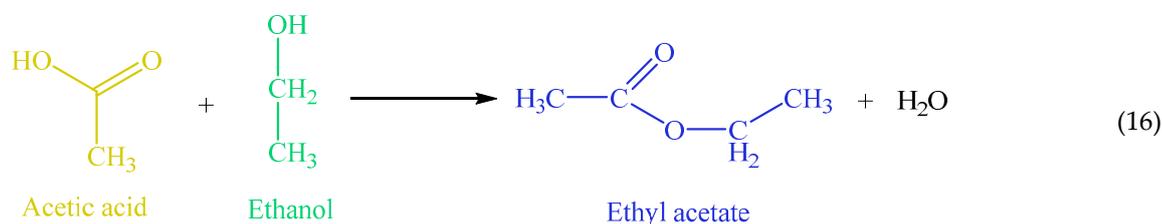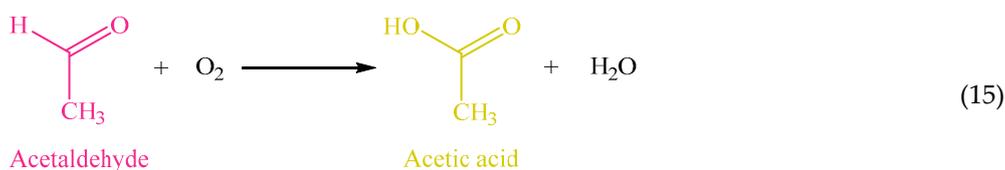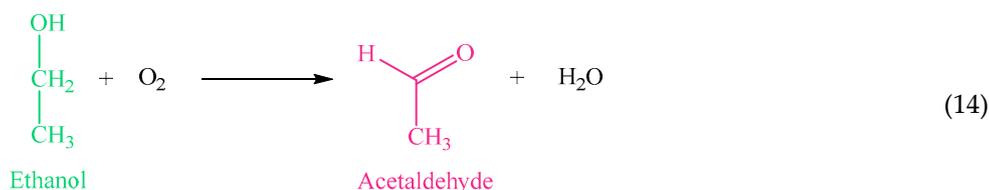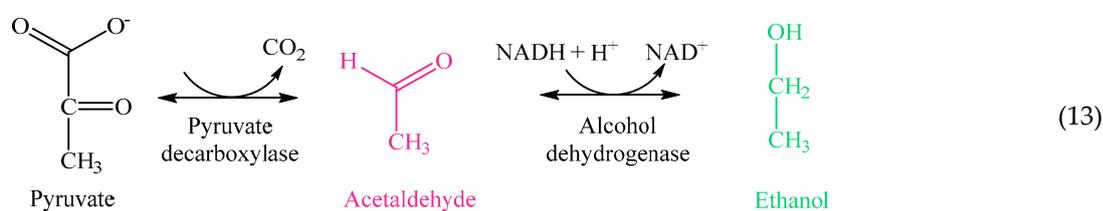
Acetic acid is a monoprotic molecule. As illustrated in the equation, the hydrogen atoms attached to acetic acid can detach and form hydronium ions [261].

$$CH_3COOH + H_2O \rightarrow H_3O^+ + CH_3COO^- \qquad (17)$$

When moisture is present, acetic acid tends to corrode metals by donating hydrogen ions to the exposed material.

6.2. Increasing Water Content

The hygroscopic nature of ethanol causes ethanol to absorb water well from the surrounding environment, even when stored in a controlled environment such as in the

laboratory. Kane et al. [262] reported that when ethanol is exposed to the atmosphere during storage and transportation, the water content in ethanol tends to increase. Cummings [263] has reported that controlling the water content of ethanol product can maintain storage stability. Ethanol surface area in a given tank, headspace volume, tank type, type of tank layer material in contact with the ethanol, and tank breathing system, affect water intake through the tank [264]. In the experiment conducted by Nakajima and Yahagi [265], E0 (Pure gasoline), E10, and E100 ethanol were exposed to a humid environment. After 30 days, it was found that the higher the ethanol content, the more moisture was absorbed from the environment, in the order of E100, E10, and E0, respectively.

6.3. Sulfite Oxidation

Sulfite is generally converted from sulfur dioxide added during the wet milling process, juice clarification, and fermentation processes [222,266]. The addition of sulfuric acid to adjust the pH during fermentation can also increase residual sulfite. Yeast metabolism is another issue that can result in the contamination of sulfite during fermentation. The amount of sulfite generated by yeast depends on the fermentation conditions, yeast strains, and sulfur content in raw materials [7].

In the distillation step, sulfite in ethanol is distilled with ethanol simultaneously because sulfite in the form of sulfur dioxide easily vaporizes with ethanol during distillation. When storing ethanol for an extended period, sulfite can be oxidized to sulfate by oxygen, as shown in Equation (18). Although there is no evidence of the oxidation of sulfite to sulfate in fuel ethanol, related evidence was found in a study on reducing sulfur dioxide in beer due to oxidation, which showed that the rate of SO_2 reduction is pseudo-first-order. The rate of SO_2 loss increases with increasing storage temperature [267].

$$2SO_3^{-2} + O_2 \rightarrow 2SO_4^{2-} \tag{18}$$
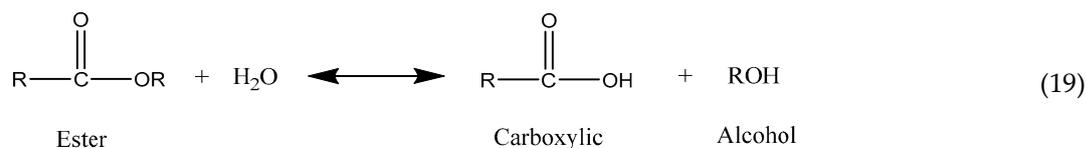
6.4. Carbon Dioxide

Carbon dioxide can dissolve in ethanol better than water, according to the order of magnitude of Henry's constants [268]. A study by General Motors (GM) concluded that ethanol contains high dissolved carbon dioxide gas because carbon dioxide is a fermentation byproduct. The presence of water can cause the formation of carbonic acid during storage time [269].

Typically, the dissolution of carbon dioxide in ethanol fuel causes the value of measured pHe to be biased, showing acidity higher than reality. Hence, acidity measurement should be determined with the ASTM D1613 (standard test method for acidity in volatile solvents and chemical intermediates used in paint, varnish, lacquer, and related products) because this method allows carbon dioxide to be removed [18].

6.5. Ester Hydrolysis

Ester is mainly yielded from yeast fermentation [270]. Volatile esters can form as fermentation byproducts during ethanol fermentation via the biosynthesis of two enzymes: acyl-CoA synthetase and alcohol acetyltransferase. The most abundant ester is ethyl acetate. Other esters comprise isoamyl acetate, isobutyl acetate, ethyl caproate, and 2-phenyl ethyl acetate.

Ramey and Ough [271] studied the factors that affect the hydrolysis reaction of the volatility ester in wine (when the concentration of ethanol is 10–14%) and found that the rate of hydrolysis mainly depends on ester types, temperature, and pH. Similarly, esters in ethanol fuel are possibly hydrolyzed during the storage of ethanol fuel. This can yield carboxylic which increases acid content.

$$R-\overset{\displaystyle O}{\overset{\|}{C}}-OR \;+\; H_2O \;\longleftrightarrow\; R-\overset{\displaystyle O}{\overset{\|}{C}}-OH \;+\; ROH \qquad (19)$$

     Ester   Carboxylic   Alcohol

6.6. Ethanol Quality Control Strategies during Storage

During storage periods, nitrogen blanketing should be applied. It can be performed for a wide range of functions.

- It reduces the water intake rate to the tank (maintains water content);
- Maintaining water content can minimize the cause of carboxylic formation from ester hydrolysis;
- Oxygen concentration, which is the cause of the oxidation reaction, can be minimized; as a result, the formation of acetaldehyde, acetic, and ethyl acetate is reduced.

In order to maintain ethanol storage stability, a corrosion inhibitor can be applied. Many available commercial corrosion inhibitors can control acidity and buffer pHe [263]. In addition to corrosion inhibitors and nitrogen blanketing, storage tank characteristics also play a significant role in maintaining ethanol quality during storage. The American Petroleum Institute (API) [19] have recommended a suitable storage tank for storing ethanol: a fixed roof tank with an internal floating cover. Compatible materials for tank construction can be carbon steel, stainless steel, aluminum, or bronze. However, carbon steel is mostly used. A suitable internal storage tank liner should be selected. For example, a specific type of epoxy compound can be used because of its compatibility with ethanol [18].

7. Comparison Study of Contamination in Ethanol Derived from Different Feedstocks

Bioethanol can contain up to 300 different organic compounds depending on feedstock type, process type, and operating conditions [10]. Moreover, ethanol contamination is also a result of the storage process. Contamination in ethanol is necessary to improve fuel ethanol quality when used in vehicle engines. Bioethanol usually contains organic impurities, water, and organic acid. Many contaminants, such as water, methanol, volatile acidity, copper, chloride, and sulfate, are listed in the specifications of fuel ethanol [15] as they can cause corrosion on engine components, fuel storage, and fuel transportation systems. To ensure fuel ethanol quality, Monteiro et al. [272] concluded that the amount of water and various contaminants (sulphate, chloride, acetate, etc.) must be monitored.

Habe et al. [10] investigated the different amounts of organic impurities, organic acid, sulfur compounds, cations, and anions in diverse ethanol samples. The ethanol sample derived from lignocellulosic ethanol had a higher number of organic impurities than the sugar- and starch-derived ethanol. Twenty-nine types of organic impurity were found in lignocellulosic ethanol, but in sugar- and starch-derived ethanol, only 16 types were detected. Commonly, in sugar and starch-based ethanol, methanol, acetaldehyde, 1-propanol, ethyl acetate, 2-methyl-1-propanol, and acetal were found to be more significant than other impurities. For lignocellulosic ethanol, the quantities of 2-methyl-1-butanol and 3-methyl-1-butanol were greater than in the sugar- and starch-based ethanol. Other important impurities found in lignocellulosic ethanol are furan-related compounds that originate from acid pretreatment, leading to acetic acid and furan-related compound formations. The types of organic impurities and organic acids found in different derivations of feedstock ethanol are shown in Figure 8.

The most organic acids found in ethanol are formic, acetic, propionic, and n-butyric acid. For lignocellulosic ethanol, the amount of acetic acid is high due to the lignocellulosic pretreatment and the autohydrolysis process. Generated residual acetic acid in the fermentation broth can remain in final ethanol after the distillation and dehydration process.
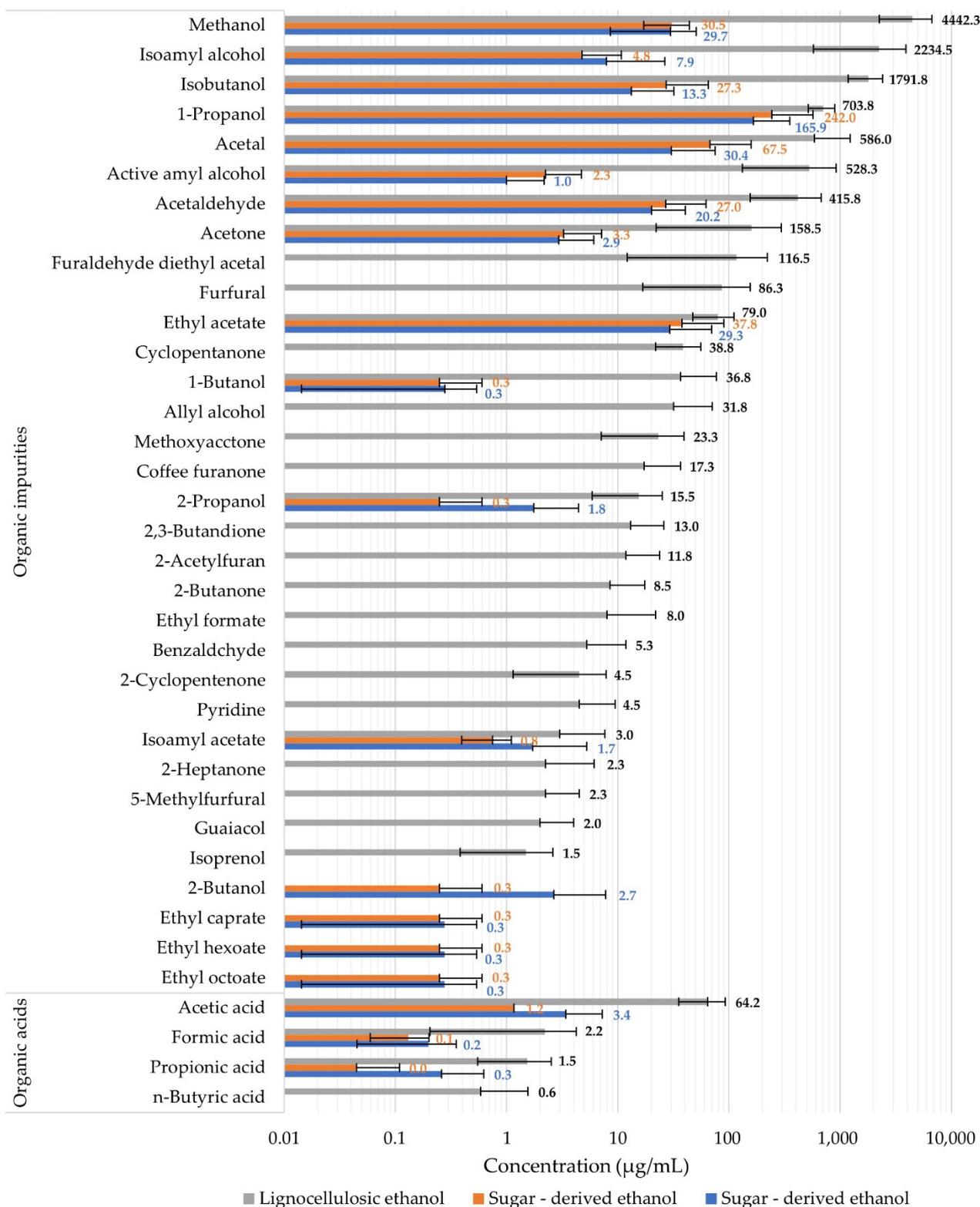
Figure 8. Organic impurities and organic acids found in ethanol derived from different feedstocks; data taken from [10].

Sulfur compounds are further impurities found in ethanol. In sugar- and starch-based ethanol, only dimethyl sulfide (DMS) and dimethyl sulfoxide (DMSO) were found as organosulfur compounds, but these organosulfurs were scarcely found in lignocellulosic

ethanol. In lignocellulose ethanol, Dimethyl disulfide (DMDS) and Thiazole were found as the sulfur compounds [10].

The amount of silicon (Si) detected in lignocellulosic ethanol was higher than in sugar- and starch-derived ethanol since wood and herbaceous plant feedstock contain ash at around 0.5–5%.

After reviewing the inorganic impurities in Brazilian ethanol [273], sugarcane ethanol was found to have a higher amount of inorganic impurities than corn ethanol. These inorganic impurities included sulfate, sodium, potassium, calcium, magnesium, and sulfur.

Starch-based ethanol can be produced by two methods. There are wet milling and dry milling methods. Weaver et al. [274] compared the ethanol compositions between corn wet milling and dry milling. Ethyl acetate and 1,1-Diethoxyethane were detected in wet milling ethanol. Thus, impurities in ethanol are not only affected by feedstocks, but by the production process too.

Besides impurities in the form of compounds, elemental traces were also found in ethanol. Sánchez et al. [16] analyzed metal and metalloid content in ethanol fuel. Trace elements in ethanol fuel are summarized in Table 6. However, the sources of these metals in ethanol fuel are difficult to identify. Some studies report that metal content in ethanol depends on the soil used for growing feedstock and the environmental conditions [275]. Furthermore, metals can contaminate ethanol fuel during production. Various metals can contaminate ethanol during storage and transport due to contact with the metallic container.

Table 6. Main elements found in ethanol fuel; data taken from [16].

Concentration	Elements
>1 mg/L	Na
10 µg/L–1 mg/L	Mg, Cr, Fe, Ni, Cu, Zn, Al, Si
<10 µg/L	Ba, V, Mo, Mn, Co, Ag, Cd, Ga, Tl, Sn, Pb, As, Bi, Se

8. Specific Guidelines to Control Ethanol Quality during Production and Storage Periods

The quality of fuel ethanol is regulated by the standard specification for denatured anhydrous ethanol because the impurities in ethanol impact vehicle engines. Second-generation ethanol has more impurities than first-generation ethanol. Furthermore, the increasing ethanol mandate requires stricter revisions of these ethanol standards.

Currently there are many research topics related to ethanol impurities in fuel ethanol [10,15–17,91]. Many reports and works of scientific research identify the effect of contaminants in fuel ethanol on vehicles engines, e.g., sulfate [7,8,14,276], acetic acid [215,276], chloride salt [276], and so on. The difference in these impurity profiles depends on raw materials, production processes, and storage procedures. With regard to fuel quality specifications in the U.S. today, the ASTM (American Society for Testing and Materials) international standard specifications for fuel ethanol have been based on traditional corn feedstock production [263]. With so many new feedstocks entering the marketplace, there will be a need to review and, if necessary, update the required quality control testing to ensure that the final blended fuel will not adversely impact vehicle system components and driving performance. There are many challenging aspects to controlling ethanol quality, as already mentioned. However, although industry guideline specifications and procedures for blended gasoline provided by the RFA are currently available [18], there are no specific guidelines related to anhydrous ethanol impurities and quality control regarding the entire production step and storage periods. Therefore, we have reviewed and proposed specific guidelines to cover ethanol quality control for both first- and second-generation fuel ethanol. In Table 7, the possible contamination points for each production step's entire storage period are summarized, along with the control strategies that can mitigate the effects of contamination.

Table 7. Summary of overall contamination in ethanol production.

Stage	Source of Contaminants		Contaminants	Concern	Control Strategies
Conversion of feedstock	Sugarcane	Juice clarification	Sulfur dioxide	• Sulfur dioxide in a dilute aqueous solution can occur in many forms ($SO_2 \cdot H_2O$, HSO_3^- and SO_3^{2-}) depending on pH [7]; • Increased sulfur dioxide content in ethanol [7,277]; • The addition of sulfur dioxide can leave residual sulfite in clarified juice [278,279].	• Sulfite can be removed by evaporation [279].
	Cassava	Cassava composition	Cysteine	• This sulfur-containing compound ends up in the DDGS fraction, not in the ethanol product stream [14,77].	-
			Methionine		
	Lignocellulosic biomass	Lignocellulosic feedstock components	Lignin	• Lignin can degrade into phenolic compounds and benzoquinone. These compounds can inhibit fermentative yeast [104,137,165,166].	• Select less recalcitrant feedstock that can be pretreated under mild conditions producing fewer inhibitors during pretreatment [104].
			Hemicellulose	• Hemicellulose can degrade into undesired products, such as furfurals and hydroxymethyl furfurals [112]; • Degradation products of hemicellulose can inhibit fermentative yeast [137].	
		Pretreatment	HMF	• These inhibitors can negatively influence both enzymatic hydrolysis and fermentation yeast [137].	• Remove inhibitors in lignocellulosic hydrolysate by suitable detoxification methods; • Change fermentation strategies; • Metabolic engineering.
			Furfural		
			Acetic acid		
			Formic acid		
			Levulinic acid		
			Phenolic compounds		
			2-furoic acid		
			Furanic compounds	• Furanic compounds, specifically 2,5-dimethyfuran and 2-methylfuran, have poor oxidative stability in blended gasoline [11]. • These compounds show potential for forming dangerous organic peroxides [11].	
			Sulfur components	• Sulfur compounds can cause catalyst deactivation when ethanol is used in chemical processes [10,90]; • The combustion of high-sulfur fuel can cause sulfur dioxide emissions [90].	• Adsorption with anion exchange resin, aluminum silicate clay, alumina silicate (alumina), activated carbon, smectite clay, barium salt [280].

Table 7. *Cont.*

Stage	Source of Contaminants	Contaminants	Concern	Control Strategies
Fermentation	Water	Calcium	• Increased osmotic stress to yeast [194]; • Calcium damage to the distillation equipment [204].	• The quality of water used in fermentation affects enzymatic activity. Thus, water testing and water treatment should be carried out.
		Magnesium	• Increased osmotic stress to yeast [194,281];	
		Sodium	• Increased osmotic stress to yeast [282,283]; • Increases the conductivity of ethanol [90,284]; • Na^+ content increases looser floc formation (smaller agglomerates) during the juice clarification process [204]; • Sodium accumulates in the vehicle combustion chamber and causes corrosion [90,285]; • Ion present in ethanol would impact the corrosion inhibitor's storage stability and effectiveness [263].	
		Chloride	• Increased osmotic stress to yeast [194]; • Chloride increases ethanol corrosivity [2,90]; • It can increase ethanol conductivity [90,276,284]; • In the presence of water, hydrochloric acid (HCl) can form [90]; • Chloride in ethanol can cause injector plugging, fuel pump failure, and intake valve deposits [263]; • It also causes failure on the fuel sender card [263]; • Ions present in ethanol would impact the corrosion inhibitor's storage stability and effectiveness [263].	
		Sulfate	• Increased osmotic stress to yeast [194]; • Sulfate content increases ethanol conductivity [90,284]; • It can increase ethanol corrosivity even in a small concentration and accelerates the corrosion of vehicle fuel system parts [285]; • Sulfates (present as SO_3 and SO_4) form a gum with petrol and cause scale formation in engine pipes [90]; • Sulfate depositing causes injector clogging in vehicle engines [90,263].	
	Nitrogen source	Ammonium sulfate	• Ammonium sulfate is possible to increase sulfate residual in ethanol; • Sulfate content in ethanol correlates with ethanol pHe and conductivity [13,90]; • Sulfate content increases ethanol corrosivity [285].	• Select suitable nitrogen source; the addition of nitrogen source in the form of sulfate possibly causes an increase in sulfate residual.
	Control of Aldehyde Emissions	Sodium bisulfite	• It can react with acetaldehyde converting to 1-hydroxy-ethane sulfonic acid salt [14]; • It reacts with acrolein resulting in sulfonic acid salt [14]; • Sodium bisulfite can decompose to sulfur dioxide and cause increasing acidity [241]; • Overused sodium bisulfite will contribute to sulfur levels and stress the yeast to produce more glycerol, thus reducing ethanol yield [286].	• Minimize sodium bisulfite used [286]; • In most ethanol plants, the emission of VOCs is controlled by a scrubber, which requires sodium bisulfite. Thus, alternative VOC removal methods might be used instead of scrubbers, such as a bio-trickling filter [233].

Table 7. *Cont.*

Stage	Source of Contaminants	Contaminants	Concern	Control Strategies
Fermentation byproducts		Carbon dioxide	• Presence of water; carbon dioxide can be converted into carbonic acid [287]; • High carbon dioxide concentration can reduce growth and general metabolic activity. The recommended carbon dioxide concentration in most industries (P_{CO_2} value) should be below 0.15–0.2 atm [288].	
		Acetaldehyde	• The reaction with sodium bisulfite produces 1-hydroxy-ethane sulfonic acid salt [14]; • It shows the inhibitory effect on fermentative yeast [120,217].	• Aldehyde in ethanol can be removed by strong base anion exchange resin in the HSO_3^- form [289]; • Minimize SO_2 addition because SO_2 addition during fermentation induces acetaldehyde production [290,291]; • Sodium bisulfite addition should be optimized when it is used to remove acetaldehydes in the scrubber [286]; • Alternative VOC removal methods might be used instead of scrubbers, such as a bio-trickling filter [233].
		Acrolein	• It reacts with sodium bisulfite and converts to sulfonic acid salt [14].	• Alternative VOC removal methods might be used instead of scrubbers, such as a bio-trickling filter [233].
		Acetic acid	• Increases the acidity of fuel ethanol [215]; • It shows the inhibitory effect on yeast [194,292]; • Acetic acid can increase ethanol corrosivity [2].	• The most common bacterial contaminants found in ethanol production are lactic acid bacteria (LAB) which can produce lactic and acetic acids. Therefore, bacterial contamination must be carefully monitored [212]. • Control fermentation condition (oxygen, medium composition, temperature) [293].
		Sulfite	• Sulfite can occur naturally as a product of yeast metabolism; • It shows the inhibitory effect on fermentative yeast [283].	• Optimize sulfur dioxide addition; • Screen fewer sulfite-producing yeasts [277].
		1-Propanol	• It has chemical interference (change cell morphology) [120,217].	• Change fermentation condition (temperature, oxygen content, medium composition) [293]; • Addition of ammonium sulfate in fermentation medium [64,65].
		Formic acid	• It shows an inhibitory effect on yeast [194,217,282,292,294,295]; • Formic acid enhances ethanol corrosivity [2].	• Control fermentation condition (pH, nitrogen level, thiamin content, SO_2 content) [296].
		Glycerol	• Glycerol affects osmotic pressure on yeast cells [120,217]; • Production of glycerol reduces ethanol yield [297].	• Metabolic engineering [298,299]; • Change the fermentation conditions (such as aeration levels, osmotic stress) [297]; • Distillation [120].

Table 7. *Cont.*

Stage	Source of Contaminants	Contaminants	Concern	Control Strategies
		Lactic acid	• It has chemical interference with cell maintenance functions [217,283].	• Control bacteria contamination [212].
		Methanol	• Methanol enhances pump and fuel sender card failure [263].	• Control microbial contamination because the methanol contamination can be linked to microbes producing pectin methylesterase (PME) that can produce methanol from pectin-rich feedstocks [300]; • Use low pectin content feedstock because pectin content affects methanol production in alcohol fermentation [300,301].
	pH regulator, antimicrobial agent	Sulfuric acid	• Sulfuric acid can react with ethanol and convert to ethyl sulfate and diethyl sulfate [13,225,226]; • Sulfate introduced from sulfuric utilization remains in the byproduct stream, not in the ethanol product stream [14]; • Sulfuric utilization increases sulfur residual in DDGS [77].	• Use acetic acid to control pH instead of sulfuric [302].
Ethanol Recovery	Distillation	Sulfite	• During ethanol distillation sulfite can vaporize with ethanol resulting in ethanol contamination [7,13,14,249].	• Treat first distillated with calcium oxide, powdered activated charcoal, or hydrogen peroxide [249].
			• Volatile compounds (other alcohols, aldehydes, ketones, fatty acids, esters, sulfite, cyclic, and heterocyclic compounds) can contaminate in distillate [91].	• Treat distillate with ozonation and physical adsorption [91].
	Dehydration	-	-	-
Ethanol storage	Sulfite oxidation	Sulfate	• In storage periods, sulfite in ethanol can be converted into sulfate resulting in ethanol pHe conductivity change over time [13,263]; • In ethanol with a high sulfate ion concentration, high conductivity ethanol can be observed [263]; • The reaction of sulfite oxidation to sulfate is the function of ethanol pHe. Ethanol pHe decreased during the reaction [13,262,303]; • Sulfate content increases the electrical conductivity of ethanol [13,263,304]; • It can increase ethanol corrosivity even in a small concentration and accelerate the corrosion of vehicle fuel system parts [276,284,285]; • Sulfates (present as SO_3 and SO_4) form a gum with petrol and result in scale in engine pipes [90]; • Sulfate depositing cause injector clogging in vehicle engine [90,263]; • Ion present in ethanol would impact the corrosion inhibitor's storage stability and effectiveness [263].	• Nitrogen blanketing prevents air and other contaminants which cause oxidative degradation; • Using corrosion inhibitor that contains antioxidants, the oxidation reaction can be minimized; • Use anion exchange resin to adsorb sulfate ions in ethanol; • Determine potential sulfate since potential sulfate can be oxidized into sulfate during the storage period [13].

Table 7. *Cont.*

Stage	Source of Contaminants	Contaminants	Concern	Control Strategies
	Water pickup	Water	• It increases water content over the storage period [90,262,305]; • Water can hydrolyze esters to produce carboxylic acid [271]; • It affects ethanol corrosivity [262,306–308]; • Water can increase ethanol conductivity [90,263,276,309]; • Water reacts with carbon dioxide produces carbonic acid [18,269,310]; • Increasing water content reduces ethanol pHe [311,312]; • Increasing water content would markedly reduce oxygen solubility in ethanol [257].	• Purging with nitrogen can prevent air and moisture in the storage tank.
	The reaction of carbon dioxide and water	Carbonic acid	• When ethanol contacts atmospheric air, it can absorb CO_2 [313]; • Carbonic formation leads to acidity overestimation [18,308,310,313,314]; • Increasing carbon dioxide in ethanol is not responsible for the corrosiveness of anhydrous ethanol on carbon steel [313]; • pHe is a function of carbonic acid content in ethanol [310].	• Purging with nitrogen can control the carbon dioxide level in the storage tank.
	Ester hydrolysis	Carboxylic acid and alcohol	• Ester hydrolysis leads to increasing acidity over storage periods [271]; • Organic acid impurity can cause fuel pump and fuel sender card failure [263].	• Purging with nitrogen prevents air and moisture involved in the ester hydrolysis.
	Ethanol oxidation	Acetaldehyde and acetic acid	• Ethanol has high oxygen solubility [257]; • Ethanol oxidation causes the forming of acetaldehyde, acetic acid, and ethyl acetate [91,256,259,260,315]; • This reaction increases ethanol acidity [215,260]; • It increases ethanol corrosivity [18]; • It can increase the water content [316]; • Organic acid impurity can cause fuel pump and fuel sender card failure [263]; • Acetic acid reduces pHe and increases conductivity of ethanol [276].	• Use nitrogen blanketing to prevent air and other contaminants which cause oxidative degradation; • Using a corrosion inhibitor that contains antioxidants, the oxidation reaction can be minimized; • Anion exchange resin can remove the acetic acid in ethanol [215].
	Esterification between acetic and ethanol	Ethyl acetate	• The corrosive action of alcoholic solutions is considerably affected by acetate, which can result from the manufacturing process, improper handling and storage, and illegal adulteration [272]; • This reaction can change electrical conductivity because hydrogen ions (H^+) and acetate anions (CH_3COO^-) are formed by acetic acid dissociation [317].	• Nitrogen blanketing can be applied to remove both oxygen and water vapor from the storage vessel and prevent oxidation which causes the formation of acetic acid; • The corrosion inhibitor can maintain ethanol pHe by neutralizing strong acids.

9. Conclusions

Among different ethanol production feedstocks, the number and quantities of organic impurities found in lignocellulosic ethanol are higher than sugar- and starch-derived ethanol. Lignocellulosic biomass requires a pretreatment process to increase enzymatic accessibility. A side effect of the pretreatment process is the formation of inhibitors. The main inhibitors of lignocellulosic hydrolysate are furan derivatives, phenolic compounds, and organic acids. These impurities can cause a reduction in yeast cell viability and consequently ethanol yield. The selection of suitable detoxification and fermentation methods, as well as metabolic engineering, can help overcome the detrimental effects of these impurities.

There are byproducts from fermentation which show inhibitory effects on yeast cells, such as organic acids (e.g., formic acid, acetic acid lactic acid), alcohol (e.g., 1-propanol, 2-methyl-1-butanol, glycerol), aldehyde, and ion concentration (Ca^{2+}, Mg^{2+}, K^+, Cl^-, SO_4^{2-}). When defined separately between different feedstocks, volatile byproducts are also found as impurities, such as other alcohols, aldehydes, ketones, fatty acids, and esters, produced in the cases of sugar and starch ethanol fermentation. In contrast, cyclic and heterocyclic compounds are volatile byproducts in lignocellulosic ethanol fermentation. However, these volatile impurities remain contaminated in ethanol after distillation. Another volatile impurity is sulfite, which is a residual effect of: (1) employing sulfuric acid as a pH regulator; (2) the addition of sulfur dioxide during the production process; and (3) undesired products produced during fermentation.

Ethanol characteristics can be changed in the ethanol storage period; this is primarily dependent on the ethanol properties, storage conditions, and the initial contaminants in the ethanol being stored. Based on the oxidation reaction during the storage period, the properties of ethanol products are changed, which can be observed by increased ethanol acidity, water content, and decreased ethanol content and pHe. Due to the hygroscopic properties of ethanol, ester as a contaminant in ethanol can be hydrolyzed into alcohol and carboxylic acid in the presence of water. Additionally, this hygroscopic nature also increases electrical conductivity and reduces ethanol content. When ethanol makes contact with atmospheric air, carbon dioxide can cause carbonic acid formation. This rising carbonic acid concentration could increase acidity. Ethanol also has high oxygen solubility, and therefore oxidation reactions can take place, yielding acetaldehyde and acetic acid. Furthermore, acetic acid can react with ethanol to form ethyl acetate. Moreover, oxygen can oxidize sulfite-containing ethanol into sulfate. Contamination with sulfate has significant impacts on vehicle engines, such as injector plugging and gum formation.

Nitrogen blanketing should be applied to maintain ethanol quality during storage by reducing the amount of water, oxygen, and carbon dioxide intake into the storage tank, because these can promote hydrolysis, oxidation, and carbonic formation. Buffering/corrosion-inhibiting additives can be applied to maintain pHe, as well as conductivity during storage periods. In the case of off-spec ethanol, treatment with anion resin exchange can be applied to remove sulfate and acetic acid.

10. Recommendation and Future Perspectives

Besides the food and fuel debate, the high price of edible feedstocks, e.g., corn, cassava, sugarcane, or beet sugar, also create highly uncertain business environments for ethanol production. Although there are currently few lignocellulosic ethanol plants, which are located in the USA, Brazil, the EU, and China [318], shares of lignocellulosic ethanol are continuously increasing. As mentioned above, lignocellulosic ethanol tends to have problems with inhibitors. There are several techniques available for solving these problems, from traditional methods to the state-of-the-art technology; however, data collection, data analysis and interpretation based on techno-economic and socio-environmental assessments, as well as inhibitor removal efficiency should be individually performed during decision-making processes.

Controlling the amount of impurities in ethanol and understanding their influence in its application are other important topics to investigate further. For example, the impurities in ethanol need stricter control with the increase in ethanol blend rate. Accordingly, some ethanol specifications need to be revised. For example, sulfate limitations may be lowered in the future due to the increasing ethanol concentration in ethanol-blended gasoline, as sulfate causes injector clogging in vehicle engines.

The types of impurities found in lignocellulosic ethanol and first-generation ethanol are different. Scientific confirmation is needed to prove which impurities in lignocellulosic ethanol can cause adverse effects on vehicle engine performance. Such findings could lead to the adoption of new specifications or the revision of existing ones to make them more compatible with second-generation ethanol. For instance, phosphorus limitations should be included in lignocellulosic ethanol because lignocellulosic ethanol contains high phosphorus content which can deactivate automotive catalytic converters. Since the concentration of acetic acid in lignocellulosic ethanol is higher than conventional ethanol, it is challenging for ethanol producers to meet the current required standards. Furan derivates are unique impurities that can be found in lignocellulosic ethanol. Although furan can be used as an alternative fuel, the chemical interactions of furan with other hydrocarbons when it is blended with gasoline should be further investigated [319].

Although the adoption of electric vehicles will lessen demand for ethanol fuel to power internal combustion engines, the widespread use of these is still minimal compared to market-ready ethanol. Moreover, higher ethanol blending requirements are being demanded in several countries worldwide [320], e.g., the USA via the Renewable Fuel Standard (RFS) program, Brazil, Thailand, and India. Therefore, setting the standard of lignocellulosic ethanol is vital to support higher ethanol blending.

Ethanol is also a promising hydrogen carrier which could be considered as a carbon-neutral and sustainable resource for green hydrogen production. Impurities in ethanol have been reported to play an important role in ethanol steam reforming. Some impurities, such as amines, methanol, and aldehydes enhance the hydrogen yield of ESR, while other impurities, such as glycerol, fusel alcohol, and ethyl acetate can cause carbon deposits on the catalyst surface and supress activity. Therefore, these kinds of impurities need to be controlled. On the contrary, acetic acid and sulfur components could increase the acidity of catalyst support and promote dehydration reaction [120].

In addition, ethanol is a versatile building block for biorefineries, and there are many chemicals that could be commercially produced from ethanol such as ethylene, polyethylene, 1,3-butadien, and ethyl acetate. However, the impurities of ethanol, such as sulfur compounds and phosphorous, are elements of concern due to their catalyst deactivation abilities.

In order to control the negative effects of the impurities in ethanol in these applications, the understanding of impurity/inhibitor formation and the control strategies in this research work are crucial.

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References

1. Gallo, J.M.; Bueno, J.; Schuchardt, U. Catalytic transformations of ethanol for biorefineries. *J. Braz. Chem. Soc.* **2014**, *25*, 2229–2243. [CrossRef]
2. Galante-Fox, J.; Von Bacho, P.; Notaro, C.; Zizelman, J. E-85 fuel corrosivity: Effects on port fuel injector durability performance. *SAE Trans.* **2007**, *116*, 989–994.
3. Sriroth, K.; Wanlapatit, S.; Piyachomkwan, K. Cassava bioethanol. In *Bioethanol*; IntechOpen: London, UK, 2012.
4. SEWPAC. *Regulation Impact Statement Fuel Quality Standard: Ethanol (E85) Automotive Fuel*; Department of Sustainability, Environment, Water, Population and Communities: Canberra, Australia, 2012. Available online: https://parlinfo.aph.gov.au/parlInfo/download/publications/tabledpapers/HSTP012629_2010-13/upload_pdf/12629_2010-13.pdf;fileType=application%2Fpdf#search=%22publications/tabledpapers/HSTP012629_2010-13%22 (accessed on 7 January 2021).
5. Cortez, L.A.B. *Sugarcane Bioethanol: R&D for Productivity and Sustainability*; Blucher: São Paulo, Brazil, 2010.
6. TTF. White Paper on Internationally Compatible Biofuel Standards. Tripartite Task Force. 2007. Available online: https://www.nist.gov/document/biofuelsreportpdf (accessed on 15 December 2020).
7. McCormick, R.L.; Alleman, T.; Yanowitz, J. *Sulfate Salts in Gasoline and Ethanol Fuels—Historical Perspective and Analysis of Available Data*; National Renewable Energy Lab. (NREL): Golden, CO, USA, 2017.
8. Stepien, Z.; Krasodomski, W. Investigation into Engine Deposit-Forming Tendency due to Sulfate Salt Contamination of Gasoline–Ethanol Blends. *Energy Fuels* **2019**, *33*, 4244–4253. [CrossRef]
9. *ASTM D5798-10a*; Standard Specification for Fuel Ethanol (Ed70-Ed85) for Automotive Spark-Ignition Engines. ASTM International: West Conshohocken, PA, USA, 2011.
10. Habe, H.; Shinbo, T.; Yamoto, T.; Sato, S.; Shimada, H.; Sakaki, K. Chemical analysis of impurities in diverse bioethanol samples. *J. Jpn. Pet. Inst.* **2013**, *56*, 414–422. [CrossRef]
11. Christensen, E.; Fioroni, G.M.; Kim, S.; Fouts, L.; Gjersing, E.; Paton, R.S.; McCormick, R.L. Experimental and theoretical study of oxidative stability of alkylated furans used as gasoline blend components. *Fuel* **2018**, *212*, 576–585. [CrossRef]
12. Haaz, E.; Fozer, D.; Toth, A.J. Development of Anhydrous Ethanol Purification: Reduction of Acetal Content and Vapor–Liquid Equilibrium Study of the Ethanol–Acetal Binary System. *ACS Omega* **2021**, *6*, 1289–1298. [CrossRef]
13. Naegeli, D.W.; Lacey, P.I.; Alger, M.J.; Endicott, D.L. Surface corrosion in ethanol fuel pumps. *SAE Trans.* **1997**, *106*, 564–571.
14. Hoekman, S.K.; Broch, A. *Investigation into Filter Plugging Due to Sulfate Salt Contamination of Ethanol, Gasoline, and Gasoline-Ethanol Blends*; Coordinating Research Council, Inc.: Alpharetta, GA, USA, 2018.
15. Styarini, D.; Aristiawan, Y.; Aulia, F.; Abimanyu, H.; Sudiyani, Y. Determination of organic impurities in lignocellulosic bioethanol product by GC-FID. *Energy Procedia* **2013**, *32*, 153–159. [CrossRef]
16. Sánchez, R.; Sánchez, C.; Lienemann, C.-P.; Todolí, J.-L. Metal and metalloid determination in biodiesel and bioethanol. *J. Anal. At. Spectrom.* **2015**, *30*, 64–101. [CrossRef]
17. Sánchez, C.; Santos, S.; Sánchez, R.; Lienemann, C.-P.; Todolí, J.-L. Profiling of Organic Compounds in Bioethanol Samples of Different Nature and the Related Fractions. *ACS Omega* **2020**, *5*, 20912–20921. [CrossRef] [PubMed]
18. RFA. *Fuel Ethanol: Industry Guidelines, Specifications, and Procedures*; Renewable Fuels Association: Washington, DC, USA, 2018.
19. *API 1626: 2010*; Storing and Handling Ethanol and Gasoline-Ethanol Blends at Distribution Terminals and Service Stations. American Petroleum Institute: Washington, DC, USA, 1985.
20. Costenoble, O. Worldwide Fuels Standards. In *Overview of Specifications and Regulations on (Bio) Fuels*; NEN–Netherlands Standardization Institute: Delft, The Netherlands, 2017.
21. *TIS 640-1*; Ethanol for Pharmaceutical Use. Thai Industrial Standards Institute (TISI): Bangkok, Thailand, 2010.
22. *TIS 640-2*; Ethanol for Industrial Use. Thai Industrial Standards Institute (TISI): Bangkok, Thailand, 2010.
23. *TIS 2324*; Denatured Ethanol for Gasohol Production. Thai Industrial Standards Institute (TISI): Bangkok, Thailand, 2006.
24. Vohra, M.; Manwar, J.; Manmode, R.; Padgilwar, S.; Patil, S. Bioethanol production: Feedstock and current technologies. *J. Environ. Chem. Eng.* **2014**, *2*, 573–584. [CrossRef]
25. Nguyen, T.L.T.; Gheewala, S.H. Life cycle assessment of fuel ethanol from cane molasses in Thailand. *Int. J. Life Cycle Assess.* **2008**, *13*, 301. [CrossRef]
26. Cardona, C.A.; Sanchez, O.J.; Gutierrez, L.F. *Process Synthesis for Fuel Ethanol Production*; CRC Press: Boca Raton, FL, USA, 2009.
27. Modesto, M.; Nebra, S.A.; Zemp, R.J. Improving the Ethanol Production From Sugar Cane Biomass. In Proceedings of the ASME 8th Biennial Conference on Engineering Systems Design and Analysis, Torino, Italy, 4–7 July 2006; pp. 203–210.
28. Dias, M.O.; Junqueira, T.L.; Cavalett, O.; Cunha, M.P.; Jesus, C.D.; Rossell, C.E.; Maciel Filho, R.; Bonomi, A. Integrated versus stand-alone second generation ethanol production from sugarcane bagasse and trash. *Bioresour. Technol.* **2012**, *103*, 152–161. [CrossRef]
29. Fang, Z. *Biofuels—Economy, Environment and Sustainability*; IntechOpen: London, UK, 2013.
30. de Castro, R.E.N.; de Brito Alves, R.M.; do Nascimento, C.A.O.; Giudici, R. Assessment of Sugarcane-Based Ethanol Production. In *Fuel Ethanol Production from Sugarcane*; IntechOpen: London, UK, 2018.

31. Gilbert, R.A.; Shine, J., Jr.; Miller, J.; Rice, R.; Rainbolt, C. The effect of genotype, environment and time of harvest on sugarcane yields in Florida, USA. *Field Crops Res.* **2006**, *95*, 156–170. [CrossRef]

32. Thai, C.C.; Doherty, W.O. The composition of sugarcane juices dervied from burnt cane and whole green cane crop. In Proceedings of the 33rd Annual Conference of the Australian Society of Sugar Cane Technologists 2011, Mackay, QLD, Australia, 4–6 May 2011; pp. 368–376.

33. Solomon, S. Post-harvest deterioration of sugarcane. *Sugar Tech* **2009**, *11*, 109–123. [CrossRef]

34. Canilha, L.; Chandel, A.K.; Suzane dos Santos Milessi, T.; Antunes, F.A.F.; Luiz da Costa Freitas, W.; das Graças Almeida Felipe, M.; da Silva, S.S. Bioconversion of sugarcane biomass into ethanol: An overview about composition, pretreatment methods, detoxification of hydrolysates, enzymatic saccharification, and ethanol fermentation. *BioMed Res. Int.* **2012**, *2012*, 989572. [CrossRef] [PubMed]

35. de Souza Dias, M.O.; Maciel Filho, R.; Mantelatto, P.E.; Cavalett, O.; Rossell, C.E.V.; Bonomi, A.; Leal, M.R.L.V. Sugarcane processing for ethanol and sugar in Brazil. *Environ. Dev.* **2015**, *15*, 35–51. [CrossRef]

36. Prati, P.; Moretti, R.H. Study of clarification process of sugar cane juice for consumption. *Food Sci. Technol.* **2010**, *30*, 776–783. [CrossRef]

37. Thai, C.C.D.; Moghaddam, L.; Doherty, W.O.S. Calcium Phosphate Flocs and the Clarification of Sugar Cane Juice from Whole of Crop Harvesting. *J. Agric. Food Chem.* **2015**, *63*, 1573–1581. [CrossRef] [PubMed]

38. Andrzejewski, B.; Eggleston, G.; Lingle, S.; Powell, R. Development of a sweet sorghum juice clarification method in the manufacture of industrial feedstocks for value-added fermentation products. *Ind. Crops Prod.* **2013**, *44*, 77–87. [CrossRef]

39. Clarke, M.A. Sugar—Chemical compound. In *Britannica*; Encyclopædia Britannica, Inc.: Chicago, IL, USA, 2021.

40. Laluce, C.; Leite, G.R.; Zavitoski, B.Z.; Zamai, T.T.; Ventura, R. Fermentation of sugarcane juice and molasses for ethanol production. In *Sugarcane-Based Biofuels Bioproducts*; Wiley: New York, NY, USA, 2016; pp. 53–86.

41. Pina, E.A.; Palacios-Bereche, R.; Chavez-Rodriguez, M.F.; Ensinas, A.V.; Modesto, M.; Nebra, S.A. Reduction of process steam demand and water-usage through heat integration in sugar and ethanol production from sugarcane–Evaluation of different plant configurations. *Energy* **2017**, *138*, 1263–1280. [CrossRef]

42. Gunther, F.A. *Residue Reviews Residues of Pesticides and Other Foreign Chemicals in Foods and Feeds*; Springer: New York, NY, USA, 1966; Volume 15.

43. Jacques, K.A.; Lyons, T.P.; Kelsall, D.R. *The Alcohol Textbook: A Reference for the Beverage, Fuel and Industrial Alcohol Industries*; Nottingham University Press: Nottingham, UK, 2003.

44. Pratt, P.L.; Bryce, J.H.; Stewart, G.G. The effects of osmotic pressure and ethanol on yeast viability and morphology. *J. Inst. Brew.* **2003**, *109*, 218–228. [CrossRef]

45. Yokoyama, S.; Matsumura, Y. *The Asian Biomass Handbook*; The Japan Institute of Energy: Tokyo, Japan, 2008.

46. Doran, P.M. Chapter 11—Unit Operations. In *Bioprocess Engineering Principles*, 2nd ed.; Doran, P.M., Ed.; Academic Press: London, UK, 2013; pp. 445–595.

47. Todd, D.K.; Mays, L. *Groundwater Hydrology*; John Willey & Sons Inc.: New York, NY, USA, 1980; Volume 535.

48. Babar, M. *Hydrogeomorphology: Fundamentals, Applications and Techniques*; New India Publishing Agency (NIPA): New Delhi, India, 2005.

49. Schilling, K.E.; Jacobson, P.J.; Libra, R.D.; Gannon, J.M.; Langel, R.J.; Peate, D.W. Estimating groundwater age in the Cambrian–Ordovician aquifer in Iowa: Implications for biofuel production and other water uses. *Environ. Earth Sci.* **2017**, *76*, 2. [CrossRef]

50. SivaRaman, H.; Chandwadkar, A.; Baliga, S.; Prabhune, A. Effect of synthetic zeolite on ethanolic fermentation of sugarcane molasses. *Enzym. Microb. Technol.* **1994**, *16*, 719–722. [CrossRef]

51. Roque-Malherbe, R.; Delgado, R.; Contreras, C.; Lago, A. Behaviour of yeast fermentation in the presence of zeolite. *Biotechnol. Lett.* **1987**, *9*, 640–642. [CrossRef]

52. Casey, E.; Mosier, N.S.; Adamec, J.; Stockdale, Z.; Ho, N.; Sedlak, M. Effect of salts on the Co-fermentation of glucose and xylose by a genetically engineered strain of Saccharomyces cerevisiae. *Biotechnol. Biofuels* **2013**, *6*, 83. [CrossRef] [PubMed]

53. Castellar, M.R.; Aires-Barros, M.R.; Cabral, J.M.; Iborra, J.L. Effect of zeolite addition on ethanol production from glucose by Saccharomyces bayanus. *J. Chem. Technol. Biotechnol. Int. Res. Process Environ. Clean Technol.* **1998**, *73*, 377–384.

54. Carioca, J.O.B.; Leal, M.R.L.V. 3.04-Ethanol Production from Sugar-Based Feedstocks. In *Comprehensive Biotechnology*, 2nd ed.; Moo-Young, M., Ed.; Elsevier: Amsterdam, The Netherlands, 2011; pp. 27–35.

55. Russell, I. Understanding yeast fundamentals. In *The Alcohol Textbook*; Nottingham University Press: Nottingham, UK, 2003; Volume 4, pp. 531–537.

56. Walker, G.M.; Walker, R.S. Enhancing yeast alcoholic fermentations. In *Advances in Applied Microbiology*; Elsevier: Amsterdam, The Netherlands, 2018; Volume 105, pp. 87–129.

57. Hill, A.E.; Stewart, G.G. Free Amino Nitrogen in Brewing. *Fermentation* **2019**, *5*, 22. [CrossRef]

58. Thomas, K.C.; Ingledew, W. Fuel alcohol production: Effects of free amino nitrogen on fermentation of very-high-gravity wheat mashes. *Appl. Environ. Microbiol.* **1990**, *56*, 2046–2050. [CrossRef] [PubMed]

59. Nigam, D.; Asthana, M.; Kumar, A. Penicillium: A Fungus in the Wine and Beer Industries. In *New and Future Developments in Microbial Biotechnology and Bioengineering*; Elsevier: Amsterdam, The Netherlands, 2018; pp. 187–200.

60. Lopes, A.C.A.; Eda, S.H.; Andrade, R.P.; Amorim, J.C.; Duarte, W.F. New Alcoholic Fermented Beverages—Potentials and Challenges. In *Fermented Beverages*; Elsevier: Amsterdam, The Netherlands, 2019; pp. 577–603.

61. Lekkas, C.; Stewart, G.; Hill, A.; Taidi, B.; Hodgson, J. The importance of free amino nitrogen in wort and beer. *Tech. Q.-Master Brew. Assoc. Am.* **2005**, *42*, 113.

62. Marullo, P.; Dubourdieu, D. Yeast selection for wine flavour modulation. In *Managing Wine Quality*; Elsevier: Amsterdam, The Netherlands, 2010; pp. 293–345.

63. Sanchez, O.J.; Cardona, C.A. Trends in biotechnological production of fuel ethanol from different feedstocks. *Bioresour. Technol.* **2008**, *99*, 5270–5295. [CrossRef] [PubMed]

64. Vidal, E.E.; de Billerbeck, G.M.; Simões, D.A.; Schuler, A.; François, J.M.; de Morais, M.A., Jr. Influence of nitrogen supply on the production of higher alcohols/esters and expression of flavour-related genes in cachaça fermentation. *Food Chem.* **2013**, *138*, 701–708. [CrossRef] [PubMed]

65. Sanchez, N.; Ruiz, R.Y.; Infante, N.; Cobo, M. Bioethanol Production from Cachaza as Hydrogen Feedstock: Effect of Ammonium Sulfate during Fermentation. *Energies* **2017**, *10*, 2112. [CrossRef]

66. Yue, G.; Yu, J.; Zhang, X.; Tan, T. The influence of nitrogen sources on ethanol production by yeast from concentrated sweet sorghum juice. *Biomass Bioenergy* **2012**, *39*, 48–52. [CrossRef]

67. Fadel, M.; Keera, A.A.; Mouafi, F.E.; Kahil, T. High level ethanol from sugar cane molasses by a new thermotolerant Saccharomyces cerevisiae strain in industrial scale. *Biotechnol. Res. Int.* **2013**, *2013*, 253286. [CrossRef]

68. Silva-Ortiz, P.; Maciel Filho, R. Comparative performance indexes for ethanol production based on autonomous and annexed sugarcane plants. *Chem. Eng. Trans.* **2018**, *65*, 625–630.

69. Lopes, M.L.; de Lima Paulillo, S.C.; Godoy, A.; Cherubin, R.A.; Lorenzi, M.S.; Giometti, F.H.C.; Bernardino, C.D.; de Amorim Neto, H.B.; de Amorim, H.V. Ethanol production in Brazil: A bridge between science and industry. *Braz. J. Microbiol.* **2016**, *47*, 64–76. [CrossRef]

70. Nikodinovic-Runic, J.; Guzik, M.; Kenny, S.T.; Babu, R.; Werker, A.; Connor, K.E. Carbon-rich wastes as feedstocks for biodegradable polymer (polyhydroxyalkanoate) production using bacteria. In *Advances in Applied Microbiology*; Elsevier: Amsterdam, The Netherlands, 2013; Volume 84, pp. 139–200.

71. Hassan, Z.M.A. Glycerol Extraction from Final Molasses from Aljunied Sugar Factory, Sudan. Ph.D. Thesis, University of Gezira, Wad Madani, Sudan, 2016.

72. Khoja, A.H.; Ali, E.; Zafar, K.; Ansari, A.A.; Nawar, A.; Qayyum, M. Comparative study of bioethanol production from sugarcane molasses by using Zymomonas mobilis and Saccharomyces cerevisiae. *Afr. J. Biotechnol.* **2015**, *14*, 2455–2462.

73. Jackson, D.S.; Shandera, D.L., Jr. Corn wet milling: Separation chemistry and technology. In *Advances in Food and Nutrition Research*; Elsevier: Amsterdam, The Netherlands, 1995; Volume 38, pp. 271–300.

74. Shandera, D.L., Jr.; Jackson, D.S. Effect of corn wet-milling conditions (sulfur dioxide, lactic acid, and steeping temperature) on starch functionality. *Cereal Chem.* **1996**, *73*, 632–637.

75. Mumm, R.; Goldsmith, P.; Rausch, K.; Stein, H. Land usage attributed to corn ethanol production in the United States: Sensitivity to technological advances in corn grain yield, ethanol conversion, and co-product utilization. *Biotechnol. Biofuels* **2014**, *7*, 61. [CrossRef]

76. Joyner, W.M. *Compilation of Air-Pollutant Emission Factors Volume 1 Stationary Point and Area Sources Fourth Edition Supplement D*; Environmental Protection Agency: Research Triangle Park, NC, USA, 1991; p. 137.

77. Zhang, Y. *Sulfur Concentration in Distiller's Dried Grains with Soluble (DDGS) and Its Impact on Palatability and Pig Performance. National Pork Board*; Report no. NPB-08–093; Available online: https://live.porkcheckoff.org/wp-content/uploads/2021/02/08-093-ZHANG-SIU.pdf (accessed on 23 December 2020).

78. Abera, S.; Rakshit, S.K. Processing technology comparison of physicochemical and functional properties of cassava starch extracted from fresh root and dry chips. *Starch-Stärke* **2003**, *55*, 287–296. [CrossRef]

79. Weber, E.J.; Cock, J.H.; Chouinard, A. *Cassava Harvesting and Processing: Proceedings*; IDRC: Ottawa, ON, Canada, 1978.

80. Kuiper, L.; Ekmekci, B.; Hamelinck, C.; Hettinga, W.; Meyer, S.; Koop, K. *Bio-Ethanol from Cassava*; Ecofys Netherlands BV: Utrecht, The Netherlands, 2007; Volume 13, pp. 9–16.

81. Marx, S. Cassava as feedstock for ethanol production: A global perspective. In *Bioethanol Production from Food Crops*; Elsevier: Amsterdam, The Netherlands, 2019; pp. 101–113.

82. Sorapipatana, C.; Yoosin, S. Life cycle cost of ethanol production from cassava in Thailand. *Renew. Sustain. Energy Rev.* **2011**, *15*, 1343–1349. [CrossRef]

83. Ademiluyi, F.; Mepba, H. Yield and properties of ethanol biofuel produced from different whole cassava flours. *ISRN Biotechnol.* **2013**, *2013*, 916481. [CrossRef] [PubMed]

84. Białas, W.; Czerniak, A.; Szymanowska-Powałowska, D. Kinetic modeling of simultaneous saccharification and fermentation of corn starch for ethanol production. *Acta. Biochim. Pol.* **2014**, *61*, 153–162. [CrossRef] [PubMed]

85. Wangpor, J.; Prayoonyong, P.; Sakdaronnarong, C.; Sungpet, A.; Jonglertjunya, W. Bioethanol production from cassava starch by enzymatic hydrolysis, fermentation and ex-situ nanofiltration. *Energy Procedia* **2017**, *138*, 883–888. [CrossRef]

86. Salcedo-Mendoza, J.; Paternina-Urzola, S.; Lujan-Rhenals, D.; Figueroa-Flórez, J. Enzymatic modification of cassava starch (Corpoica M-Tai) around the pasting temperature. *Dyna* **2018**, *85*, 223–230. [CrossRef]

87. Labout, J. Conversion of liquified starch into glucose using a novel glucoamylase system. *Starch-Stärke* **1985**, *37*, 157–161. [CrossRef]

88. Yeunyaw, P.-n.; Yuwa-amornpitak, T. Bioconversion of cassava starch to bio-ethanol in a single step by co-cultures of Amylomyces rouxii and Saccharomyces cerevisiase. *Songklanakarin J. Sci. Technol.* **2018**, *40*, 776–783.

89. Candra, K.P.; Kasma, K.; Ismail, I.; Murdianto, W.; Marwati, M.; Yuliani, Y. Optimization Method for the Bioethanol Production from Giant Cassava (Manihot Esculenta var. Gajah) Originated from East Kalimantan. *Indones. J. Chem.* **2019**, *19*, 176–182. [CrossRef]

90. Kole, C.; Joshi, C.P.; Shonnard, D.R. *Handbook of Bioenergy Crop Plants*; CRC Press: Boca Raton, FL, USA, 2012.

91. Onuki, S.; Koziel, J.A.; Jenks, W.S.; Cai, L.; Grewell, D.; van Leeuwen, J. Taking ethanol quality beyond fuel grade: A review. *J. Inst. Brew.* **2016**, *122*, 588–598. [CrossRef]

92. Saville, B.; Griffin, W.; MacLean, H. Ethanol Production Technologies in the US: Status and Future Developments. In *Global Bioethanol*; Elsevier: Amsterdam, The Netherlands, 2016; pp. 163–180.

93. Gnansounou, E.; Dauriat, A. Ethanol Fuel from Biomass: A Review. *J. Sci. Ind. Res.* **2005**, *64*, 809–821.

94. Tran, T.; Le, P.; Mai, P.; Nguyen, Q. *Bioethanol Production from Lignocellulosic Biomass*; IntechOpen: London, UK, 2019.

95. Triana, C.F. Towards Improved Ethanol Production from Lignocellulosic Biomass. Ph.D. Thesis, UCL (University College London), London, UK, 2016.

96. Jönsson, L.J.; Alriksson, B.; Nilvebrant, N.-O. Bioconversion of lignocellulose: Inhibitors and detoxification. *Biotechnol. Biofuels* **2013**, *6*, 16. [CrossRef] [PubMed]

97. Parsons, S.; McManus, M.C.; Taylor, C.M. Second-Generation Ethanol from Lignocellulose. In *Greenhouse Gas Balances of Bioenergy Systems*; Elsevier: Amsterdam, The Netherlands, 2018; pp. 193–206.

98. Hayes, M.H.; Mylotte, R.; Swift, R.S. Humin: Its composition and importance in soil organic matter. In *Advances in Agronomy*; Elsevier: Amsterdam, The Netherlands, 2017; Volume 143, pp. 47–138.

99. Saldívar, S.O.S.; Soto, F.E.A. Chemical Composition and Biosynthesis of Dietary Fiber Components. In *Science and Technology of Fibers in Food Systems*; Springer: Berlin/Heidelberg, Germany, 2020; pp. 15–43.

100. Jung, H.J.; Oh, K.K.J.A.S. Production of Bio-Based Chemicals, Acetic Acid and Furfural, through Low-Acid Hydrothermal Fractionation of Pine Wood (Pinus densiflora) and Combustion Characteristics of the Residual Solid Fuel. *Appl. Sci.* **2021**, *11*, 7435. [CrossRef]

101. Thakur, S.; Govender, P.P.; Mamo, M.A.; Tamulevicius, S.; Mishra, Y.K.; Thakur, V.K. Progress in lignin hydrogels and nanocomposites for water purification: Future perspectives. *Vacuum* **2017**, *146*, 342–355. [CrossRef]

102. Whetten, R.; Sederoff, R. Lignin biosynthesis. *Plant Cell* **1995**, *7*, 1001. [CrossRef] [PubMed]

103. Baloch, M.; Labidi, J.J.R.A. Lignin biopolymer: The material of choice for advanced lithium-based batteries. *RSC Adv.* **2021**, *11*, 23644–23653. [CrossRef]

104. Jönsson, L.J.; Martín, C. Pretreatment of lignocellulose: Formation of inhibitory by-products and strategies for minimizing their effects. *Bioresour. Technol.* **2016**, *199*, 103–112. [CrossRef]

105. Pattiya, A. Fast pyrolysis. In *Direct Thermochemical Liquefaction for Energy Applications*; Elsevier: Amsterdam, The Netherlands, 2018; pp. 3–28.

106. Zając, G.; Szyszlak-Bargłowicz, J.; Gołębiowski, W.; Szczepanik, M. Chemical characteristics of biomass ashes. *Energies* **2018**, *11*, 2885. [CrossRef]

107. de Jong, E.; Gosselink, R.J.A. Chapter 17—Lignocellulose-Based Chemical Products. In *Bioenergy Research: Advances and Applications*; Gupta, V.K., Tuohy, M.G., Kubicek, C.P., Saddler, J., Xu, F., Eds.; Elsevier: Amsterdam, The Netherlands, 2014; pp. 277–313.

108. Shrotri, A.; Kobayashi, H.; Fukuoka, A. Chapter Two—Catalytic Conversion of Structural Carbohydrates and Lignin to Chemicals. In *Advances in Catalysis*; Song, C., Ed.; Academic Press: Cambridge, MA, USA, 2017; Volume 60, pp. 59–123.

109. Isaacs, S.H. *Ethanol Production by Enzymatic Hydrolysis: Parametric Analysis of a Base-Case Process*; Solar Energy Research Institue: Golden, CO, USA, 1984.

110. Dias, M.O.S.; Cunha, M.P.; Jesus, C.D.F.; Rocha, G.J.M.; Pradella, J.G.C.; Rossell, C.E.V.; Maciel Filho, R.; Bonomi, A. Second generation ethanol in Brazil: Can it compete with electricity production? *Bioresour. Technol.* **2011**, *102*, 8964–8971. [CrossRef]

111. Baldino, C.; Berg, R.; Pavlenko, N.; Searle, S. Advanced alternative fuel pathways: Technology overview and status. *Int. Counc. Clean Transp.* **2019**, *13*, 1–31.

112. Lee, H.; Hamid, S.B.A.; Zain, S. Conversion of lignocellulosic biomass to nanocellulose: Structure and chemical process. *Sci. World J.* **2014**, *2014*, 631013. [CrossRef] [PubMed]

113. Rosales-Calderon, O.; Arantes, V. A review on commercial-scale high-value products that can be produced alongside cellulosic ethanol. *Biotechnol. Biofuels* **2019**, *12*, 240. [CrossRef]

114. Khiyami, M.A.; Pometto, A.L.; Brown, R.C. Detoxification of corn stover and corn starch pyrolysis liquors by Pseudomonas putida and Streptomyces setonii suspended cells and plastic compost support biofilms. *J. Agric. Food Chem.* **2005**, *53*, 2978–2987. [CrossRef] [PubMed]

115. Prosen, E.M.; Radlein, D.; Piskorz, J.; Scott, D.S.; Legge, R.L. Microbial utilization of levoglucosan in wood pyrolysate as a carbon and energy source. *Biotechnol. Bioeng.* **1993**, *42*, 538–541. [CrossRef]

116. Balat, M. Production of bioethanol from lignocellulosic materials via the biochemical pathway: A review. *Energy Convers. Manag.* **2011**, *52*, 858–875. [CrossRef]

117. Wang, K.; Chen, J.; Sun, S.-N.; Sun, R.-C. Steam explosion. In *Pretreatment of Biomass*; Elsevier: Amsterdam, The Netherlands, 2015; pp. 75–104.

118. Pang, S.C.; Voon, L.K.; Chin, S.F. Controlled Depolymerization of Cellulose Fibres Isolated from Lignocellulosic Biomass Wastes. *Int. J. Polym. Sci.* **2018**, *2018*, 6872893. [CrossRef]

119. Walker, D.J.; Gallagher, J.; Winters, A.; Somani, A.; Ravella, S.R.; Bryant, D.N. Process optimization of steam explosion parameters on multiple lignocellulosic biomass using Taguchi method—A critical appraisal. *Front. Energy Res.* **2018**, *6*, 46. [CrossRef]

120. Sanchez, N.; Ruiz, R.; Hacker, V.; Cobo, M. Impact of bioethanol impurities on steam reforming for hydrogen production: A review. *Int. J. Hydrogen Energy* **2020**, *45*, 11923–11942. [CrossRef]

121. Zabed, H.; Sahu, J.; Boyce, A.N.; Faruq, G. Fuel ethanol production from lignocellulosic biomass: An overview on feedstocks and technological approaches. *Renew. Sustain. Energy Rev.* **2016**, *66*, 751–774. [CrossRef]

122. López-Linares, J.C.; Ballesteros, I.; Tourán, J.; Cara, C.; Castro, E.; Ballesteros, M.; Romero, I. Optimization of uncatalyzed steam explosion pretreatment of rapeseed straw for biofuel production. *Bioresour. Technol.* **2015**, *190*, 97–105. [CrossRef] [PubMed]

123. Kucharska, K.; Rybarczyk, P.; Hołowacz, I.; Łukajtis, R.; Glinka, M.; Kamiński, M. Pretreatment of lignocellulosic materials as substrates for fermentation processes. *Molecules* **2018**, *23*, 2937. [CrossRef] [PubMed]

124. Salakkam, A.; Plangklang, P.; Sittijunda, S.; Kongkeitkajorn, M.B.; Lunprom, S.; Reungsang, A. Bio-hydrogen and Methane Production from Lignocellulosic Materials. In *Biomass for Bioenergy-Recent Trends and Future Challenges*; IntechOpen: London, UK, 2019.

125. Mergner, R.; Janssen, R.; Rutz, D.; de Bari, I.; Sissot, F.; Chiaramonti, D.; Giovannini, A.; Pescarolo, S.; Nistri, R. Lignocellulosic Ethanol Process and Demonstration. In *A Handbook Part I*; WIP Renewable Energies: Munich, Germany, 2013.

126. Travaini, R.; Otero, M.D.M.; Coca, M.; Da-Silva, R.; Bolado, S. Sugarcane bagasse ozonolysis pretreatment: Effect on enzymatic digestibility and inhibitory compound formation. *Bioresour. Technol.* **2013**, *133*, 332–339. [CrossRef] [PubMed]

127. Baruah, J.; Nath, B.K.; Sharma, R.; Kumar, S.; Deka, R.C.; Baruah, D.C.; Kalita, E. Recent trends in the pretreatment of lignocellulosic biomass for value-added products. *Front. Energy Res.* **2018**, *6*, 141. [CrossRef]

128. Harmsen, P.; Lips, S.; Bakker, R. *Pretreatment of Lignocellulose for Biotechnological Production of Lactic Acid*; Wageningen UR FBR: Wageningen, The Netherlands, 2013.

129. Refaat, A.A. 5.13—Biofuels from Waste Materials. In *Comprehensive Renewable Energy*; Sayigh, A., Ed.; Elsevier: Oxford, UK, 2012; pp. 217–261.

130. Zhang, J.; Zhou, H.; Liu, D.; Zhao, X. Chapter 2—Pretreatment of lignocellulosic biomass for efficient enzymatic saccharification of cellulose. In *Lignocellulosic Biomass to Liquid Biofuels*; Yousuf, A., Pirozzi, D., Sannino, F., Eds.; Academic Press: Cambridge, MA, USA, 2020; pp. 17–65.

131. Peral, C. Chapter 5—Biomass Pretreatment Strategies (Technologies, Environmental Performance, Economic Considerations, Industrial Implementation). In *Biotransformation of Agricultural Waste and By-Products*; Poltronieri, P., D'Urso, O.F., Eds.; Elsevier: Amsterdam, The Netherlands, 2016; pp. 125–160.

132. Limayem, A.; Ricke, S.C. Lignocellulosic biomass for bioethanol production: Current perspectives, potential issues and future prospects. *Prog. Energy Combust. Sci.* **2012**, *38*, 449–467. [CrossRef]

133. Sebayang, A.; Masjuki, H.; Ong, H.C.; Dharma, S.; Silitonga, A.; Mahlia, T.; Aditiya, H. A perspective on bioethanol production from biomass as alternative fuel for spark ignition engine. *RSC Adv.* **2016**, *6*, 14964–14992. [CrossRef]

134. da Costa Lopes, A.M.; João, K.G.; Rubik, D.F.; Bogel-Łukasik, E.; Duarte, L.C.; Andreaus, J.; Bogel-Łukasik, R. Pre-treatment of lignocellulosic biomass using ionic liquids: Wheat straw fractionation. *Bioresour. Technol.* **2013**, *142*, 198–208. [CrossRef] [PubMed]

135. Sindhu, R.; Binod, P.; Pandey, A. Biological pretreatment of lignocellulosic biomass–An overview. *Bioresour. Technol.* **2016**, *199*, 76–82. [CrossRef]

136. Muñoz, R.; Gonzalez-Fernandez, C. *Microalgae-Based Biofuels and Bioproducts: From Feedstock Cultivation to END-Products*; Woodhead Publishing: Cambridge, UK, 2017.

137. Sjulander, N.; Kikas, T. Origin, Impact and Control of Lignocellulosic Inhibitors in Bioethanol Production—A Review. *Energies* **2020**, *13*, 4751. [CrossRef]

138. Kumar, A.K.; Sharma, S. Recent updates on different methods of pretreatment of lignocellulosic feedstocks: A review. *Bioresour. Bioprocess.* **2017**, *4*, 7. [CrossRef]

139. Sandström, A.G.; Almqvist, H.; Portugal-Nunes, D.; Neves, D.; Lidén, G.; Gorwa-Grauslund, M.F. Saccharomyces cerevisiae: A potential host for carboxylic acid production from lignocellulosic feedstock? *Appl. Microbiol. Biotechnol.* **2014**, *98*, 7299–7318. [CrossRef]

140. Neureiter, M.; Danner, H.; Thomasser, C.; Saidi, B.; Braun, R. Dilute-acid hydrolysis of sugarcane bagasse at varying conditions. *Appl. Biochem. Biotechnol.* **2002**, *98*, 49–58. [CrossRef]

141. Larsson, S.; Palmqvist, E.; Hahn-Hägerdal, B.; Tengborg, C.; Stenberg, K.; Zacchi, G.; Nilvebrant, N.-O. The generation of fermentation inhibitors during dilute acid hydrolysis of softwood. *Enzym. Microb. Technol.* **1999**, *24*, 151–159. [CrossRef]

142. Cantarella, M.; Cantarella, L.; Gallifuoco, A.; Spera, A.; Alfani, F. Effect of inhibitors released during steam-explosion treatment of poplar wood on subsequent enzymatic hydrolysis and SSF. *Biotechnol. Prog.* **2004**, *20*, 200–206. [CrossRef] [PubMed]

143. Liu, Z.; Slininger, P.; Dien, B.; Berhow, M.; Kurtzman, C.; Gorsich, S. Adaptive response of yeasts to furfural and 5-hydroxymethylfurfural and new chemical evidence for HMF conversion to 2, 5-bis-hydroxymethylfuran. *J. Ind. Microbiol. Biotechnol.* **2004**, *31*, 345–352. [CrossRef]

144. Iwaki, A.; Kawai, T.; Yamamoto, Y.; Izawa, S. Biomass conversion inhibitors furfural and 5-hydroxymethylfurfural induce formation of messenger RNP granules and attenuate translation activity in Saccharomyces cerevisiae. *Appl. Environ. Microbiol.* **2013**, *79*, 1661–1667. [CrossRef]

145. Deng, F.; Cheong, D.-Y.; Aita, G.M. Optimization of activated carbon detoxification of dilute ammonia pretreated energy cane bagasse enzymatic hydrolysate by response surface methodology. *Ind. Crops Prod.* **2018**, *115*, 166–173. [CrossRef]

146. Monlau, F.; Sambusiti, C.; Antoniou, N.; Zabaniotou, A.; Solhy, A.; Barakat, A. Pyrochars from bioenergy residue as novel bio-adsorbents for lignocellulosic hydrolysate detoxification. *Bioresour. Technol.* **2015**, *187*, 379–386. [CrossRef]

147. Cannella, D.; Sveding, P.V.; Jørgensen, H. PEI detoxification of pretreated spruce for high solids ethanol fermentation. *Appl. Energy* **2014**, *132*, 394–403. [CrossRef]

148. Nguyen, N.; Fargues, C.; Guiga, W.; Lameloise, M.-L. Assessing nanofiltration and reverse osmosis for the detoxification of lignocellulosic hydrolysates. *J. Membr. Sci.* **2015**, *487*, 40–50. [CrossRef]

149. Horváth, I.S.; Sjöde, A.; Nilvebrant, N.-O.; Zagorodni, A.; Jönsson, L.J. Selection of anion exchangers for detoxification of dilute-acid hydrolysates from spruce. In Proceedings of the Twenty-Fifth Symposium on Biotechnology for Fuels and Chemicals, Breckenridge, CO, USA, 4–7 May 2003; pp. 525–538.

150. Cavka, A.; Jönsson, L.J. Detoxification of lignocellulosic hydrolysates using sodium borohydride. *Bioresour. Technol.* **2013**, *136*, 368–376. [CrossRef]

151. Marton, G.; Dencs, J.; Koukios, E.G. Integrated Technologies in the Biomass Utilization. In *Biomass for Energy and the Environment*; Chartier, P., Ferrero, G.L., Henius, U.M., Hultberg, S., Sachau, J., Wiinblad, M., Eds.; Pergamon: Oxford, UK, 1996; pp. 1782–1787.

152. Wang, L.-Q.; Cai, L.-Y.; Ma, Y.-L. Study on inhibitors from acid pretreatment of corn stalk on ethanol fermentation by alcohol yeast. *RSC Adv.* **2020**, *10*, 38409–38415. [CrossRef]

153. Ximenes, E.; Kim, Y.; Mosier, N.; Dien, B.; Ladisch, M. Deactivation of cellulases by phenols. *Enzym. Microb. Technol.* **2011**, *48*, 54–60. [CrossRef]

154. Martín, C.; Galbe, M.; Wahlbom, C.F.; Hahn-Hägerdal, B.; Jönsson, L.J. Ethanol production from enzymatic hydrolysates of sugarcane bagasse using recombinant xylose-utilising Saccharomyces cerevisiae. *Enzym. Microb. Technol.* **2002**, *31*, 274–282. [CrossRef]

155. Cho, D.H.; Lee, Y.J.; Um, Y.; Sang, B.-I.; Kim, Y.H. Detoxification of model phenolic compounds in lignocellulosic hydrolysates with peroxidase for butanol production from Clostridium beijerinckii. *Appl. Microbiol. Biotechnol.* **2009**, *83*, 1035–1043. [CrossRef] [PubMed]

156. Freitas, J.V.; Nogueira, F.G.; Farinas, C.S. Coconut shell activated carbon as an alternative adsorbent of inhibitors from lignocellulosic biomass pretreatment. *Ind. Crops Prod.* **2019**, *137*, 16–23. [CrossRef]

157. Wang, W.; Yang, S.; Hunsinger, G.B.; Pienkos, P.T.; Johnson, D.K. Connecting lignin-degradation pathway with pre-treatment inhibitor sensitivity of Cupriavidus necator. *Front. Microbiol.* **2014**, *5*, 247. [CrossRef] [PubMed]

158. Zha, Y.; Muilwijk, B.; Coulier, L.; Punt, P.J. Inhibitory compounds in lignocellulosic biomass hydrolysates during hydrolysate fermentation processes. *J. Bioprocess Biotech.* **2012**, *2*, 112–122. [CrossRef]

159. Monisha, T.R.; Ismailsab, M.; Masarbo, R.; Nayak, A.S.; Karegoudar, T.B. Degradation of cinnamic acid by a newly isolated bacterium *Stenotrophomonas* sp. TRMK2. *3 Biotech* **2018**, *8*, 368. [CrossRef]

160. Guo, Z.-P.; Olsson, L. Physiological responses to acid stress by Saccharomyces cerevisiae when applying high initial cell density. *FEMS Yeast Res.* **2016**, *16*, fow072. [CrossRef]

161. Stagge, S.; Cavka, A.; Jönsson, L.J. Identification of benzoquinones in pretreated lignocellulosic feedstocks and inhibitory effects on yeast. *AMB Express* **2015**, *5*, 62. [CrossRef]

162. Yan, Z.; Gao, X.; Gao, Q.; Bao, J. Mechanism of tolerance to the lignin-derived inhibitor p-benzoquinone and metabolic modification of biorefinery fermentation strains. *Appl. Environ. Microbiol.* **2019**, *85*, e01443-19. [CrossRef] [PubMed]

163. Steinbach, D.; Kruse, A.; Sauer, J. Pretreatment technologies of lignocellulosic biomass in water in view of furfural and 5-hydroxymethylfurfural production-a review. *Biomass Convers. Biorefin.* **2017**, *7*, 247–274. [CrossRef]

164. Świątek, K.; Gaag, S.; Klier, A.; Kruse, A.; Sauer, J.; Steinbach, D. Acid Hydrolysis of Lignocellulosic Biomass: Sugars and Furfurals Formation. *Catalysts* **2020**, *10*, 437. [CrossRef]

165. Klinke, H.B.; Ahring, B.K.; Schmidt, A.S.; Thomsen, A.B. Characterization of degradation products from alkaline wet oxidation of wheat straw. *Bioresour. Technol.* **2002**, *82*, 15–26. [CrossRef]

166. Martín, C.; Klinke, H.B.; Marcet, M.; García, L.; Hernandez, E.; Thomsen, A.B. Study of the phenolic compounds formed during pretreatment of sugarcane bagasse by wet oxidation and steam explosion. *Holzforschung* **2007**, *61*, 483–487. [CrossRef]

167. Larsson, S.; Reimann, A.; Nilvebrant, N.-O.; Jönsson, L.J. Comparison of different methods for the detoxification of lignocellulose hydrolyzates of spruce. *Appl. Biochem. Biotechnol.* **1999**, *77*, 91–103. [CrossRef]

168. Mitchell, V.D.; Taylor, C.M.; Bauer, S. Comprehensive analysis of monomeric phenolics in dilute acid plant hydrolysates. *BioEnergy Res.* **2014**, *7*, 654–669. [CrossRef]

169. Pan, G.X.; Bolton, J.L.; Leary, G.J. Determination of Ferulic and p-Coumaric Acids in Wheat Straw and the Amounts Released by Mild Acid and Alkaline Peroxide Treatment. *J. Agric. Food Chem.* **1998**, *46*, 5283–5288. [CrossRef]

170. Akin, D.; Mitchell, R.; Vogel, K. Cell-Wall Composition and Accessibility to Hydrolytic Enzymes is Differentially Altered in Divergently Bred Switchgrass (*Panicum virgatum* L.) Genotypes. *Appl. Biochem. Biotechnol.* **2008**, *150*, 1. [CrossRef]

171. Ludwig, C.H.; Sarkanen, K. *Lignins: Occurrence, Formation, Structure and Reactions*; Wiley-Interscience: New York, NY, USA, 1971.

172. Saa, J.M.; Morey, J.; Rubido, C. Oxidative degradation approach to p-quinones. *J. Org. Chem.* **1986**, *51*, 4471–4473. [CrossRef]

173. Chandel, A.K.; da Silva, S.S.; Singh, O.V. Detoxification of lignocellulosic hydrolysates for improved bioethanol production. *Biofuel Prod.-Recent Dev. Prospect.* **2011**, *10*, 225.

174. Palmqvist, E.; Hahn-Hägerdal, B. Fermentation of lignocellulosic hydrolysates. I: Inhibition and detoxification. *Bioresour. Technol.* **2000**, *74*, 17–24. [CrossRef]

175. Fernandes, D.L.A.; Pereira, S.R.; Serafim, L.S.; Evtuguin, D.V.; Xavier, A.M.R.B. *Second Generation Bioethanol from Lignocellulosics: Processing of Hardwood Sulphite Spent Liquor*; Pinheiro Lima, M.A., Ed.; IntechOpen: London, UK, 2012; pp. 123–152.

176. Pan, L.; He, M.; Wu, B.; Wang, Y.; Hu, G.; Ma, K. Simultaneous concentration and detoxification of lignocellulosic hydrolysates by novel membrane filtration system for bioethanol production. *J. Clean. Prod.* **2019**, *227*, 1185–1194. [CrossRef]

177. Weng, Y.H.; Wei, H.J.; Tsai, T.Y.; Lin, T.H.; Wei, T.Y.; Guo, G.L.; Huang, C.P. Separation of furans and carboxylic acids from sugars in dilute acid rice straw hydrolyzates by nanofiltration. *Bioresour. Technol.* **2010**, *101*, 4889–4894. [CrossRef]

178. Liu, S.; Amidon, T.E.; David Wood, C. Membrane filtration: Concentration and purification of hydrolyzates from biomass. *J. Biobased Mater. Bioenergy* **2008**, *2*, 121–134. [CrossRef]

179. Sagehashi, M.; Nomura, T.; Shishido, H.; Sakoda, A.J.B.t. Separation of phenols and furfural by pervaporation and reverse osmosis membranes from biomass–superheated steam pyrolysis-derived aqueous solution. *Bioresour. Technol.* **2007**, *98*, 2018–2026. [CrossRef]

180. Domínguez, E.; Río, P.G.d.; Romaní, A.; Garrote, G.; Domingues, L. Hemicellulosic Bioethanol Production from Fast-Growing Paulownia Biomass. *Processes* **2021**, *9*, 173. [CrossRef]

181. Fonseca, B.G.; Moutta, R.d.O.; Ferraz, F.d.O.; Vieira, E.R.; Nogueira, A.S.; Baratella, B.F.; Rodrigues, L.C.; Hou-Rui, Z.; da Silva, S.S. Biological detoxification of different hemicellulosic hydrolysates using Issatchenkia occidentalis CCTCC M 206097 yeast. *J. Ind. Microbiol. Biotechnol.* **2011**, *38*, 199–207. [CrossRef]

182. Palmqvist, E.; Hahn-Hägerdal, B.; Szengyel, Z.; Zacchi, G.; Rèczey, K. Simultaneous detoxification and enzyme production of hemicellulose hydrolysates obtained after steam pretreatment. *Enzym. Microb. Technol.* **1997**, *20*, 286–293. [CrossRef]

183. Yang, B.; Dai, Z.; Ding, S.-Y.; Wyman, C.E.J.B. Enzymatic hydrolysis of cellulosic biomass. *Biofuels* **2011**, *2*, 421–449. [CrossRef]

184. Amarasekara, A.S. *Handbook of Cellulosic Ethanol*; John Wiley & Sons: Hoboken, NJ, USA, 2013.

185. Hamelinck, C.N.; Van Hooijdonk, G.; Faaij, A.P. Ethanol from lignocellulosic biomass: Techno-economic performance in short-, middle-and long-term. *Biomass Bioenergy* **2005**, *28*, 384–410. [CrossRef]

186. Wolfaardt, F.J.; Leite Fernandes, L.G.; Cangussu Oliveira, S.K.; Duret, X.; Görgens, J.F.; Lavoie, J.-M. Recovery approaches for sulfuric acid from the concentrated acid hydrolysis of lignocellulosic feedstocks: A mini-review. *Energy Convers. Manag. X* **2021**, *10*, 100074. [CrossRef]

187. Tanjore, D.; Richard, T.L. A systems view of lignocellulose hydrolysis. In *Advances in Bioprocess Technology*; Springer: Berlin/Heidelberg, Germany, 2015; pp. 387–419.

188. Parawira, W.; Tekere, M. Biotechnological strategies to overcome inhibitors in lignocellulose hydrolysates for ethanol production. *Crit. Rev. Biotechnol.* **2011**, *31*, 20–31. [CrossRef] [PubMed]

189. Claassen, P.; Van Lier, J.; Contreras, A.L.; Van Niel, E.; Sijtsma, L.; Stams, A.; de Vries, S.; Weusthuis, R. Utilisation of biomass for the supply of energy carriers. *Appl. Microbiol. Biotechnol.* **1999**, *52*, 741–755. [CrossRef]

190. Sutton, M.; Barr, S. *Food Biotechnology*; ED-Tech Press: Waltham Abbey, UK, 2018.

191. Stanbury, P.F.; Whitaker, A.; Hall, S.J. Chapter 4—Media for industrial fermentations. In *Principles of Fermentation Technology*, 3rd ed.; Stanbury, P.F., Whitaker, A., Hall, S.J., Eds.; Butterworth-Heinemann: Oxford, UK, 2017; pp. 213–272.

192. Novozymes. Yeast Micronutrient and Growth Factor Requirements. 2017. Available online: https://www.novozymes.com/-/media/Project/Novozymes/Website/website/document-library/Advance-your-business/Bioenergy/Yeast-Micronutrient-Requirements-2017.pdf (accessed on 18 February 2021).

193. Chandrasena, G. Role of Metal Ions in Fermentative Metabolism of Yeast. Ph.D. Thesis, University of Abertay, Dundee, Scotland, 1996.

194. Ishola, M.M. Novel Application of Membrane Bioreactors in Lignocellulosic Ethanol Production: Simultaneous Saccharification, Filtration and Fermentation (SSFF). Ph.D. Thesis, University of Borås, Swedish Centre for Resource Recovery, Borås, Sweden, 2014.

195. Chaudhary, A.; Karita, S. Screening of yeast isolates from flowers for effective ethanol production. *Turk. J. Biol.* **2017**, *41*, 890–900. [CrossRef] [PubMed]

196. Bluestein, H.; Greenglass, B.; Holmberg, W. *Ethanol Fuels: Reference Guide*; Solar Energy Research Institue: Golden, CO, USA, 1982.

197. Ingledew, W. Yeasts: Physiology, nutrition and ethanol production. In *Alcohol Text Book*, 5th ed.; Nottingham University Press: Nottingham, UK, 2009; pp. 101–114.

198. Walker, G.; Birch, R.; Chandrasena, G.; Maynard, A. Magnesium, calcium, and fermentative metabolism in industrial yeasts. *J. Am. Soc. Brew. Chem.* **1996**, *54*, 13–18. [CrossRef]

199. Anthony, A.; Nwabueze, U. Simultaneous effect of divalent cation in hydrolyzed cassava starch medium used by immobilized yeast for ethanol production. *Afr. J. Food Sci.* **2009**, *3*, 217–222.

200. Stehlik-Tomas, V.; Gulan Zetić, V.; Stanzer, D.; Grba, S.; Vahčić, N. Zinc, copper and manganese enrichment in yeast Saccharomyces cerevisae. *Food Technol. Biotechnol.* **2004**, *42*, 115–120.

201. de Nicola, R.; Hall, N.; Bollag, T.; Thermogiannis, G.; Walker, G.M. Zinc accumulation and utilization by wine yeasts. *Int. J. Wine Res.* **2009**, *1*, 85–94. [CrossRef]

202. Jones, R.P.; Gadd, G.M. Ionic nutrition of yeast—physiological mechanisms involved and implications for biotechnology. *Enzym. Microb. Technol.* **1990**, *12*, 402–418. [CrossRef]

203. Kispal, G.; Sipos, K.; Lange, H.; Fekete, Z.; Bedekovics, T.; Janáky, T.; Bassler, J.; Aguilar Netz, D.J.; Balk, J.; Rotte, C. Biogenesis of cytosolic ribosomes requires the essential iron–sulphur protein Rli1p and mitochondria. *EMBO J.* **2005**, *24*, 589–598. [CrossRef] [PubMed]

204. Durán, M.A.; Dantur, K.; Ruiz, R.M.; Romero, E.R.; Zossi, S.; Gusils, C. Effect of the clarification pH of sorghum juice on the composition of essential nutrients for fermentation. *FEMS Microbiol. Lett.* **2018**, *365*, fny083. [CrossRef] [PubMed]

205. Raheja, G.; Gill, R.K.; Kumar, S.; Mahmood, S.; Mahmood, A. Effect of sodium on Saccharomyces cerevisiae invertase activity. *Indian J. Exp. Biol.* **1998**, *36*, 493–496. [PubMed]

206. Connolly, C. Bacterial contaminants and their effects on alcohol production. In *The Alcohol Textbook*; Nottingham University Press: Nottingham, UK, 1999; Volume 317, p. 334.

207. Mahboubi, A.; Cayli, B.; Bulkan, G.; Doyen, W.; de Wever, H.; Taherzadeh, M. Removal of Bacterial Contamination from Bioethanol Fermentation System Using Membrane Bioreactor. *Fermentation* **2018**, *4*, 88. [CrossRef]

208. Skinner, K.A.; Leathers, T.D. Bacterial contaminants of fuel ethanol production. *J. Ind. Microbiol. Biotechnol. Bioprocess Eng.* **2004**, *31*, 401–408. [CrossRef]

209. Basso, L.C.; Basso, T.O.; Rocha, S.N. Ethanol production in Brazil: The industrial process and its impact on yeast fermentation. *Biofuel Prod.-Recent Dev. Prospect.* **2011**, *1530*, 85–100.

210. Madigan, M.T.; Martinko, J.M.; Parker, J. *Brock Biology of Microorganisms*; Prentice Hall: Upper Saddle River, NJ, USA, 1997; Volume 11.

211. Hofvendahl, K.; Hahn–Hägerdal, B. Factors affecting the fermentative lactic acid production from renewable resources1. *Enzym. Microb. Technol.* **2000**, *26*, 87–107. [CrossRef]

212. Beckner, M.; Ivey, M.L.; Phister, T.G. Microbial contamination of fuel ethanol fermentations. *Lett. Appl. Microbiol.* **2011**, *53*, 387–394. [CrossRef]

213. Gans, J.; Wolinsky, M.; Dunbar, J. Computational improvements reveal great bacterial diversity and high metal toxicity in soil. *Science* **2005**, *309*, 1387–1390. [CrossRef]

214. Bischoff, K.M.; Liu, S.; Leathers, T.D.; Worthington, R.E.; Rich, J.O. Modeling bacterial contamination of fuel ethanol fermentation. *Biotechnol. Bioeng.* **2009**, *103*, 117–122. [CrossRef]

215. Lv, H.; Sun, Y.; Zhang, M.; Geng, Z.; Ren, M. Removal of Acetic Acid from Fuel Ethanol Using Ion-Exchange Resin. *Energy Fuels* **2012**, *26*, 7299–7307. [CrossRef]

216. Oliva-Neto, P.d.; Dorta, C.; Carvalho, A.F.A.; Lima, V.d.; Silva, D.d. The Brazilian technology of fuel ethanol fermentation—yeast inhibition factors and new perspectives to improve the technology. *Mater. Processes Energy Commun. Curr. Res. Technol. Dev.* **2013**, *1*, 371–379.

217. Maiorella, B.; Blanch, H.W.; Wilke, C.R. By-product inhibition effects on ethanolic fermentation by Saccharomyces cerevisiae. *Biotechnol. Bioeng.* **1983**, *25*, 103–121. [CrossRef] [PubMed]

218. Campbell, I. Chapter 4—Yeast and fermentation. In *Whisky*; Russell, I., Bamforth, C.W., Stewart, G.G., Eds.; Academic Press: San Diego, CA, USA, 2003; pp. 115–150.

219. Sanchez, N.; Cobo, M.; Rodriguez-Fontalvo, D.; Uribe-Laverde, M.Á.; Ruiz-Pardo, R.Y. Bioethanol Production from Sugarcane Press-Mud: Assessment of the Fermentation Conditions to Reduce Fusel Alcohol. *Fermentation* **2021**, *7*, 194. [CrossRef]

220. Sanchez, N.; Ruiz, R.Y.; Cifuentes, B.; Cobo, M. Controlling sugarcane press-mud fermentation to increase bioethanol steam reforming for hydrogen production. *Waste Manag.* **2019**, *98*, 1–13. [CrossRef]

221. Gomes, M.T.; Rocha, T.A.; Duarte, A.C.; Oliveira, J.P. Determination of sulfur dioxide in wine using a quartz crystal microbalance. *Anal. Chem.* **1996**, *68*, 1561–1564. [CrossRef]

222. Ilet, D. Aspects of the analysis, role, and fate of sulphur dioxide in beer: A review. *Tech. Q.* **1995**, *32*, 213–221.

223. Cartwright, C.P.; Rose, A.H.; Calderbank, J.; Keenan, M.H. Solute transport. *Yeasts* **1989**, *3*, 5–56.

224. da Silva-Neto, J.M.; Covre, E.A.; Rosa, B.C.; Ceccato-Antonini, S.R. Can ethanol partially or fully replace sulfuric acid in the acid wash step of bioethanol production to fight contamination by Lactobacillus fermentum? *Braz. J. Chem. Eng.* **2020**, *37*, 323–332. [CrossRef]

225. Theodore, S.; Sai, P. Esterification of ethanol with sulfuric acid: A kinetic study. *Can. J. Chem. Eng.* **2001**, *79*, 54–64. [CrossRef]

226. Bennett, G. *The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals*, 11th ed.; Budavari, S., Ed.; Merck and Co. Inc.: Rahway, NJ, USA, 1989; 2274p, ISBN 911910-28-X.

227. Pimentel, J.; Richardson, K. Eliminating the Need of Acidification in Bioethanol Production. U.S. Patent Application 14/077,113, 15 May 2014.

228. Nielsen, J.C.; de Oliveira Lino, F.S.; Rasmussen, T.G.; Thykær, J.; Workman, C.T.; Basso, T.O. Industrial antifoam agents impair ethanol fermentation and induce stress responses in yeast cells. *Appl. Microbiol. Biotechnol. Bioeng.* **2017**, *101*, 8237–8248. [CrossRef] [PubMed]

229. Blasco, L.; Viñas, M.; Villa, T.G. Proteins influencing foam formation in wine and beer: The role of yeast. *Int. Microbiol.* **2011**, *14*, 61–71. [PubMed]

230. Keith Westwood, Y.-Y.F. Katarzyna WOLINSKA, Agnieszka RACJA-FERREIRA. Antifoam Agents. Worldwide Applications WO2013021177A1, 14 February 2013.

231. Routledge, S. The Effect of Antifoams upon Recombinant Protein Production in Yeast. Ph.D. Thesis, Aston University, Birmingham, UK, 2012.
232. Grosz, R.; Stephanopoulos, G. Physiological, biochemical, and mathematical studies of micro-aerobic continuous ethanol fermentation by Saccharomyces cerevisiae. I: Hysteresis, oscillations, and maximum specific ethanol productivities in chemostat culture. *Biotechnol. Bioeng.* **1990**, *36*, 1006–1019. [CrossRef] [PubMed]
233. Duerschner, C.A. Biofiltration of Volatile Organic Compounds Emitted at Ethanol Plants. Ph.D. Thesis, University of Nebraska, Lincoln, NE, USA, 2019.
234. Sonnek, D.W.; Loest, G.W. Multi-Stage Scrubbing System and Method for Ethanol Vent Streams. U.S. Patent 8,512,448, 20 August 2013.
235. Woldemariam, D.; Kullab, A.; Khan, E.U.; Martin, A. Recovery of ethanol from scrubber-water by district heat-driven membrane distillation: Industrial-scale technoeconomic study. *Renew. Energy* **2018**, *128*, 484–494. [CrossRef]
236. Gryta, M.; Morawski, A.W.; Tomaszewska, M. Ethanol production in membrane distillation bioreactor. *Catal. Today* **2000**, *56*, 159–165. [CrossRef]
237. Hussain, M.A.M.; Pfromm, P.H. Reducing the Energy Demand of Cellulosic Ethanol through Salt Extractive Distillation Enabled by Electrodialysis. *Sep. Sci. Technol.* **2013**, *48*, 1518–1528. [CrossRef]
238. Singh, R.S.; Pandey, A.; Gnansounou, E. *Biofuels: Production and Future Perspectives*; CRC Press: Boca Raton, FL, USA, 2016.
239. Bartocci, A. New Ethanol Scrubber Reduces Plant Capital & Operating Cost. In Proceedings of the 2009 International Fuel Ethanol Workshop and Expo, Denver, CO, USA, 15–18 June 2009.
240. Brady, D.; Pratt, G.C. Volatile organic compound emissions from dry mill fuel ethanol production. *J. Air Waste Manag. Assoc.* **2007**, *57*, 1091–1102. [CrossRef]
241. Ishida, S.; Oshima, N.; Kurita, K.; Suzuki, I.; Ohno, H. Process for Treating Aldehydes. U.S. Patent US429765A, 30 September 1975.
242. Onuki, S.; Koziel, J.A.; van Leeuwen, J.H.; Jenks, W.S.; Grewell, D.; Cai, L. Ethanol production, purification, and analysis techniques: A review. In Proceedings of the 2008 Providence, Providence, RI, USA, 29 June–2 July 2008; p. 1.
243. Friedl, A. Bioethanol from Sugar and Starch. In *Energy from Organic Materials (Biomass): A Volume in the Encyclopedia of Sustainability Science and Technology*, 2nd ed.; Kaltschmitt, M., Ed.; Springer: New York, NY, USA, 2019; pp. 905–924.
244. Stenberg, K.; Tengborg, C.; Galbe, M.; Zacchi, G.; Palmqvist, E.; Hahn-Hägerdal, B. Recycling of process streams in ethanol production from softwoods based on enzymatic hydrolysis. *Appl. Biochem. Biotechnol.* **1998**, *70*, 697. [CrossRef]
245. Stout, B.A. *Handbook of Energy for World Agriculture*; Elsevier: Amsterdam, The Netherlands, 2012.
246. Nguyen, T.L.T.; Gheewala, S.H. Fuel ethanol from cane molasses in Thailand: Environmental and cost performance. *Energy Policy* **2008**, *36*, 1589–1599. [CrossRef]
247. Mangmeechai, A.; Pavasant, P. Water footprints of Cassava-and Molasses-based ethanol production in Thailand. *Nat. Resour. Res.* **2013**, *22*, 273–282. [CrossRef]
248. Schill, S.R. Sulfur Compounded in Ethanol Regulations. *Ethanol Prod. Mag.* **2016**. Available online: http://www.ethanolproducer.com/articles/13513/sulfur-compounded-in-ethanol-regulations (accessed on 12 October 2020).
249. Zhang, Q.; Du, J.; Jin, Y.; Zhao, Z.; Li, Y. SO_2 reduction in distilled grape spirits by three methods. *J. Inst. Brew.* **2013**, *119*, 314–320. [CrossRef]
250. Edwards, T.J.; Newman, J.; Prausnitz, J.M. Thermodynamics of aqueous solutions containing volatile weak electrolytes. *AIChE J.* **1975**, *21*, 248–259. [CrossRef]
251. Huang, H.-J.; Ramaswamy, S.; Tschirner, U.W.; Ramarao, B. Separation and Purification processes for lignocellulose-to-bioalcohol production. In *Bioalcohol Production*; Elsevier: Amsterdam, The Netherlands, 2010; pp. 246–277.
252. Ramos, W.B.; Figueiredo, M.F.; Brito, R.P. Optimization of extractive distillation process with a single column for anhydrous ethanol production. In *Computer Aided Chemical Engineering*; Elsevier: Amsterdam, The Netherlands, 2014; Volume 33, pp. 1411–1416.
253. Ortuño-Boter, D.; Plesu, V.; Ruiz, A.E.B.; Ruiz, J.B.; Iancu, P.; Llorens, J. Enhanced Distillation Based on Feed Impurities. In *Computer Aided Chemical Engineering*; Elsevier: Amsterdam, The Netherlands, 2016; Volume 38, pp. 1923–1928.
254. Morigami, Y.; Kondo, M.; Abe, J.; Kita, H.; Okamoto, K. The first large-scale pervaporation plant using tubular-type module with zeolite NaA membrane. *Sep. Purif. Technol.* **2001**, *25*, 251–260. [CrossRef]
255. Innospec. *DCI-11 Corrosion Inhibitor for Gasoline-Alcohol Fuels*; Innospec: Leuna, Germany, 2007.
256. Jeuland, N.; Montagne, X.; Gautrot, X. Potentiality of ethanol as a fuel for dedicated engine. *Oil Gas Sci. Technol.* **2004**, *59*, 559–570. [CrossRef]
257. Moyo, F. Effects of Water on the Stress Corrosion Cracking of Carbon Steel in Ethanolic Media. Ph.D. Thesis, University of the Witwatersrand, Johannesburg, South Africa, 2013.
258. Lehninger, A. *Translation of the Second Edition of: Biochemistry, the Molecular Basis of Cell Structure and Function*; Worth Publishers Inc.: New York, NY, USA, 1972.
259. Batista, F.R.; Meirelles, A.J. A strategy for controlling acetaldehyde content in an industrial plant of bioethanol. *IFAC Proc. Vol.* **2009**, *42*, 928–933. [CrossRef]
260. Caetano, L.G.; Takeuchi, R.M.; Santos, A.L.; de Oliveira, M.F.; Stradiotto, N.R. Voltammetric determination of ethyl acetate in ethanol fuel using a Fe^{3+}/Nafion®-coated glassy carbon electrode. *Fuel* **2013**, *106*, 837–842. [CrossRef]
261. Singh, R. Ethanol corrosion in pipelines. *Mater. Perform.* **2009**, *48*, 53–55.

262. Kane, R.; Eden, D.; Maldonado, J.G.; Srinivasan, S.; Brongers, M.P.; Agarwal, A.K.; Beavers, J. *Stress Corrosion Cracking of Carbon Steel in Fuel Grade Ethanol: Review, Experience, Survey, Field Monitoring, and Laboratory Testing: Downstream Segment*; American Petroleum Institute: Washington, DC, USA, 2007.

263. Cummings, J. *Effects of Fuel Ethanol Quality on Vehicle System Components*; 0148-7191; SAE Technical Paper; SAE International: Warrendale, PA, USA, 2011.

264. Biernat, K. *Storage Stability of Fuels*; IntechOpen: London, UK, 2015.

265. Nakajima, K.; Yahagi, Y. Corrosive Wear of Steel With Gasoline-Ethanol-Water. *J. Lubr. Technol.* **1983**, *105*, 552–557. [CrossRef]

266. Saska, M.; Zossi, B.S.; Liu, H.-l. Removal of colour in sugar cane juice clarification by defecation, sulfitation and carbonation. *Int. Sugar J.* **2010**, *112*, 258.

267. Ilett, D.R.; Simpson, W.J. Loss of sulphur dioxide during storage of bottled. *Food Res. Int.* **1995**, *28*, 393–396. [CrossRef]

268. Dalmolin, I.; Skovroinski, E.; Biasi, A.; Corazza, M.; Dariva, C.; Oliveira, J.V. Solubility of carbon dioxide in binary and ternary mixtures with ethanol and water. *Fluid Phase Equilib.* **2006**, *245*, 193–200. [CrossRef]

269. Bureman, P. Good Ethanol Storage Practices. *Biofuels Int.* **2012**. Available online: https://www.slideshare.net/pebureman/ethanol-storage-best-practices (accessed on 22 September 2020).

270. Schreier, P.; Drawert, F.; Junker, A. Identification of volatile constituents from grapes. *J. Agric. Food Chem.* **1976**, *24*, 331–336. [CrossRef]

271. Ramey, D.D.; Ough, C.S. Volatile ester hydrolysis or formation during storage of model solutions and wines. *J. Agric. Food Chem.* **1980**, *28*, 928–934. [CrossRef]

272. Monteiro, M.; Ambrozin, A.; Santos, A.; Contri, P.; Kuri, S. Evaluation of metallic corrosion caused by alcohol fuel and some contaminants. In *Materials Science Forum*; Trans Tech Publications Ltd.: Freienbach, Switzerland, 2010; pp. 1024–1029.

273. Mathur, I. Differences between Corn and Sugar Cane Derived Ethanol for Fuels. Available online: https://www.haltermannsolutions.com/differences-corn-sugar-cane-derived-ethanol-fuels (accessed on 15 September 2019).

274. Weaver, J.W.; Skaggs, S.A.; Spidle, D.L.; Stone, G.C. *Composition and Behavior of Fuel Ethanol, Volume EPA/600/R-09/037*; Athens, G.A., Ed.; US Environmental Protection Agency: Washington, DC, USA, 2009.

275. Donati, G.L.; Amais, R.S.; Schiavo, D.; Nóbrega, J.A. Determination of Cr, Ni, Pb and V in gasoline and ethanol fuel by microwave plasma optical emission spectrometry. *J. Anal. At. Spectrom.* **2013**, *28*, 755–759. [CrossRef]

276. Luecke, J.; McCormick, R.L. Electrical conductivity and pHe response of fuel ethanol contaminants. *Energy Fuels* **2014**, *28*, 5222–5228. [CrossRef]

277. Lamon, J. Sulfites Production and Management by Wine Yeast During Alcoholic Fermentation. Available online: https://www.winemak-in.com/en/publications/sulfites-production-and-management-by-wine-yeast-during-alcoholic-fermentation (accessed on 7 February 2021).

278. Bernardi, R.; JORGE, L.M.d.M.; Paraiso, P.R. Kinetic modeling of sugarcane juice clarification by ozonation in batch reactor with ozone saturation control. *Food Sci. Technol.* **2019**, *39*, 81–84. [CrossRef]

279. Vijayanand, A.; Soumyadip, G. Method of Desulphitation of Carbohydrates. Worldwide Applications WO2016090213A1, 9 June 2016.

280. Binder, T.P.; Beggin, J.R.; Hilaly, A.K.; Karcher, L.P.; Solheim, L.P.; Soper, J.G.; Zenthoefer, B.L. Method for Removing Sulfur Compounds from an Alcohol Stream. U.S. Patent Application 11/784,052, 11 October 2007.

281. Udeh, H.; Kgatla, T.; Jideani, A. Effect of mineral ion addition on yeast performance during very high gravity wort fermentation. *Int. J. Biol. Biomol. Agric. Food Biotechnol. Eng.* **2014**, *8*, 1208–1216.

282. Piotrowski, J.S.; Zhang, Y.; Sato, T.; Ong, I.; Keating, D.; Bates, D.; Landick, R. Death by a thousand cuts: The challenges and diverse landscape of lignocellulosic hydrolysate inhibitors. *Front. Microbiol.* **2014**, *5*, 90. [CrossRef]

283. Elena, P.; Gabriela, R.; Traian, H. Current approaches to efficient biotechnological production of ethanol. *Innov. Rom. Food Biotechnol.* **2009**, *4*, 1–11.

284. WWFC. *Ethanol Guidelines*; World Wide Fuel Charter Committee 2009; Available online: http://oica.net/wp-content/uploads/ethanol-guideline-final-26mar09.pdf (accessed on 11 December 2021).

285. IFQC. *Setting a Quality Standard for Fuel Ethanol*; International Fuel Quality Center: Houston, TX, USA, 2004.

286. Slunecka, T. Protecting Distillers Grains from Sulfur Build-Up. Available online: http://www.ethanolproducer.com/articles/5660/protecting-distillers-grains-from-sulfur-build-up (accessed on 18 February 2021).

287. Slaughter, J. The effects of carbon dioxide on yeasts. In *Biotechnology Applications in Beverage Production*; Springer: Dordrecht, Germany, 1989; pp. 49–64.

288. Jones, R.P.; Greenfield, P.F. Effect of carbon dioxide on yeast growth and fermentation. *Enzym. Microb. Technol.* **1982**, *4*, 210–223. [CrossRef]

289. Marcus, Y.; Glikberg, S. Recommended methods for the purification of solvents and tests for impurities: Methanol and ethanol. *Pure Appl. Chem.* **1985**, *57*, 855–864. [CrossRef]

290. Byrne, S.; Howell, G. *Acetaldehyde: How to Limit Its Formation during Fermentation*; Aust, N.Z., Ed.; Grapegrower Winemaker: Sydney, NSW, Australia, 2017; pp. 68–69.

291. Jackowetz, J.N.; Dierschke, S.; Mira de Orduña, R. Multifactorial analysis of acetaldehyde kinetics during alcoholic fermentation by Saccharomyces cerevisiae. *Food Res. Int.* **2011**, *44*, 310–316. [CrossRef]

292. Qian, X.; Malmali, M.; Wickramasinghe, S. Membranes for the removal of fermentation inhibitors from biofuel production. In *Membrane Technologies for Biorefining*; Elsevier: Amsterdam, The Netherlands, 2016; pp. 219–240.

293. Dzialo, M.C.; Park, R.; Steensels, J.; Lievens, B.; Verstrepen, K.J. Physiology, ecology and industrial applications of aroma formation in yeast. *FEMS Microbiol. Rev.* **2017**, *41*, S95–S128. [CrossRef]

294. Ibraheem, O.; Ndimba, B.K. Molecular adaptation mechanisms employed by ethanologenic bacteria in response to lignocellulose-derived inhibitory compounds. *Int. J. Biol. Sci.* **2013**, *9*, 598. [CrossRef]

295. Larsson, S. Ethanol from Lignocellulose-Fermentation Inhibitors, Detoxification and Genetic Engineering of Saccharomyces Cerevisiae for Enhanced Resistance. Ph.D. Thesis, Lund University, Lund, Sweden, 2000.

296. Whiting, G. Organic acid metabolism of yeasts during fermentation of alcoholic beverages—A review. *J. Inst. Brew.* **1976**, *82*, 84–92. [CrossRef]

297. Bideaux, C.; Alfenore, S.; Cameleyre, X.; Molina-Jouve, C.; Uribelarrea, J.-L.; Guillouet, S.E. Minimization of glycerol production during the high-performance fed-batch ethanolic fermentation process in Saccharomyces cerevisiae, using a metabolic model as a prediction tool. *Appl. Environ. Microbiol.* **2006**, *72*, 2134–2140. [CrossRef]

298. Navarrete, C.; Nielsen, J.; Siewers, V. Enhanced ethanol production and reduced glycerol formation in fps1Δ mutants of Saccharomyces cerevisiae engineered for improved redox balancing. *AMB Express* **2014**, *4*, 86. [CrossRef]

299. Zhang, A.; Chen, X. Improve Ethanol Yield Through Minimizing Glycerol Yield in Ethanol Fermentation of Saccharomyces cerevisiae. *Chin. J. Chem. Eng.* **2008**, *16*, 620–625. [CrossRef]

300. Ohimain, E.I. Methanol contamination in traditionally fermented alcoholic beverages: The microbial dimension. *Springerplus* **2016**, *5*, 1607. [CrossRef] [PubMed]

301. Blumenthal, P.; Steger, M.C.; Einfalt, D.; Rieke-Zapp, J.; Quintanilla Bellucci, A.; Sommerfeld, K.; Schwarz, S.; Lachenmeier, D.W. Methanol Mitigation during Manufacturing of Fruit Spirits with Special Consideration of Novel Coffee Cherry Spirits. *Molecules* **2021**, *26*, 2585. [CrossRef] [PubMed]

302. Yang, X.; Wang, K.; Wang, H.; Zhang, J.; Tang, L.; Mao, Z. Control of pH by acetic acid and its effect on ethanol fermentation in an integrated ethanol–methane fermentation process. *RSC Adv.* **2016**, *6*, 57902–57909. [CrossRef]

303. Wilkinson, P.M.; Doldersum, B.; Cramers, P.H.M.R.; Van Dierendonck, L.L. The kinetics of uncatalyzed sodium sulfite oxidation. *Chem. Eng. Sci.* **1993**, *48*, 933–941. [CrossRef]

304. Chapman, E.; Cummings, J.; Conran, D. Effects of gasoline and ethanol fuel corrosion inhibitors on powertrain intake valve deposits. *SAE Int. J. Fuels Lubr.* **2013**, *6*, 63–79. [CrossRef]

305. Sridhar, N.; Price, K.; Buckingham, J.; Dante, J. Stress corrosion cracking of carbon steel in ethanol. *Corrosion* **2006**, *62*, 687–702. [CrossRef]

306. Pedraza-Basulto, G.; Arizmendi-Morquecho, A.; Miramontes, J.C.; Borunda-Terrazas, A.; Martinez-Villafane, A.; Chacón-Nava, J. Effect of water on the stress corrosion cracking behavior of API 5L-X52 steel in E95 blend. *Int. J. Electrochem. Sci.* **2013**, *8*, 5421–5437.

307. Hauffe, K.; Bender, R. Unalloyed steels and cast steel. In *Corrosion Handbook*; Wiley: New York, NY, USA, 2008.

308. Moreira, A.R.; Panossian, Z.; Pimenta, G.S.; de Oliveira, V. Effect of Total Acidity and Water Content on Ethanol Corrosiveness (E100). In Proceedings of the CORROSION 2015, Dallas, TX, USA, 15–19 March 2015.

309. Lou, X.; Singh, P.M. Role of water, acetic acid and chloride on corrosion and pitting behaviour of carbon steel in fuel-grade ethanol. *Corros. Sci.* **2010**, *52*, 2303–2315. [CrossRef]

310. Golisz, S.R.; Yang, J.S.; Johnson, R.D. Understanding the effect of CO2 on the pHe of fuel ethanol. *Fuel* **2017**, *199*, 1–3. [CrossRef]

311. Lou, X.; Yang, D.; Singh, P.M. Effect of ethanol chemistry on stress corrosion cracking of carbon steel in fuel-grade ethanol. *Corrosion* **2009**, *65*, 785–797. [CrossRef]

312. Sowards, J.W.; Weeks, T.S.; McColskey, J.D. The influence of simulated fuel-grade ethanol on fatigue crack propagation in pipeline and storage-tank steels. *Corros. Sci.* **2013**, *75*, 415–425. [CrossRef]

313. Moreira, A.R.; Panossian, Z.; Pimenta, G.S.; de Oliveira, V.; Brugnelli, R.A. Effect of Inert Gases (Pure Nitrogen, Nitrogen And Carbon Dioxide Mixtures) On the Corrosiveness of Anhydrous Ethanol (E100). In Proceedings of the CORROSION 2011, Houston, TX, USA, 28 March 2011.

314. Belincanta, J.; Alchorne, J.; Teixeira da Silva, M. The brazilian experience with ethanol fuel: Aspects of production, use, quality and distribution logistics. *Braz. J. Chem. Eng.* **2016**, *33*, 1091–1102. [CrossRef]

315. Saharin, S.B. Vaporization and Autoignition Characteristics of Ethanol and 1-Propanol Droplets: Influence of Water. Ph.D. Thesis, Université de Bourgogne, Dijon, France, 2013.

316. Matějovský, L.s.; Macák, J.; Pospíšil, M.; Staš, M.; Baroš, P.; Krausová, A. Study of Corrosion Effects of Oxidized Ethanol–Gasoline Blends on Metallic Materials. *Energy Fuels* **2018**, *32*, 5145–5156. [CrossRef]

317. Somboon, T.; Phatchana, R.; Tongpoothorn, W.; Sansuk, S. A simple and green method for determination of ethanol in liquors by the conductivity measurement of the uncatalyzed esterification reaction. *LWT* **2022**, *154*, 112593. [CrossRef]

318. Hoang, T.-D.; Nghiem, N. Recent Developments and Current Status of Commercial Production of Fuel Ethanol. *Fermentation* **2021**, *7*, 314. [CrossRef]

319. Eldeeb, M.A.; Akih-Kumgeh, B. Recent Trends in the Production, Combustion and Modeling of Furan-Based Fuels. *Energies* **2018**, *11*, 512. [CrossRef]
320. Sarkar, B.; Mridha, B.; Pareek, S. A sustainable smart multi-type biofuel manufacturing with the optimum energy utilization under flexible production. *J. Clean. Prod.* **2022**, *332*, 129869. [CrossRef]