Chapter 1

Comparative of Lignocellulosic Ethanol Production by *Kluyveromyces marxianus* and *Saccharomyces cerevisiae*

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Abstract

The world faces a progressive depletion of its energy resources, mainly fossil fuels based on non-renewable resources. At the same time, the consumption of energy grows at high rates, and the intensive use of fossil fuels has led to an increase in the generation of gaseous pollutants released into the atmosphere, which has caused changes in the global climate. The lignocellulosic bioethanol is considered as a promising alternative for use as fuel ethanol. However, one of the main problems in producing ethanol is toxic compounds generated during hydrolysis of lignocellulosic wastes; these compounds cause a longer lag phase and irreversible cell damage to the microorganisms used in the fermentation step. These conditions of fermentation affect the productivity and the economic feasibility of the lignocellulosic ethanol production process. In this context, many efforts had been carried out to improve the capacity of volumetric ethanol productivity of the yeast. The yeast *Saccharomyces cerevisiae* is commonly employed in industrial ethanol production. However non-*Saccharomyces* yeast as *Kluyveromyces marxianus* can produce alcohols at similar or higher levels than *S. cerevisiae* and on inhibitory conditions.

Keywords: *Kluyveromyces marxianus*, *Saccharomyces cerevisiae*, ethanol, productivity, physiology

1. Introduction

Petroleum-based fuels have been widely used by the human being in daily life and industry for hundreds of years; this has generated a depletion of fossil fuel reserves. In addition, the burning of fossil fuels has caused global climate changes (such as global warming). These concerns have led to the search and development of alternative fuels that are environmentally friendly, renewable, and sustainable. Biofuels such as bioethanol are considered an excellent alternative to mitigate these problems because the production of bioethanol uses renewable resources (such as lignocellulosic biomass) as a raw material; this reduces the dependence on fossil resources and produces cleaner combustion that contributes in a positive way to the environment. Lignocellulosic biomass, in the form of agro-industrial waste, has enormous potential as source energy, precisely as a precursor to bioethanol. The problem of agro-industrial waste in the ethanol production, the potential advantages for the environment, the main stages of production of bioethanol, and a comparison of different microorganisms (yeasts) used for their possible production at the industrial level will be discussed in this chapter. In addition, other bioalcohols that are generated during the production of bioethanol that can be used as high value-added products will be shown.

2. Impact of agro-industrial waste in the ethanol production

The industry has become an essential part of human life; however, development activities produce much waste throughout the world. Large-scale production of agriculture has led to the release of huge quantities of residues. Industries in the agricultural sector generate a large number of residues (in the form of solids, liquids, and gases) throughout the year. Those residues can be used as animal feed, burned (causing an increase in air pollution), or deposited in landfills. The generation of these residues can result in several environmental problems and may cause contamination of air (generating CO_2), contamination of surface water (groundwater seepage). Their elimination is a problem for the producing industries. Therefore, the recovery and re-utilization (recycling) of agro-industrial waste is of interest to industries. This large volume of residues, together with their slow degradation capacity, has stimulated research activities focused on the determination of the possible uses of this waste with a dual purpose. On the one hand, the elimination of an environmental problem (when the waste is discharged) causes the deterioration of the environment since it contains potential toxic compounds, and on the other, the search to produce other value-added products.

Agro-industrial wastes are generated from crop residues or animal feed and include materials such as cereals (corn, rice, wheat, sorghum), bagasses (cane, agave), wheat straw, husk, leaves, seed, stem, and others. Millions of tons of agricultural waste are generated all over the world. **Table 1** shows the generation of agro-industrial wastes according to the region across the world. Rice straw/husk, wheat straw/husk, and sugarcane bagasse are the biggest agro-industrial wastes produced. Brazil, China, India, and the United States account for 60% of the crop residues produced [3]. The agricultural production of maize dominates in the

Residue	Africa	America	Asia	Europe	Oceania	Subtotal	Reference
Crops/grains							
Maize	63.58	445.34	245.75	85.10	0.53	840.3	[1]
Oats	0.20	5.08	0.98	11.95	19.62	37.83	[1]
Rice	34.49	55.49	948.30	6.49	0.31	1045.08	[1]
Rye	0.10	3.77	2.54	34.50	0.81	41.72	[1]
Sorghum	31.66	33.76	14.69	1.06	2.40	83.57	[1]
Sugar cane	22.42	241.15	156.15	0.00	8.39	428.11	[1]
Wheat	33.18	169.19	439.04	305.75	33.89	981.05	[1]
Wheat straw	5.34	62.64	145.20	132.59	8.57	354.34	[1]
Lignocellulosic biomass							
Bagasse	11.73	87.62	74.88	0.01	6.49	180.73	[2]
Corn straw	0.00	140.86	33.90	28.61	0.24	203.61	[2]
Oat straw	0.00	3.04	0.27	6.83	0.47	10.61	[2]
Rice straw	20.9	37.2	667.6	3.9	1.7	731.3	[2]
Sorghum straw	0.00	0.00	9.67	0.35	0.32	10.34	[2]

Table 1. Quantities of potential agro-industrial waste (million tons) available for bioethanol production.

United States and also in China (in less quantity); rice production is mainly found in China and India; cereal production such as wheat is dominant in Europe (outside), China, and India (as they are the major producers of wheat and rice in the world); sugar cane production takes place in Brazil (as major producer) and India. The large portion of agricultural residues generated every year showed that maize has a residue potential of more than 900 million tons of waste; wheat and rice have a residue potential of more than 600 and 400 million tons of waste, respectively. Sugar cane and soybean potentials are in a range of 450 and 350 million tons of waste, respectively [3]. Other studies have also reported that the global production of rice straw is 600–900 million tons per year [4].

The agro-industrial waste contains part of lignocellulosic material; this material is composed by cellulose, hemicellulose, and lignin along with smaller amounts of pectin, proteins, and ashes [5]. Cellulose is the main structural component of the cell wall of plants, and it is in an organized fibrous structure (crystalline), constituting 30–50% of the cell wall. This linear polymer is composed of D-glucose subunits linked together by β 1–4 glycosidic bonds forming cellobiose molecules. These long chains (called elementary fibrils) are linked together by hydrogen bonds and Van der Waals forces that cause cellulose to pack into microfibrils. Hemicellulose and lignin cover the microfibrils forming a matrix. Hemicellulose is a heteropolysaccharide that covers the surface of cellulose fibers and contributes 10–40% of the biomass of the plant, has an irregular structure, and is chemically bound to the lignin in the cell wall. The main characteristic that differentiates hemicellulose from cellulose is that

hemicellulose has branches with short side chains that consist of different sugars. These monosaccharides include pentoses (xylose and arabinose), hexoses (glucose, mannose, and galactose), and uronic acids (4-O-methyl-glucuronic, D-glucuronic acid, and D-galacturonic acid) and are linked by β 1–4 glucosidic bonds and occasionally by β 1–3 bonds [6]. Lignin provides rigidity to the cell wall of plants and contributes 15–30% of the biomass of the plant. It is an aromatic polymer of three-dimensional structure quite complex, very branched, and amorphous. Three phenyl propionic alcohols exist as monomers of lignin: coniferyl alcohol, p-coumaryl alcohol, and sinapyl alcohol. Alkyl-aryl, alkyl-alkyl, and aryl-aryl ether linkages hold these phenolic monomers together.

The composition of these components can vary from one plant species to another. For example, wood has a higher amount of cellulose, while straw and wheat leaves increase the content of hemicellulose [7]. Besides, the relationships between the various components within a single plant vary with age, growth stage, and other culture conditions [6]. Practically, all biomass residues produced in agricultural and industrial activities, and even urban waste, have high concentrations of available lignocellulosic materials. Lignocellulosic materials represent the most abundant renewable resources on earth and therefore have been a great deal of interest in utilizing as energy resource. In the last decades, the bioconversion of lignocellulosic materials in products of commercial interest (biofuels, bioalcohols) has been searched. Agro-industrial wastes, which are byproducts of key industrial and economical activities, are attractive raw materials for the production of renewable fuels, like bioethanol. The great advantage of using these materials is that they are natural, biodegradable, and can often be extracted from waste or as byproducts of the agricultural or food industry. The advantage associated with these products is double, since the cost of the raw material becomes cheap and, also, an added value is given to the industrial waste or byproduct. The use of lignocellulosic biomass as a raw material for the production of biofuels (such as bioethanol) has been associated with a concept of biorefinery, which is key to the production of ethanol at an industrial level. However, the bioconversion of lignocellulosic wastes into useful products is an enormous environmental challenge.

3. Lignocellulosic ethanol

Currently, the world faces a progressive depletion of its energy resources, mainly fossil fuels based on non-renewable resources. At the same time, the consumption of energy grows at high rates and the intensive use of fossil fuels has increased the generation of gaseous pollutants released into the atmosphere, which has caused changes in the global climate. Through the use of renewable energy resources, it is possible to find part of the solution to the energy requirements in a friendly way for the environment. The global potential of bioenergy is represented in energy crops and lignocellulosic waste [1, 8]. The conversion of these raw materials into biofuels is an option for the exploitation of alternative sources of energy and the reduction of polluting gases [9]. In addition, the use of biofuels has important economic and social effects.

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Bioenergy companies focus on first-generation biofuels and involve the use of grains and raw materials directly related to human consumption (grains, sugarcane, etc.). The use of these raw materials implies that the processes to produce first-generation fuels are not sustainable and at the same time are considered not ethic (it comes from feedstocks directly related to human or animal feed). On the other hand, second-generation biofuels are those that use non-food raw materials such as waste from agro-industries (straw, bagasse, husks, effluents). The second-generation biofuels market is based on the basic principle that the majority will be produced from agro-industrial waste, which implies the creation of sustainable developments and clean and friendly companies with the environment. Governments envision the second-generation biofuels market as an innovative way to reduce costs in the disposal of waste materials and improve the production of clean, renewable, and sustainable energies, which in the long term partially replace the use of fossil fuels such as petroleum.

Nowadays, bioethanol is the most attractive and important renewable fuel in terms of capacity and market value [10, 11]. Globally, bioethanol production has increased considerably in recent years and has gained importance in many parts of the world. The largest producer of bioethanol is located in America, and Asia stands second while Europe follows the list (**Figure 1**). Several reports have indicated the world production capacity of bioethanol; in 2012 and 2013, it was approximately 234 billion liters per year [12]. More than 128.5 billion liters of bioethanol were produced worldwide during 2014 [13], while for 2016, it increased to almost 144 billion liters [14]. Brazil and the United States are the main producers of first-generation bioethanol (which represent around 60% of world production) (**Figure 1**) [2]. In the United States, ethanol production increased from 175 million gallons to 15,800 million gallons in almost 20 years [12]. For 2017, the annual production of bioethanol was 27,050 million liters (the United States, Brazil, Europe, and China being the main contributors).

The production of second-generation bioethanol is shown in **Table 2**. The production of bioethanol from agro-industrial waste has taken much interest about the first generation, due,

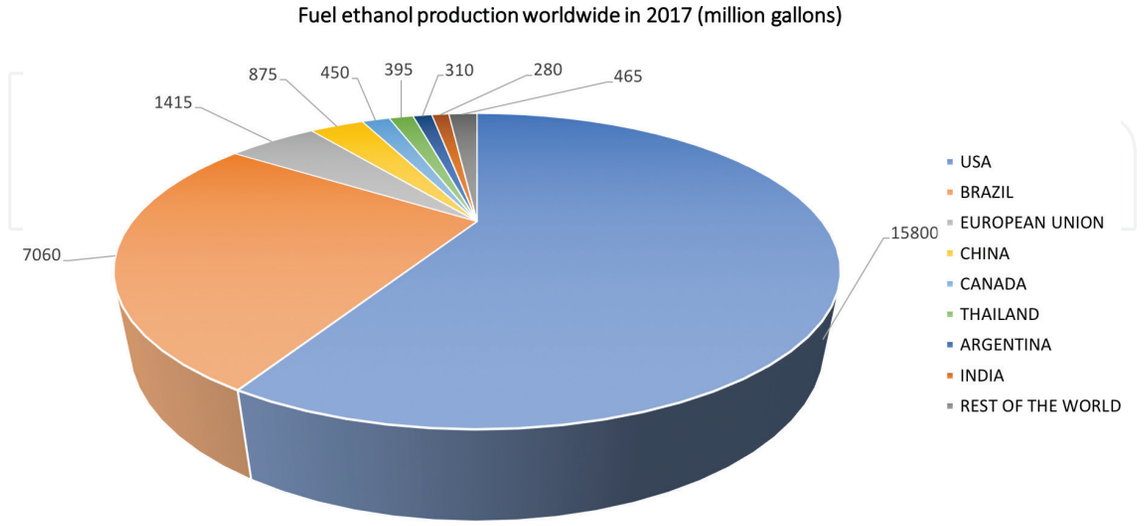

Figure 1. Worldwide fuel ethanol production in 2017.

Residue	Africa	America	Asia	Europe	Oceania	Subtotal
Waste crop						
Barley	0.12	0.045	0.83	1.35	0.13	2.47
Corn	2.17	4.29	6.82	1.09	0.01	14.38
Oat	0.02	0.044	0.04	0.30	0.001	0.405
Rice	0.71	1.61	14.4	0.02	0.02	16.76
Sorghum	1.55	0.21	0.37	0.003	0.0004	2.13
Sugar cane	0.23	0.55	0.82	—	0.0001	1.60
Wheat	0.55	0.78	6.78	2.70	0.54	11.35
Subtotal (1)	5.35	7.529	30.06	5.463	0.7015	49.103
Lignocellulosic biomass						
Bagasse	3.33	24.87	21.3	0.0004	1.84	51.34
Barley straw	—	3.2	0.61	13.7	0.60	18.11
Corn stover	—	40.47	9.75	8.23	0.07	58.52
Oat straw	—	0.799	0.07	1.79	0.12	2.77
Rice straw	5.86	10.41	186.8	1.10	0.47	204.64
Sorghum straw	—	2.61	—	0.10	.09	2.8
Wheat straw	1.57	18.39	42.6	38.9	2.51	103.97
Subtotal (2)						
Total (1 + 2)	16.11	108.278	291.19	69.2834	6.4015	491.2535

Table 2. Potential bioethanol production (gigaliters) from agro-industrial wastes by continent [1, 2].

the main feedstock for the production are residues, in that way avoiding the use of extensions of cultivable land to bioethanol production. In addition, as mentioned earlier, a product of added value is obtained from residues which also help to avoid a problem of waste accumulation. The selection of the agro-industrial feedstock is in function of the agricultural production and the interests of each country for transferring value to the produced wastes. Kim and Dale [2] indicated that area about 73.9 Tera grams (Tg) of dry wasted crops in the world could potentially produce 49.1 gigaliters (GL) per year of bioethanol. Also mentioned was that the lignocellulosic biomass could produce up to 442 GL per year of bioethanol and the total potential bioethanol production from crop residues and wasted crops is 491 GL per year (about 16 times higher than the world ethanol production in 2003).

Bioethanol presents some important differences about conventional fuels derived from petroleum. The main one is the high oxygen content, which constitutes about 35% by mass of ethanol. The characteristics of bioethanol allow a cleaner combustion (it emits low levels of non-combusted hydrocarbons, such as carbon monoxide (CO), oxides of nitrogen (NOx), and other reactive organic gases that pollute the air) [12, 15]. Also, it improves the performance of

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the engines, which contributes to reduced pollutant emissions (with low emission of CO_2 to the atmosphere), even when mixed with gasoline [9, 16]. In this way, bioethanol (and biofuels) helps to reduce greenhouse gas (GHG) emissions and consequently mitigates the climate change. Also, the use of agro-industrial waste for the production of bioethanol helps to reduce their accumulation which is of great environmental concern. Farrell et al. [17] estimated (using the displacement method) a reduction of the 88% greenhouse gas emissions using lignocellulosic ethanol (from switchgrass). Schmer et al. [18] reported that ethanol from switchgrass reduces GHG emissions by 94% compared to GHG emissions from gasoline. Various studies have reported a reduction of 63–118% in life-cycle GHG emissions using ethanol from lignocellulosic feedstock in comparison to fossil fuel [9, 19–24].

The main steps that are involved in the production of bioethanol from lignocellulosic wastes are 1) pre-treatment, 2) hydrolysis and 3) fermentation (**Figure 2**). An adequate process of pre-treatment and hydrolysis can increase the concentrations of fermentable sugars, which potentially would help to obtain greater quantities of bioethanol.

3.1. Pre-treatment

The aim of the pre-treatment is to disintegrate the lignocellulosic complex to make it more accessible for the hydrolysis stage; in this way, it helps decrease the crystallinity of the cellulose, increase the surface area of the biomass, remove the hemicellulose, and break the seal of the lignin. This process increases the porosity of the pretreated material and makes cellulose more accessible to enzymes so that the conversion of carbohydrates into fermentable sugars is achieved quickly and with higher yields. The pre-treatment methods can be divided into different categories: physical (grinding), chemical (alkaline, dilute acids, oxidizing agents, organic solvents), biological, or a combination of these.

3.2. Hydrolysis

After pre-treatment, cellulose and hemicellulose are released and converted to monomers which are known as saccharification (containing mainly glucose and pentose); the reaction can be catalyzed by diluted acids, concentrated acids, or enzymes (cellulases and hemicellulases). Acid hydrolysis (both concentrated and diluted) occurs in two stages, to take advantage of the differences between hemicellulose and cellulose. The first involves, essentially, the hydrolysis of the hemicellulose, conducted in accordance with the pre-treatment conditions discussed earlier. In the second stage, higher temperatures are applied, seeking to optimize the hydrolysis of the cellulose fraction [25]. The process with concentrated acid uses high temperatures and pressures, with reaction times of seconds to a few minutes, which facilitates the use of continuous processes. On the other hand, the processes with diluted acid develop under less severe conditions, but with typically long reaction times [26]. In the enzymatic process, hydrolysis is catalyzed by enzymes generically cellulases and requires at least three key enzymes, endoglucanases, exoglucanases, and β-glucosidases. Enzymatic hydrolysis is a prolonged process because the enzymes are hampered by the structural parameters of the substrate (hemicellulose/lignin) and the surface area. However, enzymatic hydrolysis has certain advantages over acid hydrolysis: the first is that by not using chemicals, it is an ecological

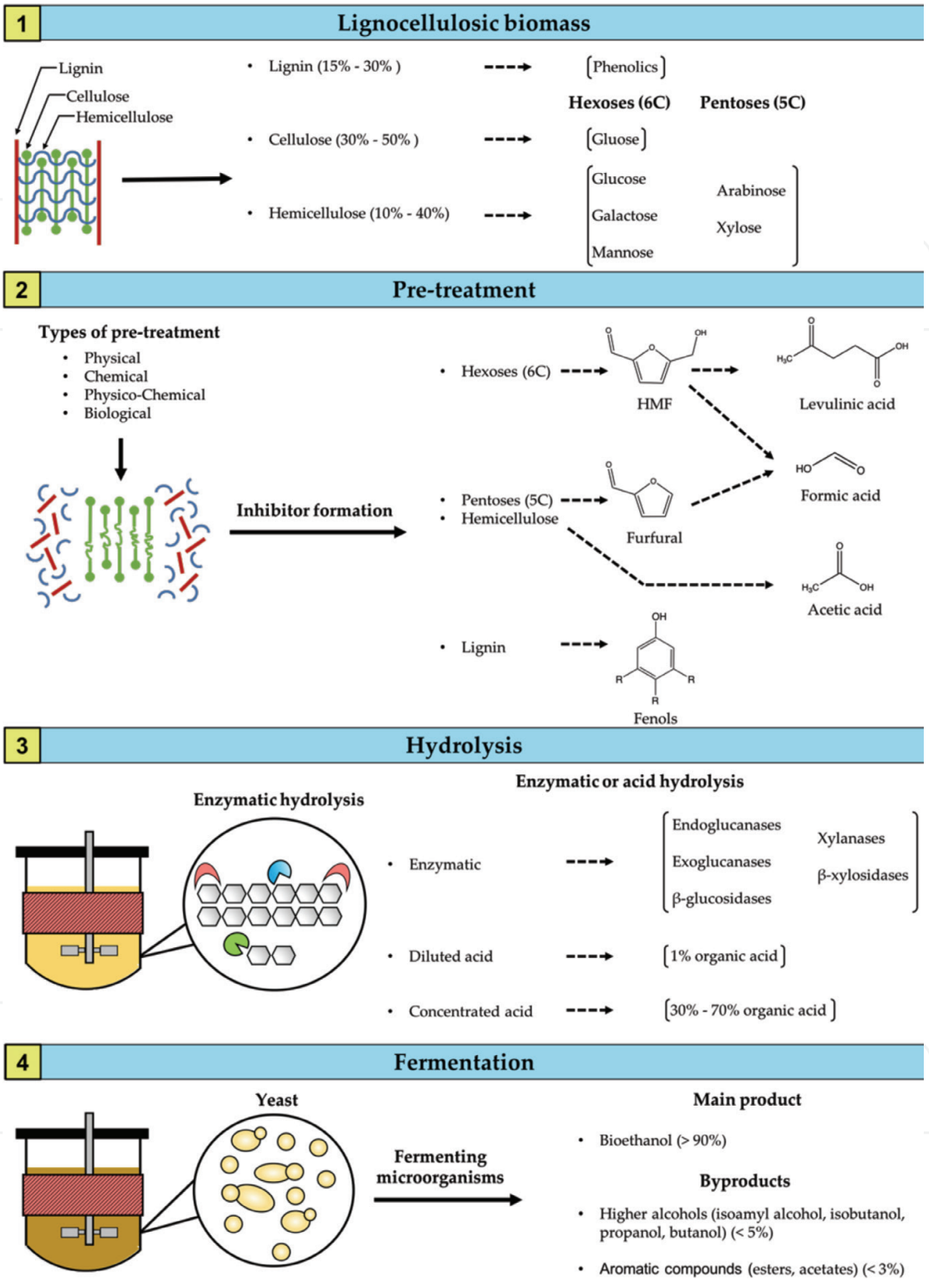

Figure 2. Schematic diagram of bioethanol production from lignocellulosic biomass. 1: Composition of lignocellulosic biomass; 2: Effects of pre-treatment on lignocellulosic biomass and main/representative inhibitory compounds generated during the process. Pre-treatment is required to separate the cellulose, hemicellulose and lignin fractions; 3: Hydrolysis of pre-treated lignocellulosic material. Hydrolysis helps to convert polymeric carbohydrates (cellulose and hemicellulose) into fermentable sugars; 4: Fermentation process. The fermentation process by microorganisms converts the soluble sugars released during hydrolysis into ethanol and byproducts.

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alternative, a second is that they are carried out under moderate environmental conditions of temperature and pH, which reduces their cost in comparison with acid hydrolysis; they also avoid corrosion problems, and additionally, toxic byproducts (inhibitors) are not formed.

3.3. Fermentation

After hydrolysis, the product of the sugars is fermented to ethanol by microorganisms. The microorganisms can be either bacteria or yeast that can use one or more of the sugars present in the lignocellulosic material pretreated and hydrolyzed.

The typical configuration used for the fermentation of lignocellulosic hydrolysates involves a sequential process in which hydrolysis (cellulose/hemicellulose) and fermentation are carried out in different units. This configuration is known as separate hydrolysis and fermentation (SHF). SHF has the advantage that each step can be carried out under optimal conditions of hydrolysis (45–50°C) and fermentation (30–35°C). SHF has the disadvantage that enzymes (cellulases/β-glucosidases) are inhibited by the glucose released during hydrolysis; therefore, to obtain optimum yields, it requires a lower charge of solids and the addition of a higher load of enzymes. In addition, due to the fact that the process is carried out in different steps, it has the disadvantage that increases its cost, which compromises its economic viability. An alternative to this process is the known simultaneous saccharification and fermentation (SSF), where hydrolysis and fermentation are carried out in a single unit. SSF considerably reduces cellulase inhibition problems (which improves yields and production bioethanol). Also, during the SSF method, the risk of contamination can be reduced (due to the ethanol generated), and the use of a single unit reduces the costs of the process. However, it has the disadvantage that each stage cannot be optimized (limiting and restricting the process). A variant of SSF is simultaneous saccharification and co-fermentation (SSCF) and non-isothermal simultaneous saccharification and fermentation (NSSF). SSCF consists of the enzymatic hydrolysis and co-fermentation of pentoses and hexoses in a single unit; the co-fermenting microorganisms need to be compatible regarding operating pH and temperature. In NSSF, hydrolysis and fermentation occur simultaneously in two different units at their optimum temperature; the hydrolysis reaction liquid passes to the fermenter. An alternative is consolidated bioprocessing (CBP), also known as direct microbial conversion (DMC). In this method, all the biological transformations involved in the production of bioethanol (enzyme production (cellulase), biomass hydrolysis (saccharification), fermentation of hexoses, and fermentation of pentoses) occur in a single unit with one or more microorganisms. This method is attractive because it reduces the number of units, the cost of the products, and simplifies its operation.

Currently, the tendency of these methods focuses on carrying out the processes simultaneously in a smaller number of steps. The microorganisms are fundamental pieces in the production of lignocellulosic bioethanol. Microorganisms capable of fermenting both sugars with high yield are required. One of the main problems in the production of bioethanol from lignocellulosic hydrolysates is that not all microorganisms can assimilate and ferment pentoses to ethanol; the microorganisms conventionally used in the fermentation of bioethanol present this inconvenient. As mentioned earlier, hemicellulose can represent a large part of the lignocellulosic material, which can lead to a large number of available pentoses for fermentation.

For this reason, the development and search for microorganisms capable of efficiently fermenting pentose to ethanol have led to a great research effort. Genetic engineering has been used to add pentose metabolic pathways in yeast and other microorganisms to effectively utilize the sugars present in lignocellulosic hydrolysates. In addition, several types of research have sought to improve the performance of microorganisms that already can ferment both sugars. Although success has been achieved in this regard, the fermentation of mixtures of the sugars of the lignocellulosic biomass has not yet reached a commercially viable level.

4. Fermentative behavior of *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* in the bioethanol production

S. cerevisiae is the main microorganism employed in ethanol production due to its high ethanol productivity, high ethanol tolerance, and the ability to ferment a wide range of sugars. However, there are some adverse effects in yeast fermentation which inhibit ethanol production such as high temperature, inhibitory compounds from the hydrolysis of lignocellulosic residues (furans, aldehydes, and organic acids), high concentrations of carbon and ethanol source, and the ability to ferment pentose sugars. Different types of yeast strains have been used in the fermentation process for ethanol production (**Table 3**) including hybrid, recombinant, and wild-type yeasts. The production of bioethanol depends on several factors, such as the concentration of sugars, pH, temperature, the concentration of inhibitor compounds, fermentation time, cell growth rate, agitation, and inoculum size [40].

K. marxianus, a non-conventional thermotolerant yeast, is potentially useful for the production of ethanol and other products because they can assimilate diverse sugars including xylose, arabinose, sucrose, raffinose, and inulin in addition to several hexoses [41]. In addition, *K. marxianus* has a faster growth and ethanol production at higher temperatures [42]. Flores et al. [43] reported several strains of *K. marxianus* capable of producing ethanol from *Agave tequilana* fructans (ATF) at high temperatures, which evidence the high potential of *K. marxianus* for the simultaneous saccharification and fermentation for bioethanol production. Other investigations have also reported that *K. marxianus* shows a better behavior to produce ethanol under inhibitory conditions (furans) compared to a commercial strain of *S. cerevisiae* due to the best response and resistance to inhibitors (**Table 4**) [27].

The presence of inhibitors is another problem faced by yeast during the fermentation of lignocellulosic hydrolysates. The presence of inhibitory compounds originated in pre-treatment, and hydrolysis processes of lignocellulosic biomass can strongly inhibit the fermentation stage and generate irreversible cellular damage (which negatively affect yeast metabolism). Furan aldehydes such as 2-furaldehyde (furfural) and 5-hydroxymethylfurfural (HMF) are products of degradation of pentoses and hexoses released from hemicellulose and cellulose, respectively. Acetic, formic, and levulinic acids are the most common acids present in lignocellulosic hydrolysates. Acetic acid is formed by deacetylation of hemicelluloses, while formic

Yeast strain	Type of strain	Waste/medium	Sugar concentration (g L^{-1})	Ethanol concentration (g L^{-1})	Qp	Reference
S. cerevisiae ERD	Commercial	Mineral medium	40	18.2	1.51	[27]
S. cerevisiae	Laboratory	Sugarcane bagasse	30	13.1	0.18	[28]
S. cerevisiae AR5	Laboratory	Sugarcane bagasse	18.5	2.2	0.09	[29]
S. cerevisiae ERD	Laboratory	Sugarcane bagasse	18.5	4.8	0.17	[29]
S. cerevisiae AR5	Laboratory	Wheat straw	14.5	2.4	0.21	[29]
S. cerevisiae ERD	Laboratory	Wheat straw	14.5	2.2	0.20	[29]
S. cerevisiae RL-11	Laboratory	Spend coffee grounds	195.0	11.7	0.49	[30]
S. cerevisiae MTCC 173	Laboratory	Sorghum stover	200.0	68.0	0.94	[31]
S. cerevisiae KL17	Wild-type	Galactose and glucose	500.0	96.9	3.46	[32]
S. cerevisiae CHY1011	Wild-type	Cassava starch	195.0	89.9	1.35	[33]
S. cerevisiae RPRT90	Mutated	Ipomea carnea	72.1	29.0	1.03	[34]
K. marxianus K213	Laboratory	Water hyacinth	23.3	7.34	0.31	[35]
K. marxianus CECT10875	Laboratory	Wheat straw	—	36.2	0.50	[36]
K. marxianus SLP1	Laboratory	Sugarcane bagasse	18.5	9.0	0.29	[29]
K. marxianus OFF1	Laboratory	Sugarcane bagasse	18.5	5.1	0.20	[29]
K. marxianus SLP1	Laboratory	Wheat straw	14.5	3.1	0.25	[29]
K. marxianus OFF1	Laboratory	Wheat straw	14.5	2.3	0.20	[29]
K. marxianus UMIP2234.94	Wild type	Glucose	20.0	5.7	0.23	[37]
K. marxianus 10,875	Laboratory	Wheat straw	—	11.1	0.20	[38]
K. marxianus DBKKUY-103	Laboratory	Sorghum juice	194	85.16	1.42	[39]

Qp, volumetric ethanol productivity (g ethanol produced L^{-1} h^{-1}).

Table 3. Bioethanol production from different carbon sources.

and levulinic acids are products of HMF breakdown/degradation. In addition, formic acid can be formed from furfural under acidic conditions at elevated temperatures. A wide range of phenolic compounds is generated due to the decomposition of lignin and also by the degradation of carbohydrates during acid hydrolysis. Furans are considered the most representative

Stress condition	Lag phase	μ	Rs	Yx/s	qs	Qs	Yp/s	qp	Qp
	ERD								
Control	0	0.14	3.83	0.059	2.47	1.55	0.49	1.21	0.30
HMF (7 g L^{-1})	0	0.07	1.74	0.043	1.67	1.62	0.45	0.75	0.29
Furfural (3 g L^{-1})	6	0.08	1.87	0.047	1.76	1.65	0.46	0.82	0.30
HMF (3.5 g L^{-1}) + furfural (1.5 g L^{-1})	6	0.12	1.52	0.045	2.78	1.65	0.46	1.29	0.30
HMF (7 g L^{-1}) + furfural (3 g L^{-1})	—	—	—	—	—	—	—	—	—
	SLP1								
Control	0	0.16	3.20	0.053	3.03	1.66	0.40	1.22	0.26
HMF (7 g L^{-1})	12	0.10	1.32	0.049	2.11	1.59	0.43	0.91	0.27
Furfural (3 g L^{-1})	6	0.07	1.75	0.048	1.60	1.55	0.48	0.78	0.30
HMF (3.5 g L^{-1}) + furfural (1.5 g L^{-1})	6	0.06	1.25	0.046	3.46	1.48	0.49	1.73	0.29
HMF (7 g L^{-1}) + furfural (3 g L^{-1})	—	—	—	—	—	—	—	—	—

Lag phase (h); μ, specific growth rate (h^{-1}); Rs, substrate consumption rate (g L^{-1} h^{-1}); Yx/s, biomass substrate yield (g dry cell weight g substrate $utilized^{-1}$); qs, specific substrate consumption rate (g substrate consumed g dry cell $weight^{-1}$ h^{-1}); Qs, volumetric substrate uptake rate (g substrate consumed L^{-1} h^{-1}); Yp/s, ethanol yield on substrate (g ethanol produced g substrate $utilized^{-1}$); qp, specific ethanol productivity (g ethanol produced g dry cell $weight^{-1}$ h^{-1}); Qp, volumetric ethanol productivity (g ethanol produced L^{-1} h^{-1}).

Table 4. Physiological parameters of ***Saccharomyces cerevisiae*** ethanol red (ERD) and ***Kluyveromyces marxianus*** SLP1 under different inhibitor conditions in mineral medium (at 100 rpm, 30°C, pH 4.5) (taken from [27]).

and inhibitory toxic compounds of the fermentative capacities of yeasts. Furfural significantly reduces cell proliferation, ethanol production, inhibits several enzymes that are essential to central metabolism, including dehydrogenases, or by damaging and blocking the synthesis of DNA, RNA, proteins, carbohydrate metabolism, and changes in the cell wall. On the other hand, HMF damages membranes and nucleic acids, generates oxidative stress, and reduces NADH/NADPH and inhibition enzymes [44, 45].

Furfural and HMF have been studied mostly in *S. cerevisiae* showing decreases in ethanol yield. These compounds affect the physiology of the yeast and often result in a decreased viability, a lower metabolite yield, and a diminished productivity during biofuels generation [45]. *K. marxianus* has also been considered as a host for bioethanol production from lignocellulosic biomass hydrolysates [29]. It has been reported that inhibitors from the lignocellulosic material are related to the levels of reactive oxygen species (ROS). A typical 2-Cys peroxiredoxin from *K. marxianus* Y179 (KmTPX1) was identified, and its over expression was achieved in *S. cerevisiae* 280. Strain TPX1 with overexpressed KmTPX1 gene showed enhanced tolerance to oxidative stresses [46]. Considerable decreases in cell growth and ethanol yields have been observed at high concentrations of furans in *K. marxianus* (**Table 5**) (Sandoval et al., unpublished data).

Furfural (g/L)	**Kinetic parameters**		
	Yp/s	**Yp/x**	**Qp**
Control	0.42 ± 0.012	0.67 ± 0.006	0.17 ± 0.013
0.5	0.29 ± 0.022	0.63 ± 0.012	0.11 ± 0.063
1	0.24 ± 0.013	0.23 ± 0.030	0.10 ± 0.001
2	NG	NG	NG
3	NG	NG	NG
4	NG	NG	NG
HMF (g/L)	**Yp/s**	**Yp/x**	**Qp**
Control	0.42 ± 0.019	0.22 ± 0.026	0.17 ± 0.016
1	0.18 ± 0.023	0.12 ± 0.048	0.05 ± 0.005
2	0.18 ± 0.035	0.01 ± 0.023	0.05 ± 0.002
4	0.17 ± 0.027	0.09 ± 0.003	0.04 ± 0.013
6	0.15 ± 0.008	0.09 ± 0.004	0.04 ± 0.006
8	0.12 ± 0.003	0.08 ± 0.012	0.04 ± 0.012
10	0.10 ± 0.013	0.06 ± 0.011	0.04 ± 0.007

Mineral medium (glucose 20 g L^{-1}), 30°C, pH 4.5 and 100 rpm. Yp/s, ethanol yield on substrate (g ethanol produced g substrate utilized^{-1}); Yp/x, ethanol yield on biomass (g ethanol produced g dry cell weigh^{-1}); Qp, volumetric ethanol productivity (g ethanol produced L^{-1} h^{-1}); NG, no growth detected.

Table 5. Kinetic parameters of fermentation by ***Kluyveromyces marxianus*** SLP1.

5. Higher alcohols and aromatic compounds production from *S. cerevisiae* and *K. marxianus*

In addition to the production of bioethanol, other byproducts (such as fusel oil) can be generated during the metabolic processes of the yeast in fermentation. Fusel oil is a mixture of higher alcohols (from amino acid metabolism) mainly composed by isoamyl alcohol, isobutanol, propanol, butanol, and other aromatic compounds, such as esters and acetates. Higher alcohols can be used as fuel or energy source (when burned) [47]. Esters can also be obtained from higher alcohols of fuel oil, which are used as solvents, flavoring agents, medicinal, and plasticizers [48]. Isoamyl esters (such as isoamyl acetate) are aromatic compounds that are widely used in the food and beverage industry (because they have important compounds of flavor and fragrance) [49]. Other aromatic compounds produced by yeasts, such as acetates, also show commercial applications and have been used as components in flavorings (ethyl acetate, isobutyl acetate, amyl acetates, and isoamyl acetate) and additives for perfume (isopropyl acetates, acetates of octyl, and methyl acetates). Ethyl acetate is one of the most important esters and can be used as a chemical solvent, resins, adhesives, and paints [50].

Many of the studies link the formation of various aromatic compounds such as fusel alcohols and fusel acids to the degradation of branched-chain and aromatic amino acids, via the Ehrlich pathway [51, 52]. The synthesis of higher alcohols synthesized in the yeast catabolism was proposed by Ehrlich 1907; from this finding, the Ehrlich pathway has been used as a basis for the modification of routes and enzymes involved in the formation of higher alcohols. Ehrlich pathway proceeds in three steps: first, the amino acid is transaminated to create an α-keto acid; next, this α-keto acid is decarboxylated to an aldehyde; and finally, the aldehyde is reduced to an alcohol or oxidized to a fusel acid, depending on the redox status of the cells. In addition to their synthesis via the Ehrlich pathway, α-keto acids are intermediates in the biosynthesis of certain amino acids and therefore constitute a link between anabolic and catabolic processes. Depending on metabolic fluxes, unbalanced amino acid synthesis can also contribute to the synthesis of aromatic molecules [53].

K. marxianus is considered a yeast with high potential for the generation of compounds of industrial value from lignocellulosic waste; in addition to consuming different sugars from waste, it can convert the available sugars into molecules of industrial interest such as higher alcohols and aromatic molecules, mainly acetate esters. **Table 6** shows the production of ethanol and other metabolites at industrial level of some strains of yeast [43, 54]. Other studies at laboratory level have reported that *S. cerevisiae* and *K. marxianus* can produce volatile compounds, even in the presence of furans (**Table 7**) [27].

Usually, in Mexico, the strains of *K. marxianus* are isolated from environments with extreme conditions (such as fermentation process of mezcal or tequila); this allows the yeast to develop specific mechanisms to survive in those hostile environments. Therefore, some strains of *K. marxianus* could be a good candidate to be used at an industrial level to produce lignocellulosic ethanol.

Strain	Ethanol	Higher alcohols	Esters	Aldehydes	Methanol
S. cerevisiae (AR5)*	48.0	26.612	1.742	1.027	31.07
K. marxianus (GRO6)*	54.5	33.769	2.023	0.868	27.907
K. marxianus (DU3)+	47.5	84.68	84.9	25.8	3.8
K. marxianus (SLP1)+	44.9	62.08	112.7	27.3	3.6
K. marxianus (OFF1)+	49.7	101.61	120.7	25.6	4.3
K. marxianus (DH)+	46.0	67.71	101.9	23.7	3.8
K. marxianus (DZ5)+	47.1	81.53	105.3	12.6	3.7
K. marxianus (DA5)+	49.9	75.2	106.3	33.3	3.8
K. marxianus (DL)+	45.9	75.4	118	17.7	4.1

*Hydrolysis and separate fermentation (SHF).

+Simultaneous saccharification and fermentation (SSF).

Table 6. Generation of ethanol (g L^{-1}), higher alcohols (mg L^{-1}), esters (mg L^{-1}), aldehydes (mg L^{-1}), and methanol (mg L^{-1}) at the end of fermentation.

Compound (mg L^{-1})	Strain				
	AR5	**ERD**	**MC4**	**SLP1**	**OFF1**
Aldehydes					
Acetaldehyde	31.42	20.47	30.05	33.59	25.30
Esters					
Ethyl acetate	1.21	1.32	1.24	4.11	4.29
Isoamyl acetate	0.00	0.00	0.00	0.00	0.00
Ethyl lactate	0.65	1.22	1.17	0.59	0.61
Ethyl hexanoate	6.41	7.34	6.77	6.93	8.19
Ethyl octanoate	1.03	0.90	0.89	1.00	1.01
Alcohols					
Ethanol	4910	5120	5300	5270	4340
1-propanol	5.92	5.57	5.54	6.89	6.63
Isobutanol	4.51	5.03	3.58	20.48	16.87
Butanol	5.04	3.32	3.76	1.78	1.43
Amyl alcohol	21.63	19.88	22.06	47.78	41.74
2-phenyl-ethanol	0.34	0.33	0.38	0.32	0.34

Table 7. Ethanol and volatile compounds produced by strains ***S. cerevisiae*** and ***K. marxianus*** in defined medium with glucose (20 g L^{-1}) as the sole carbon source (100 rpm, pH 4.5, 30°C) (taken from [27]).

6. Conclusion

The use of bioethanol as fuel has helped to reduce the consumption of fossil fuels and the problems of pollution worldwide; also, the use of lignocellulosic waste for the production of second-generation bioethanol benefits the environment. However, to produce bioethanol, one of the critical points in the process is fermentation, due to the lack of robust microorganisms that can efficiently convert lignocellulosic hydrolysate sugars to ethanol. In the last years, the yeast *K. marxianus* has been widely tested in diverse biotechnological applications, including the production of bioethanol and has shown that it can match the bioethanol yields of commercial *S. cerevisiae* strains, and in many cases, it can be superior. The capacities of *K. marxianus* to be thermotolerant have a high growth rate, consume different carbon sources, and be resistant to toxic compounds, allowing it to have higher yields and ethanol production than *S. cerevisiae*; therefore is considered that this yeast has a good potential for lignocellulosic bioethanol production at the industrial level.

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Conflict of interest

The authors declare no conflict of interest.

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