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Review/Artículo de revisión

Lignocellulose fermenting microorganisms as a sustainable alternative for fuel ethanol production

Los microorganismos fermentadores de lignocelulosa como una alternativa sostenible para la obtención de bioetanol

Ávila-Mascareño, M.F.[1], *Gallegos-Máynez, L.L.*[1], *Gonzalez-Vazquez, I. I.*[1], *Parra-Cota, F.I.* [2], *de los Santos Villalobos, S.* [1*]

[1] *Instituto Tecnológico de Sonora, 5 de febrero 818 Sur, C.P. 85000, Cd. Obregón, Sonora, México*
[2] *Campo Experimental Norman E. Borlaug, C.P. 85000, Cd. Obregón, Sonora, México.*

ABSTRACT

Globally, the energy and production sectors generate a critical exploitation of fossil fuels. The largest reserves have a period for their exploitation depending on factors such as their geological and engineering fundamentals, initial costs, recovery factors, reservoir limits, recovery mechanisms, and volumetric estimates, among others. In addition, the number of oil reservoirs to meet current demand is in decline. One of the alternatives to mitigate the problems of crude oil depletion is to replace it with another that is economically, environmentally, and socially sustainable. The agricultural industry produces tons of residues per year, these residues are formed by lignocellulose, which is the main component of the cell wall of plants, by removing lignin it is possible to release the glucose contained in the cellulose of the biomass and use it as a source in the ethanol production. There are microorganisms capable of metabolizing plant lignin to release this substance, therefore this study reviews the advances that have been generated in the search for more sustainable fuels, specifically bioethanol using microorganisms.

KEY WORDS: Ethanol, biofuels, lignocellulose, microorganisms, agricultural residues.

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***Corresponding Author:**

Sergio de los Santos-Villalobos. Dpto de Ciencias Agronómicas y Veterinarias. Instituto Tecnológico de Sonora. 5 de febrero, 818 sur, Cajeme. C.P. 85000, Ciudad Obregón, Sonora, México. Teléfono: 410-90-00. Ext. 2124.
E-mail: sergio.delossantos@itson.edu.mx

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RESUMEN

A nivel mundial el sector energético y productivo generan una explotación crítica de los combustibles fósiles. Las reservas más grandes tienen un margen de tiempo para su explotación dependiendo de factores como sus fundamentos geológicos, de ingeniería, gastos iniciales, factores de recuperación, límites de yacimiento, mecanismos de recuperación y estimaciones volumétricas, entre otros. Además, el número de yacimientos de petróleo para satisfacer la demanda actual está en retroceso. Una de las alternativas para mitigar los problemas del agotamiento del crudo es sustituirlo con otro que sea económica, ambiental y socialmente sostenible. La industria agrícola produce toneladas de residuos al año, estos residuos están formados por lignocelulosa, la cual, es el principal componente de la pared celular de las plantas, mediante la remoción de lignina es posible liberar la glucosa contenida en la celulosa de la biomasa y utilizarla como fuente en la producción de etanol. Existen microorganismos capaces de metabolizar la lignina de la planta para la liberación de esta sustancia, por lo que el presente estudio hace una revisión de los avances que se han generado en la búsqueda de combustibles más sostenibles, específicamente el bioetanol utilizando microorganismos.

PALABRAS CLAVE: Etanol, biocombustibles, lignocelulosa, microorganismos, residuos agrícolas.

Introduction

Since the early decades of the 20th century, oil has been a determining factor in the establishment of Mexico's economy, industry, development, and foreign relations (Álvarez-Rivera, 2023). The oil fields that are currently exploited were formed millions of years ago due to the accumulation and decomposition of large amounts of organic matter (plant and animal) contained and buried within sedimentary rocks, subjected to certain levels of temperature, pressure, and quality of the organic matter; there are different types of deposits: marine deposits are potentially generators of liquid oil, while continental ones generate gas (Prado-González, 2021). These deposits are distributed throughout our country, primarily in Campeche, Tabasco, Veracruz, Tamaulipas, Oaxaca, and other states of the Mexican Republic, and are driven by an increasing and rapid demand resulting from the decline in hydrocarbon production (Estrada-Estrada *et al*., 2013).

The United States is the world's largest oil consumer (López, 2008), with a daily demand of 21 million barrels, of which Mexico contributes 1.5 million (Gil-Valdivia, 2008). In Mexico, economic

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tension has arisen despite the increase in the export crude price in recent years (Ramírez-Salas & Vargas-Zamora, 2023), due to the country's dependence on fossil fuels for the industrial, energy, and transportation sectors, which results in higher energy costs (Quezada González *et al*., 2024). Regarding the energy sector in Mexico, according to the Federal Electricity Commission and the Ministry of Energy, two-thirds of the electricity consumed is produced in thermoelectric plants using fossil fuels derived from oil, such as diesel, fuel oil, heavy oils, and natural gas (Pedrozo-Acuña, 2021).

Currently, many studies have reported the depletion of reserves of the so-called "black gold" (Rodríguez-Álvarez, 2022). The main hydrocarbon deposit in Mexico is the Akal field, with the Cantarell deposit located in Campeche. This deposit allowed the Akal field to contribute more than 30 % of the country's total crude production (800 Mbpd) from 1979 to 2000. By 2006, it accounted for 50 % of total production with 1,500 Mbpd. However, by 2008, its production had dropped by half, and by 2018, reported values were less than 50 Mbpd, continuing this downward trend (Galicia-Pineda & Arciniega-Esparza, 2023).

Additionally, there is a refining problem in Mexico, which is insufficient to meet domestic demand, along with a lack of technologies and infrastructure to process crude oil. As a result, the country has been forced to import more than a third of its crude, generating a trade deficit of more than 5 billion dollars in 2006 (Gil-Valdivia, 2008).

Besides the economic problems and depletion of fossil fuels, there is the enormous environmental impact they generate, as combustion, extraction, processing, and transportation cause pollution of air, water, and soil (Benavente-Ysart & Benito-Olalla, 2021). The negative impact on the air is due to greenhouse gas emissions, with fossil fuels being responsible for emitting 80 % of global CO_2 (Meloni *et al*., 2022). Moreover, they have direct consequences on global warming and health, as well as on water and soil, which are primarily affected by oil spills and the disposal of burned oils, negatively impacting flora, fauna, microorganisms, and soil fertility (Vergara-Salas, 2023).

By reducing the use and dependence on these fuels (both in the energy and transportation sectors), CO_2 emissions and other pollutants would be reduced. It is estimated that the use of second-generation bioethanol (obtained from agro-industrial waste) instead of conventional bioethanol would reduce net CO_2 emissions by 70-90 % (Hackenberg, 2008). Given this situation, the generation and/or adoption of clean, efficient, and environmentally sustainable technologies represent a viable alternative to meet current energy demand and, in the long term, reduce environmental, social, and economic costs. One of the viable alternatives to partially or completely replace fossil fuels is the use of biofuels.

Biofuel refers to any type of fuel derived from biomass, that is, from living organisms such as animals and plants (or their metabolic waste). The generation and use of biofuels is presented as a tool that helps reduce the concentration of greenhouse gases, such as CO_2, which are released into the atmosphere in other conventional production processes (Delgado-Alvarado *et al*., 2023). The production of biofuel, such as ethanol, depends on the raw material used and can be classified into two types: i) first generation, composed of sources with high sugar and starch content, and ii) second generation, derived from agricultural waste (Jiménez-Jiménez *et al*., 2020).

Bioethanol production is crucial for mitigating the decline in the availability of conventional fossil fuels, as it offers a sustainable and renewable alternative. In this context, the following review presents advancements in methods for obtaining cellulose from agricultural waste (lignocellulosic biomass), with a particular focus on the use of biological pretreatments, such as fungi and bacteria, to optimize bioethanol production.

Discussion

Challenges for Mexico

Bioethanol is obtained from organic material, using different sugars as a source of carbon. The main countries that produce it are Brazil, which uses the sucrose from sugarcane, and the United States, which uses glucose from corn. Together, these two countries produce more than 94 billion liters of bioethanol per year, accounting for 85 % of global production (Torroba, 2020). For Mexico, there are several challenges in bioethanol production. First, the ethanol produced is directed toward alcoholic beverages and the industry as a solvent (Ibarra-Díaz, 2020). Second, the raw materials from which sugars are obtained for fermentation (agave, molasses, grains) generate a limited amount of ethanol that cannot be used on a large scale. Additionally, using sugarcane or corn is not a viable option for the country, as these crops are part of Mexico's food base, and it is not possible to expand agricultural land for these crops to meet both food needs and bioethanol production for fuel.

First-generation biofuels are produced from crops. For example, from corn (*Zea mays*), sugarcane (*Saccharum officinarum L.*), sugar beet (*Beta vulgaris*), and sorghum (*Sorghum spp.*), while biodiesel can be derived from plants such as oil palm (*Elaeis guineensis*), jatropha (*Jatropha curcas*), soybeans (*Glycine max*), canola (*Brassica napus*), safflower (*Carthamus tinctorius*), and sunflower (*Helianthus annuus*). Meanwhile, second-generation biofuels are mainly obtained from agricultural, agro-industrial, and forestry residues (Cisneros-López *et al*., 2020).

First-generation bioethanol has been produced worldwide since 2000, with production increasing from 13 to 94 billion liters in 2014. In Mexico, efforts are underway to commercially produce bioethanol, with plans to blend it at 5.8 % with gasoline, primarily obtained from sugarcane juice in San Luis Potosí and Veracruz, and from sorghum in Tamaulipas (PEMEX, 2015). Several agricultural wastes have been identified in Mexico as having the potential to produce second-generation bioethanol, such as crop residues (corn, sugarcane, rice, barley, sorghum, and wheat) and agro-industrial by-products (sugarcane bagasse, corn cobs, rice husks, coffee, and sunflower). The country could generate millions of liters of bioethanol annually from these residues, with sugarcane leading the way at 3,405 million liters (UNCTAD, 2012).

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One of the main challenges is the selection of the agricultural raw material and the processing method. Materials rich in simple sugars, such as sugarcane and sweet sorghum, as well as those rich in starch, such as potatoes, sweet potatoes, and cereals, are considered first-generation and require the use of water, enzymes, and microorganisms for fermentation. In these cases, the cost of the raw material can represent up to 80 % of the total fuel cost (Chuck-Hernández *et al*., 2011).

The use of lignocellulose is one of the economically and socially responsible alternatives for bioethanol production. This material is found in agro-industrial waste such as wheat straw, sugarcane bagasse, and residues from corn, rice, and cassava. IIn this way, it not only repurposes waste (which is typically burned) but also minimizes greenhouse gas emissions and generates an essential product for the population (Macías-Alcívar & Zambrano-García, 2023).

Lignocelulosic biomass

Lignocellulose is the main component of plant cell walls, formed by layers of cellulose and hemicellulose (complex polymers) closely bonded to lignin, as shown in Figure 1. Cellulose is the union of glucose molecules, whose polymeric chains are organized into highly ordered crystalline regions, accompanied by amorphous zones, forming a very stable material that is insoluble in water. Similarly, hemicellulose is made up of a group of branched heteropolysaccharides primarily composed of short, branched sugar chains, including pentoses (typically D-xylose and L-arabinose), hexoses (such as D-galactose, D-glucose, and D-mannose), as well as uronic acids (glucuronic acid, 4-O-methyl galacturonic acid, and galacturonic acid) and deoxyhexoses (rhamnose and fucose). Hemicellulose is easier to solubilize and hydrolyze than cellulose (Chávez-Vilcahuamán & Poma-Fierro, 2021).

Lignin, on the other hand, is an aromatic polymer with a complex, branched, and amorphous structure formed by three phenylpropane units resulting from the enzymatic polymerization of sinapyl, coniferyl, and p-coumaric alcohols. The monomeric units that makeup lignin are bonded by carbon-carbon bonds and ether-type linkages (Maceda *et al*., 2021).

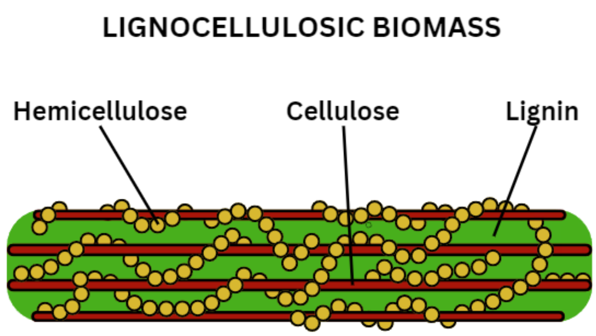

Figure 1. Lignocellulosic biomass composition.

Source: Own elaboration based on Gnansounou & Dauriat (2005).

The materials that constitute lignocellulosic material are present in varying ranges depending on the plant in question. In the case of wood composition, the most commonly found ranges are Cellulose: 38-50 %; Hemicellulose: 23-32 %; and Lignin: 15-25 % (Timoteo-Cruz, 2023). In the case of wheat straw, the lignocellulosic material contains 14.4 % lignin, 38.7 % cellulose, and 30.0 % hemicellulose (Ramos-Sevilla, 2017).

Physical and chemical pre-treatments for lignin removal

Initially, lignocellulosic raw material is contained in a complex and rigid structure, with cellulose fibers of high crystallinity, within a hemicellulose matrix and wrapped in a lignin wall, forming a very rigid material. For this reason, a pre-treatment is needed to remove lignin and hemicellulose, reduce the crystallinity of the cellulose, and increase the porosity of the material, eliminating substances that hinder hydrolysis.

There are different pre-treatments: i) physical, which uses mechanical grinding where the material is crushed, and pyrolysis of cellulose, which is based on the heating rate within a temperature range of 200-400 °C (Vaca Guevara, 2023). It is considered to prepare the material for a subsequent exothermic process that results in CO_2, CO, H_2O, and carbonization (Albis-Arrieta *et al*., 2021); ii) Chemical methods, such as ozonolysis, which uses a reduction process in lignin content through large amounts of ozone to degrade lignin and hemicellulose without producing toxic waste (Villarreal-Villarreal, 2021). In addition to chemical methods, the oxidative delignification method uses an oxidizing agent, which can be hydrogen peroxide, to increase susceptibility to enzymatic hydrolysis as it removes nearly half of the lignin and most of the hemicellulose (Amasifuen-Rengifo, 2022).

Other chemical pre-treatments use, for example, NaOH, a compound that has an extraction efficiency of 41.5 % cellulose, 22.5 % hemicellulose, and 11.2 % lignin. Meanwhile, the use of glycerol and sulfuric acid has an efficiency of 28.82% cellulose, 29.24 % hemicellulose, and 32.55 % lignin. On the other hand, the use of alkaline hydrogen peroxide reports an efficiency of 31.74 % cellulose, 34.95 % hemicellulose, and 4.46 % lignin (Chen *et al*., 2021). To reach an industrial scale using chemical methods, it is necessary to address the issues associated with high-cost products and the disposal of waste that hinders the industrial production of cellulosic ethanol.

Biological pre-treatments for lignin removal

Biological pre-treatments use lignin-degrading microorganisms through an oxidative process that produces CO_2 and water as final products. A biological pre-treatment increases the accessibility of the cellulolytic material, favoring subsequent hydrolysis and fermentation. The disadvantage of these methods is the limited capacity of microorganisms to tolerate ethanol, as well as the tendency of enzymes to be inhibited during hydrolysis (Ventura-Ibañez, 2020).

In this way, it has been reported that the minimal consumption of these sugars is achieved through the use of white rot fungi, and fermentation times are also reduced. This is due to the

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degradation of lignin by the removal of barriers that protect cellulose, as well as the increased accessibility for enzymatic hydrolysis (Mendoza-Morales & Rincón-Díaz, 2021).

These processes offer several advantages, such as cost-effectiveness, the absence of the need for additional (harmful) chemicals or equipment, and their sustainability with the environment, avoiding the partial or total formation of toxic or inhibitory compounds for microorganism activity (Cazuriaga-Durán, 2023). Therefore, various studies have been developed focusing on isolating and identifying microorganisms with enhanced lignocellulose degradation capabilities, to incorporate them into biological pre-treatments, as reported in Table 1.

In biological pre-treatment, microorganisms such as brown, white, and soft-rot fungi are used to degrade specific lignocellulosic components like lignin and hemicellulose. These include *Sclerotium rolfsii, Phanerochaete chrysosporium, Volvariella volvacea, Schizophyllum commune, Pycnoporus sanguineus, Bjerkandera adusta*, and some ascomycetes such as *Trichoderma reesei*, and species of *Aspergillus* and *Penicillium* (Cuervo *et al*., 2009). The microorganism used for biological pre-treatment must have a higher affinity for lignin and degrade it faster than the carbohydrates. The cellulose and hemicellulose are hydrolyzed by enzymes to subsequently convert cellulose into glucose and hemicellulose into monosaccharides (pentoses), and finally, a fermentation process is carried out (Grijalva-Vallejos, 2013).

The use of genetically modified microorganisms to improve fermentation efficiency and ethanol tolerance presents significant regulatory challenges. For ethanol production, the desired capacity must be met, supported by economic and financial evaluations that include capital investments and economic returns, as well as the integration of quality systems such as ISO 9001 and environmental standards like ISO 14000 (Ramos-Soto *et al*., 2020). Many countries have strict regulations regarding the use of genetically modified organisms, which limits the approval of certain strains that could enhance production efficiency. These challenges include biological safety, public acceptance, and regulation regarding environmental impact. The approval process for new strains or genetic engineering technologies can be long and costly, delaying their implementation.

An example of genetically modified microorganisms is *Saccharomyces cerevisiae*, which is effective in fermenting hexoses but does not metabolize pentoses such as arabinose and xylose, which are present in lignocellulosic biomass. Xylose, which constitutes about 35 % of the sugars in this biomass, is a potential source for obtaining high-value products such as xylitol. Through genetic engineering, recombinant strains of *S. cerevisiae* have been developed with improved capacity to ferment xylose. The process involved silencing the GAL80 gene to enhance xylose uptake and assimilation, enabling the recombinant strains to consume up to 18 % xylose without producing ethanol. The parental strain 202-3 increased its xylose consumption from 7 % to 14 %, and xylitol production rose by 345 %. The recombinant strain R2-MAPL achieved a 20 % xylose consumption with a 196 % increase in xylitol production. The B2G-MAPL strain achieved a 28 % xylose consumption with a 337 % increase in xylitol production, demonstrating that the strategies significantly improved performance in both variables (Patiño-Lagos, 2021).

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Table 1. Microorganisms used for biological pretreatment.

Bacteria/Fungi	Lignocellulose	Characteristic
P. chrysosporium[1]	Sugarcane bagasse	It degrades 5.8 % of the lignin present and increases the amount of cellulose by 0.2 %. It reaches its maximum activity on day 20 and then decreases.
Cladosporium sp[1]	Sugarcane bagasse	It exhibits a value of 15.9 % for lignin degradation and a 2 % increase in the amount of cellulose. Its maximum activity occurs on day 15.
Pleurotus spp[2]	Wheat straw	It degrades hemicellulose by 97.22 %, cellulose by 89.97 %, and the lignin fraction by 93.54 %.
Pleurotus spp[2]	Cotton fiber	It degrades hemicellulose by 54.45 %, cellulose by 85.27 %, and lignin by 0 %.
Fusarium sp [3]	Sugarcane bagasse	It degrades lignin by 5.2 % and increases the percentage of cellulose by 1.4 %. Its maximum activity occurs on day 15.
Fusarium concolor[3]	Wheat straw	It delignifies wheat straw in 5 days.
Coriolus versicolor[3]	Bamboo	There is a decrease in the amount of lignin and hemicellulose, along with an increase of up to 37 % in the saccharification rate after treatment.
Gloeophyllum trabeum* and *Fomitopsis pinicola[3]	Pine and spruce	Increase in saccharification.
Sphingomonas paucimobilis* and *Bacillus circulans[3]	-	They increase the release of sugars by up to 94 % from office paper.
P. sanguineus and B. adusta[3]	Wheat straw	They tolerate high-temperature conditions and operate across a wide pH range, a useful property for the cellulose hydrolysis process.

Sotelo-Navarro *et al*., (2012)[1], Delfín-Alcalá & Durán de Bazúa (2003)[2], Cuervo *et al*., (2009)[3].

Enzymatic hydrolysis

Enzymatic hydrolysis is primarily carried out by a group of enzymes known as cellulases, which are a mixture of various enzymatic activities whose combined action results in the degradation of cellulose (Fernandes-Klajn, 2017). Various fungi and bacteria produce this type of enzyme, such as the genera *Trichoderma*, *Phanerochaete*, and *Fusarium*. These can produce this enzymatic complex in large quantities and extracellularly, facilitating its separation in culture media (Escudero-Agudelo, 2022).

There are three types of cellulases: endoglucanases, which break down cellulose and form oligosaccharides; exoglucanases, which generate glucose and cellobiose; and glucosidases or carbohydrases, which hydrolyze cellobiose to produce glucose molecules (Gamarra-Mendoza, 2024). The cellulase enzymatic complex used during the hydrolysis of the pretreated material is made up of a set of carbohydrases capable of breaking down the glycosidic bonds of polysaccharides, releasing glucose and other monosaccharides for fermentation (Manrique-Hernandez, 2019). For example, white-rot basidiomycete fungi produce extracellular enzymes such as lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase, which are key enzymes for lignin degradation (Muñoz-Duarte, 2012).

Ethanol-fermenting microorganisms

Once the hydrolysis phase is completed, the alcoholic fermentation stage continues, in which sugars are degraded into ethanol by microorganisms through various fermentation strategies (Lara-Román, 2023). An ideal microorganism has the following characteristics: i) high ethanol productivity and yield, ii) the ability to reduce the number of undesirable by-products, iii) tolerance to inhibitors generated by hydrolysis, iv) high tolerance to ethanol and osmotic stress caused by the concentration of fermentable sugars (Leighton-Rendón *et al*., 2023).

The most commonly used genus in ethanol production is *Saccharomyces*, due to its ability to quickly convert sugars into ethanol. In addition to its high osmotolerance, resilience to temperature changes, and strong acceptance in the industry. However, in this case, *Saccharomyces cerevisiae* is not optimal for hemicellulose hydrolysates, as it presents high aeration costs, excessive biomass production, and is incapable of metabolizing pentoses, which is one of the main sugars in hemicellulose (Ramírez-Soto & López de Ávila, 2024). Hence, microorganisms that can carry out the fermentation process to produce ethanol from hemicellulose hydrolysates have been studied. Among the promising species are *Escherichia coli, Klebsiella oxytoca, Zymomonas mobilis*, and *S. cerevisiae* modified through metabolic pathway engineering (Dien *et al*., 2003), whose main metabolic characteristics are described below.

i. *Escherichia coli*

Strains of this species exhibit favorable characteristics for this process, such as their ability to ferment a large amount of sugars and the extensive knowledge of their industrial use. The requirements for their growth are minimal, and they have a higher tolerance to inhibitors compared to *S. cerevisiae* and *Z. mobilis* (Zaldivar & Ingram, 1999; Zaldivar *et al*., 1999). Furthermore, in its anaerobic metabolism, wild strains of *E. coli* form acetyl-CoA from pyruvate, and by using it, the alcohol dehydrogenase (ADHE) enzyme produces acetaldehyde, which is then converted to ethanol. However, this fermentative pathway is unbalanced, as one NADH is generated for each molecule of pyruvate, requiring two NADH molecules to convert pyruvate into ethanol. Thus, to maintain energy balance in *E. coli*, an equal amount of acetyl-CoA must be converted to acetate, which reduces ethanol yields (Ingram *et al*., 1998).

Alternatively, ethanologenic strains of *E. coli* have been constructed, including KO11, which contains the PET operon (production of ethanol) on its chromosome. This operon carries genes that code for the enzymes pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADBH) from *Z. mobilis*. These enzymes manage to increase the glucose or xylose conversion yield to ethanol by up to 27 % (Huerta-Beristain *et al*., 2005). However, when cultivating this strain using hemicellulose hydrolysates from sugarcane bagasse, ethanol yields are less than 70 % of the theoretical value, compared to 95 % in rich media. This is due to carbon being diverted to the production of organic acids (Huerta-Beristain *et al*., 2008). Moreover, although the ethanol productivity of strain KO11 is similar to that of yeast in batch cultures, its ethanol tolerance is much lower, which is why mutants have been generated to increase it (Jarboe *et al*., 2007).

ii. *Zymomonas mobilis*

This is a Gram-negative bacterium that exhibits higher specific sugar consumption rates and ethanol production speeds than yeasts, producing less biomass and showing greater ethanol tolerance (up to 120 g/l). This is due to the anaerobic metabolism of glucose through the Entner-Doudoroff (ED) pathway, instead of the Embden-Meyerhof-Parnas (EMP) pathway. The ED pathway generates half the ATP and, consequently, produces less biomass and increases the carbon directed towards fermentation (Dien *et al*., 2003).

Z. mobilis has the disadvantage of only fermenting glucose, fructose, and sucrose. Therefore, it has been genetically modified to ferment sugars such as xylose and arabinose. For example, Zhang *et al*. (1995) genetically modified *Z. mobilis* and introduced four genes from *E. coli*: xylose isomerase (xylA), xylose kinase (xylB), transketolase (tktA), and transaldolase (talB), resulting in the *Z. mobilis* pZB5 strain, which gained the ability to ferment xylose with an 86 % yield for ethanol production. Similarly, Deanda *et al*. (1996) constructed a *Z. mobilis* pZB206 strain that ferments arabinose to ethanol with a 98 % yield relative to the theoretical value, through a plasmid expressing five *E. coli* genes: L-arabinose isomerase (araA), L-ribulose kinase (araB), L-ribulose-5-phosphate-4-epimerase (araD), transketolase (tktA), and transaldolase (talB).

iii. *Klebsiella oxytoca*

A bacteria isolated from paper and wood, capable of growing at a pH of 5.0 and a temperature of 35 °C, using hexoses and pentoses for its growth, as well as cellobiose and cellotriose (Freer & Detroy, 1983), making it suitable for ethanol production from cellulose.

Ethanol production by this bacterial species is carried out through the pyruvate formate lyase (PFL) pathway. By introducing the PET operon (with the PDC and ADH genes from *Z. mobilis*), the ethanol concentration produced was increased to 90 % (Ohta *et al*., 1991). Furthermore, this strain ferments xylose as quickly as glucose (2 g/l h), which is twice as fast as *E. coli* KO11. Additionally, the *K. oxytoca* P2 strain has been obtained through mutagenesis, integrating the PET operon into its chromosome with the ability to metabolize glucose (100 g/l) or cellobiose (100 g/l) in 48 hours, producing 44-45 g/l of ethanol (Wood & Ingram, 1992).

iv. *Saccharomyces cerevisiae*

This species is known as the yeast of choice for ethanol production, capable of fermenting glucose, mannose, fructose, sucrose, and maltose through the EMP pathway (Guaraca-Vallejo, 2023). However, it faces the challenge of producing ethanol from certain pentoses, such as arabinose and xylose, found in hemicellulose. For this reason, various metabolic pathway engineering studies have been conducted to enable the metabolism of these carbon sources.

To metabolize xylose, the approach used is to introduce a heterologous enzyme that converts xylose from hemicellulose into xylulose, which can then be metabolized by *S. cerevisiae*. Xylose isomerase can be used for this purpose, as it is capable of converting D-xylose into D-xylulose through an oxidation-reduction reaction. In this regard, the xylose isomerase from *Piromyces sp*. E2 has been successfully expressed in the modified strain *S. cerevisiae* RWB 217, which, in addition to expressing the xylA gene, overexpresses genes involved in converting xylose into glycolytic intermediates (van Maris *et al*., 2006).

This strain is capable of growing under anaerobic conditions, producing ethanol, CO_2, glycerol, and biomass, with the highest specific ethanol production rate using xylose (0.46 g/g cell-h). Similarly, an alternative was used to achieve arabinose fermentation in *S. cerevisiae*, through the overexpression of the arabinose utilization pathway found in bacteria, where certain enzymes convert arabinose to ribulose, along with the overexpression of a gene encoding for a yeast galactose permease (GAL2), through metabolic evolution under oxygen-limited conditions (van Maris *et al*., 2006). The results obtained were neither the most efficient nor favorable, as the strain did not show a high specific ethanol production rate or high yield. However, this method remains the most promising alternative for ethanol production from arabinose.

Many microorganisms are capable of efficiently metabolizing different substrates. Exploring their development and resistance to variations in temperature, pH, and pressure is necessary to enable the implementation of more flexible and faster processes that promote ethanol production and increase the productivity of biorefineries.

Conclusions

Bioethanol represents one of the most promising alternatives to replace fossil fuels, offering a viable and well-founded option to mitigate the depletion of crude oil resources. Its viability is based on the advantages of its production process, as it does not generate high CO_2 emissions or other greenhouse gases. The production of second-generation bioethanol offers an advantage by not impacting the agricultural industry (without altering food production), through the use of lignocellulosic waste naturally generated by agriculture.

This biotechnological process is effective, although its main challenge lies in finding the ideal microorganism to ferment the different types of sugars present in lignocellulosic biomass, which not only achieves high ethanol production rates but also minimizes the generation of by-products. Molecular biology, combined with metabolic pathway engineering, has facilitated the development of improved strains of microorganisms, increasing ethanol yield compared to wild strains, enabling the fermentation of a broader variety of sugars, and enhancing resistance to high concentrations of ethanol and inhibitors.

Within the perspectives of bioethanol, in recent years, third-generation bioethanol has been studied, produced from algae, which have a high capacity for lipid and sugar production, making it more efficient and less costly. The future of bioethanol is promising, and there are still many aspects to explore and eventually develop it as a reliable alternative for energy security. As technologies advance and processes are optimized, it is expected that bioethanol production costs will decrease, making it more competitive with fossil fuels, especially in a scenario of fluctuating oil prices.

Authors' contribution

- Conceptualization of the work: Ávila-Mascareño, M.F., Gallegos-Máynez, L.L., de los Santos Villalobos, S.
- Writing and preparation of the manuscript: Ávila-Mascareño, M.F., Gallegos-Máynez, L.L., Gonzalez Vazquez, I. I., Parra-Cota, F.I., de los Santos Villalobos, S.
- Writing, review, and editing: Ávila-Mascareño, M.F., Gallegos-Máynez, L.L., Gonzalez Vazquez, I. I., Parra-Cota, F.I., de los Santos Villalobos, S.
- Project administration: Parra-Cota, F.I., de los Santos Villalobos, S.
- Fund acquisition: Parra-Cota, F.I., de los Santos Villalobos, S.

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Conflict of interest

The authors declare that they have no conflict of interest.

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