

Review Article

Opportunities to Production of Biofuel from Grains and to Improve the Factors Increasing the Yield of Bioethanol in a Short Time

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Abstract

When biofuels are used as an energy source, they are accepted as a renewable energy source that is safe for the environment and can replace fossil fuels at the same time. Additionally, using of organic materials obtained from plants and animals as biofuel is more advantageous compared to the formation of fossil fuels over hundreds of years. Therefore, biomass containing within itself substances such as sugar, starch, oil and cellulose, which have high energy availability, are considered as raw materials. While biodiesel is obtained from organic materials containing oil, ethanol and similar biofuels are generally obtained from organic materials containing sugar and starch. All these production stages vary depending on factors such as each plant species and variety, the amount of sugar contained in plants or sugar structures, the fermentation microorganisms used or the pretreatments applied. In this study, information is given about the stages of bioethanol production from grains and the issues affecting bioethanol yield.

Keywords: Bioethanol, Fermentation, Grain

1. Introduction

Globally, 80% of energy is produced from non-renewable energy sources (fossils) [1]. Fuels such as coal, diesel fuel, gasoline, oil and natural gas continue to be used in many sectors of the world such as electricity, transportation, heating and industry. Numerous air pollutants emerged by the use of fossil fuels cause serious harms to human health and nature [2][3]. Scientists report that air pollution due to fossil fuels is also the cause of neurological damage and many other diseases in humans [4]. It is important to use and develop renewable energy sources in order to reduce the damage to nature and

human beings. Biofuels are one of the alternatives used to meet the energy requirement [5].

Biomass is considered a renewable energy source due to its short life cycle, and biofuels derived from biomass have the potential to replace fossil fuels [6]. Biofuels are safe for the environment [5]. The use of organic materials obtained from plants and animals as biofuels [7] is more advantageous compared to the formation of fossil fuels over hundreds of years. One of the other reasons for the need to acquire energy from alternative sources is the depletion of fossil fuels [5].

Biofuel is obtained by using organic products such as sugar, starch, oil, cellulose from plants with high energy availability or raw materials such as forest products [8]. The parts of plant woody and cellulosic and dried animal residues directly burned constitute primary biofuels; substances produced indirectly from plant and animal materials constitute secondary biofuels. Furthermore, secondary biofuels are being analyzed in three different generations. The first generation biofuels are biodiesels obtained from cooking oil such as ethanol and animal oil obtained from food products quite rich in starch. And the second generation biofuels are bioethanol and soybean obtained from non-food cellulosic biomass and biodiesels obtained from oilseeds with high oil content oily seeds such as jatropha. Biofuels obtained from microorganisms constitute the third generation [9]. In this case, while biodiesel is obtained from organic materials containing oil, ethanol and similar biofuels are generally obtained from organic materials containing sugar and starch. Bioethanol (C_2H_5OH), is colorless, flammable and oxidized hydrocarbons. It is fermented from sugar, starch and cellulosic biomass and used in many fields today [10]. The plant groups that provide the most ethanol to obtain fuel in the world consist of grains such as sugar cane, corn, wheat and barley [11]. Triticale, a cereal with high starch content and high amyolytic activity, is one of the best raw materials for bioethanol [12]. In addition, materials such as straw remaining after the harvest of grain crops are a good source of ethanol through technical knowledge and technology [13]. Today, the countries that produce the most bioethanol with 84% of the total production are the United States and Brazil. The United States provides 94% of the total bioethanol it produces from corn starch [11], while Brazil provides mostly from sugarcane [14]. Recent developments in bioethanol production in the world are generally on the increasing amount of ethanol in biomass. Additionally, the focus is on technical development to minimize ethanol loss. These technical developments; It includes raw material modification with genetic methods, advancement of cellulose enzyme techniques and various studies on a cellular basis [14][15].

With a more extensive and detailed explanation, the following topics are examined in the continuation of this article; Bioethanol production from grains, factors affecting bioethanol yield and technological developments and finally the study is concluded.

2. Production of bioethanol from grains

The higher the rate of fermentable sugar in plants, the higher the amount of bioethanol will increase accordingly [16]. Each plant is exposed to different processes due to its raw material (such as starch, sugar, cellulose) [17]. Accordingly, the critical points affecting the bioethanol yield were determined at different stages in each process [18].

Basically, these stages (figure 1) are based on the principle of obtaining bioethanol from carbohydrate extracts by processing raw materials such as sugar cane, corn, wheat, potatoes, barley and wood [19]. However, when these plants are divided into three different classes as sugar-containing, starch-containing and lignocellulosic plants, there are some changes in standard procedures. Therefore, this situation affects the yield [20].

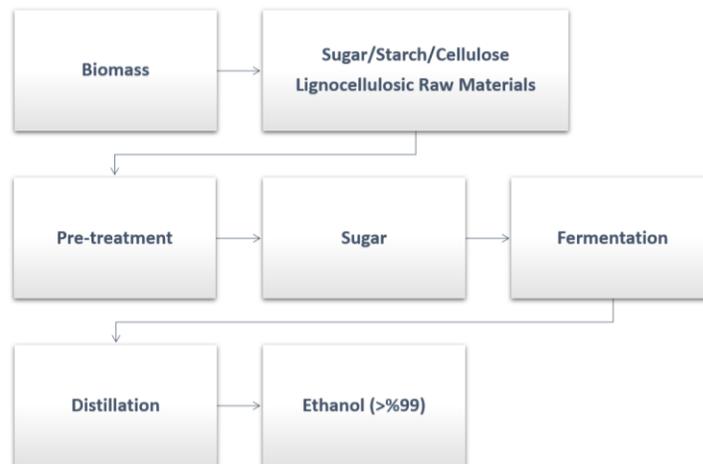


Figure 1. Bioethanol production stages [21]

2.1. Production of bioethanol from lignocellulosic raw materials

Lignocellulose plants, which are formed by the combination of cellulose, hemicellulose and lignin in their structures, are a kind of raw material of bioethanol [22]. These structures come together at different rates to form lignocellulosic plants with different properties [23].

The fact that they are in different ratios is one of the reasons that affect the bioethanol yield. In addition, the compound of hemicellulose and lignin accumulates around the cellulose, forming a protective structure (figure 2) [24]. It is also observed that this structure changes in different plant species. For this reason, each method is determined according to the plant species and the highest yield is targeted.

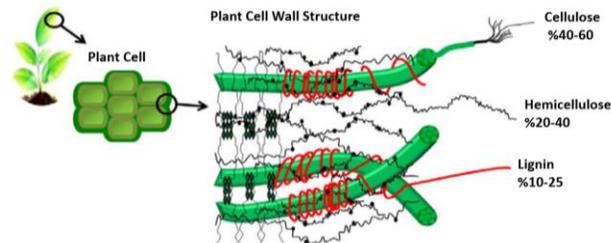


Figure 2. Plant cell wall components [25].

Lignin and hemicellulose, which are integrated around cellulose, cause problems in ethanol production due to this structure [26]. Because the sugar to be processed is hidden in these structures, reducing the ethanol yield. For this reason, these structures should be well separated from each other [27]. Therefore, lignocellulosic plants are subjected to slightly different pretreatments (figure 3) than others (plants with high starch and sugar content) [28]. Studies on the most suitable pretreatments and application methods to be selected are still ongoing. The main purpose of the pre-processing done here is; It is a good separation of lignin, hemicellulose and cellulose from each other and expanding the surface area. In this way, sugar can be fermented better [24].

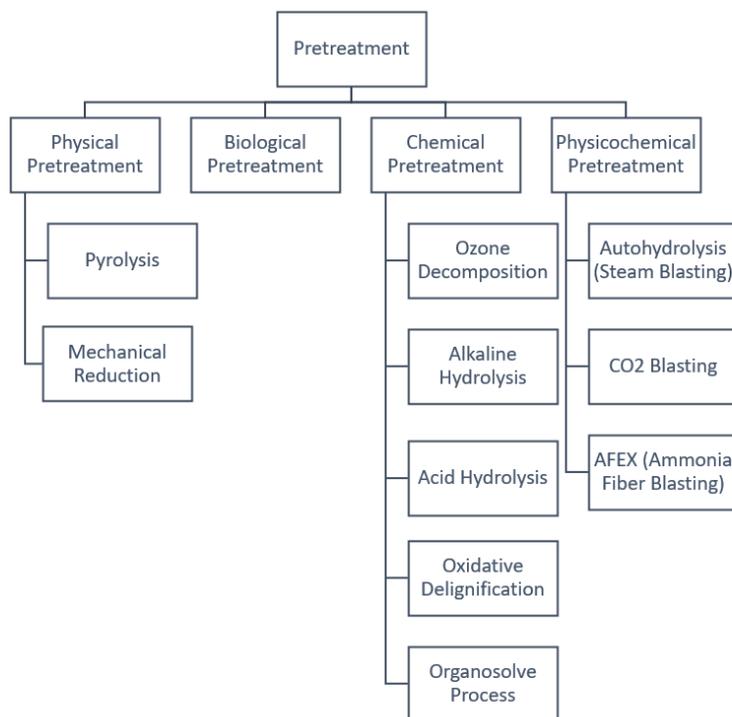


Figure 3. Pre-processing applications [24]

The chemical content of the lignocellulosic plant material is determined by natural polymer structures (lignin, cellulose and hemicellulose) formed by the combination of simple sugar structures in a certain order (Figures 2 and 4) [29]. Different chemical bonds are effective in the formation of these polymeric structures [30]. These bonds can be converted into monomeric sugars by breaking down in a controlled manner with enzymes or chemical processes [31]. Cellulose solvents in the structure of lignocellulosic material; It should be soluble at low temperatures, chemically stable, should not have toxic effects on enzymatic hydrolysis and microbial fermentation steps, have a high solubility capacity and a rapid diffusion rate in a solid lignocellulosic composition [32]. Unlike cellulose, hemicellulose is not chemically homogeneous [33]. While hemicelluloses based in hard woods contain abundant xylan, hemicelluloses based in soft woods contain more glucomannan [34]. For instance; xylan in rice bran; It contains 46% xylose, 44.9% arabinose, 6.1% galactose, 1.9% glucose and 1.1% anhydrouronic acid [35]. In wheat, arabinoxylan; It contains 65.8% xylose, 33.5% arabinose, 0.1% mannose, 0.1% galactose and 0.3% glucose. In the structure of xylan in corn fibers, it contains 48-54% xylose, 33-35% arabinose, 5-11% galactose and 3-6% glucuronic acid [36]. For these reasons, cellulose and hemicellulose structures are also subjected to different procedures [37]. In addition, lignin, which is another structure, does not contain sugar units chemically, so it cannot be converted into liquid fuels by fermentation [38]. One of the

main factors that increase the ethanol yield will be determined in detail of the cellulose or hemicellulose contents of the lignocellulosic raw material to be used according to the plant species [39].

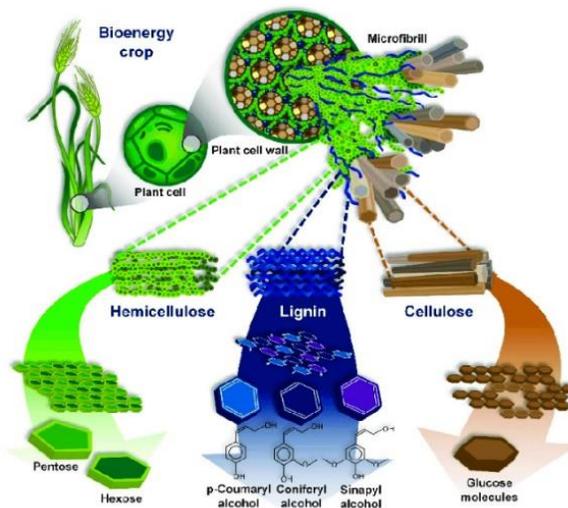


Figure 4. Composition of lignocellulosic raw material [40]

In order for the pre-treatment to be considered successful, the sugar formed must be made suitable for fermentation [41]. Therefore, the formation of sugars should be increased or converted into a suitable structure for hydrolysis, carbohydrate loss should be prevented, by-product formation should be prevented in subsequent hydrolysis and fermentation processes, it should prevent the formation of by-product inhibitors in subsequent processes and should not adversely affect the cost [16]. The products to be obtained by pre-treatment constitute the main part of the actual production [42].

2.2. Production of bioethanol from raw materials with high starch or sugar content

Starch is polymers formed by the linking of glucose units with glycosic bonds. These glycosic bonds are broken at low pH [43]. Starch polymers contain two types of glucose units, amylose and amylopectin [33]. Amylose consists of 6000 glucose units, while amylopectin is a short-chain structure consisting of 10-45 glucose units, but with side chains [44][45]. Commercially important starch granules are 2-100 μm [46]. It is synthesized as storage material in various parts of plants (such as root, seed, tuber, etc.) [47]. Some plants are rich in starch. These are plants that do not contain direct sugar, such as corn, barley, wheat, sorghum, and contain starch, which is a form of sugar. Starch in

each plant contains glucose units of different lengths. For instance, it is 1,000-6,000 lengths in potatoes and tropical plants, while it is 100-200 in wheat and corn [48][25].

Before bioethanol production, microorganisms cannot directly affect starch. Therefore, starch needs to be pretreated (figure 3; table 2). In this regard, besides the standard procedures, new studies are still in progress. The aim here is to increase the amount of fermentable sugars and to provide high ethanol yield. Thus, starch polymers are pretreated with enzymes or acids for hydrolysis [49]. However, there are many negative situations during hydrolysis [50]. In conclusion, the production of bioethanol from plants with high sugar content is based on revealing the sugar in it. It is made by crushing the product, wetting it and applying some chemical processes [51]. Plants such as sugar cane, sweet sorghum and sugar beet are in this group [52]. Production of sugar and bioethanol from these plants is the oldest uncomplex method [51][53][54]. Sugar obtained from biomass continues with the production of bioethanol with the help of microorganisms, distillation of bioethanol and dehydration stages (Figure 1) [55]. The bioethanol which is obtained can be used as a fuel either alone or as a mixture. The most common usage is E-10 (10% bioethanol, 90% gasoline) and E-85 (85% bioethanol, 15% gasoline)[51][55].

2-Factors affecting bioethanol yield and technological developments

In bioethanol obtained by fermentation, the yield depends on many factors. The content and distribution of the raw material, different pre-treatment methods, different fermentation applications and current technological developments are effective in this [56]. In general, grains, sugar cane 215 l/ tonne; sorghum 416 l/tonne; corn 360-380 l/tonne; wheat 376-340 l/ tonne; barley 250-345 l/tonne; rye 356 l/tonne; oats 264 l/tonne; triticale 367 l/ tonne; paddy 434 l/tonne; straw residues 308 l/tonne; it provides 260 l/ton yield from other lignocellulosic plants (table 1). These values continue to be developed through critical and innovative studies. Each plant is evaluated as a different raw material. Because the amount of sugar which contains and the sugar structures are different. Even within the same species or cultivars, these amounts vary more or less. For instance, in Brazil, great success has been achieved by using sugarcane varieties with high bioethanol yield as breeding material [57][58]. Other issues that help the rapid development of bioethanol production in Brazil in a short time are known as innovations in technology (such as the creation of new facilities) and increasing the use of bioethanol by the government. Increasing the rate of bioethanol in gasoline, changing its prices in the market and creating new usage areas have created new demands in this regard and the need for bioethanol has increased rapidly [58][59][60].

Table 1. Bioethanol and field yield values in cereals

Plant	Bioethanol Yield (L/Ton)	Field Yield (Kg/Daa)
Sugar cane	215 ^[66]	4,894 ^[61]
Sorghum	416 ^[64]	556 ^[61]
Maize	380 ^[61] ; 360 ^[51]	940 ^[61]
Wheat	376 ^[62] ; 340 ^[51]	296 ^[61]
Barley	250 ^[51] ; 345 ^[62]	268 ^[61]
Rye	356 ^[63]	283 ^[61]
Oat	264 ^[62]	278 ^[61]
Triticale	367 ^[63] ; 368 ^[62]	341 ^[61]
Paddy rice	434 ^[65]	782 ^[61]
Straw remains	308 ^[25]	431 ^[67]
Lignocellulosic plants	280 ^[51]	-

Another important step that affects the bioethanol yield is the combination of pretreatment step and application. Pre-processes; It is examined in four parts (figure 3 and table 2) as physical, chemical, physicochemical and biological [68]. The decomposition of the material by biological pretreatment is usually done by microorganisms such as fungi. Enzyme and microorganism activities are mostly used in this process. Furthermore, the type of microorganism to be selected according to the structure of the raw material is also very important. Because, as a structure, lignocellulose hydrolysates contain xylose, mannose, galactose, arabinose and oligosaccharides besides glucose [16], some microorganisms cannot affect pentose sugars (5 carbons) [69]. Therefore, different microorganisms are used in lignocellulosic plants. In physical pretreatments, it is usually subjected to mechanical processes (such as grinding, crushing, etc.) in order to break it into smaller pieces [18]. Thus, the surface area and enzymatic accessibility increase [19].

Chemical pretreatments, categorized in two groups as acidic and alkaline, are used more widely than others. Sometimes only basic/acidic solution and different concentrations are used, sometimes both are used [25]. For example, in a study using corn waste and sugar beet pulp; The effects of particle size, extraction conditions (temperature, alkali density and time) and extraction methods (extraction with acid addition, extraction with direct alkali addition and extraction with alkali addition after fractionation) on hemicellulose yield were analyzed. As a result, extraction with alkali gives 40.2% purity, while extraction using acid gives 27.4% purity [70][71]. It is therefore important to choose the best method suitable for the content of the lignocellulosic material. In

physicochemical pretreatments, there are combined applications in which both chemical and physical methods are applied. Methods such as microwave applications, oxidative pretreatments, steam blasting are used. In conclusion, the method chosen according to the chemical content of the lignocellulosic raw material has very different effects on the ethanol yield [72]. For this reason, it is necessary to choose the best method, taking into account the cellulose or hemicellulose structures [73].

Table 2. Types of pretreatment applications

Cereals	Pretreatment	Temperature	Time	Other	Literature
<i>Sugar cane</i>	Steam blast	160–260°C	15 min.	0,6–4,8 MPa	[74][75][76]
<i>Sugar cane</i>	Hot water	120–230°C	1–80 min.	-	[76][77][78][79][80]
<i>Sugar cane</i>	Alkaline application	53,2–120°C	4–65,6 hour	-	[74][76][82]
<i>Sugar cane</i>	Organosolve	150–200°C	30–90 min.	35–70%	[74][76][77][80][83]
<i>Sugar cane</i>	Dilute acid	100–120°C	40–120 min.	1,8–10%	[76][80][84]
<i>Sugar cane</i>	Concentrated acid	80°C	90 min.	18–40%	[76][84]
<i>Sugar cane</i>	ionic liquid	60–140°C	5–360 min.	3–10%	[76][81][85]
<i>Sugar cane</i>	(1) dilute acid + (2) Microwave	130-190°C (2)	5–10 dk (2)	(1) 1,56 %, 0,2M, pH 0.68; (2) 2,45 GHz; 900 W	[76][86]
<i>Sorghum</i>	Sodium hydroxide	30-40°C	1-3 day	%1-%2 (NaOH)	[89]
<i>Sorghum</i>	Sulfuric acid	121°C	1 hour	40.5% (v/v) (H ₂ SO ₄); 1:10 g/mL	[90][91]

<i>Sorghum</i>	Physical application	Squeeze, washing with hot water, air drying, heat drying (50-60°C), grinding and sieving (<0.5 mm)			[90][92]
<i>Sorghum</i>	Physical application	Squeeze, air drying, grinding and sieving, oven drying (105°C; 12 hours)			[90][92]
<i>Sorghum</i>	Physical application	Cutting, milling, air drying (105°C-24 hours), grinding and sieving			[90][93]
<i>Sorghum</i>	Physical application	Overnight drying (60°C) and grinding (<1 mm)			[90][94]
<i>Sorghum</i>	Physical application	Cutting (1–5 mm) and oven drying (12 hours)			[90][95]
<i>Sorghum</i>	Physical application	Stalk cutting, double milling, drying overnight at 80°C, chopping (<1 cm)			[90][96]
<i>Maize</i>	Physical application	Milling (5120min.), 30°C temperature			[97][99]
<i>Maize</i>	Ultrasound app.	-	40sec.	Ultrasound application to cornstarch slurry	[97][101]
<i>Maize</i>	Dilute alkali application	121°C	20min.	Mixture containing 2% (w/w) sodium hydroxide + 10% (w/w) biomass (total 500g) and then left to dry at 60°C (10-20% humidity). Finally, it was neutralized with hydrochloric acid.	[103]
<i>Maize</i>	Dilute acid	160 °C	10min.	Mixture containing 1% (w/w) sulfuric acid + 10% (w/w) biomass, Then neutralized with sodium hydroxide. The moisture was filtered. Left to dry at 60°C (10-20% humidity). It was stored at +4°C until use.	[103]
<i>Wheat</i>	Sodium hydroxide	37 °C	5 gün	4% NaOH (g/g TS)	[104][106]

Wheat	Sodium hydroxide	40°C	24 hour	10% NaOH (g/g TS)	[104][105]
Wheat	Ultrasound app.	-	15-35min.	Alkali application + ultrasound application (for wheat straw)	[97][100]
Barley	Steam blast	180 °C	30 min.	Steam explosion in a 10 liter reactor	[110]
Barley	Chemical + Physical applications	It was soaked overnight in 2 liters of 2.88% w/v phosphoric acid, filtered using a mechanical press, and then delivered directly to the steam explosion vessel at 160°C for 30 minutes. When the target temperature (160°C) was reached, a 30-minute processing time was started.			[111]
Rye	Sulfuric acid	121 °C	1 hour	2% (H ₂ SO ₄)	[87]
Rye	Sulfuric acid	130–200°C	5–60 min.	%0,5–1,5 (H ₂ SO ₄)	[88]
Paddy rice	Physical application	-	120min.	Ball milling application	[97][98]
Paddy rice	Sodium hydroxide	-	-	4–10% (g/g TS) NaOH	[104][107]
Paddy rice	Calcium hydroxide	25°C	6 gün	%9.8 Ca(OH) ₂ (g/gTS)	[104][108]
Paddy rice	Sodium hydroxide	20 °C	-	10% NaOH	[104][109]
Agricultural Wastes	Pyrolysis	97°C	2,5 hour	Slight acid hydrolysis (1N Sulfuric acid)	[97][102]

In addition to the plant material that affects the bioethanol yield and the pretreatment applied, other ongoing steps in this process should also be examined. Biomass continues to go through different processes after being subjected to various pretreatments. The sugar obtained at the beginning must be converted to ethanol by microorganism activity. For this, the enzyme-substrate relationship is another important issue. It is reported that both the pretreatment step and the microbial steps, enzymes are

often not completely effective on the substrate, sufficient product cannot be obtained and even yield losses are reported. For this reason, the improvement of enzyme solutions is also among innovative studies. With many studies, prevention of sugar loss, effective pretreatment and enzyme application are provided [11][112]. For example, a single enzyme usually is not used, instead of this, additional enzymes and different additives are added. For the hydrolysis of barley straw, xylase, PEG4000 and Tween 80 blends in addition to the commercial cellulase blend support the notion that the yield increases [113]. It is reported that the combination of xylase and PEG4000 provides the highest glucose and xylose yields, as the addition of xylase alone did not provide any significant improvement [114]. In addition to the commercial cellulase used in triticale straw hydrolysis, commercial hemicellulase and β -glucosidase activity reacted for longer than expected and the addition of hemicellulase increased glucan formation from 51.94 to 80.74% [11][115]. These enzymes used in fermentation and their source microorganisms; Examples include cellulase from *T. Reesei*, β -Glycosidase from *E. Coli*, cellulase from *A. Niger*, cellulase from *B. Licheniformis*, amyloglucosidase from *A. Niger* [116] [117] [118]. In order for these microorganisms to be used industrially, they must also have certain properties. These are, ethanol yield should be higher than 90%, product tolerance at least 40 g/L, productivity 1 g/(L·h), simple requirements, strong, ability to grow in undiluted hydrolysates resistant to inhibitors and they have structures that can grow in an acidic and high temperature environment. The most common microorganisms used for bioethanol production are *Saccharomyces cerevisiae* yeasts and *Zymomonas mobilis* bacteria. These microorganisms can only ferment pentose sugars [11][119]. Therefore, different enzyme solutions should be developed for other sugar structures.

Today, innovative studies are carried out on the determination of genes that affect ethanol production and new gene expressions, transfer of these characteristics to new progeny, plant breeding, enzyme and microorganism activity used in fermentation. Balta (2021), It is reported that modulation of the expression of ethanol producing and coordinated genes is necessary to increase the production of a simple compound such as ethanol [120]. *S. cerevisiae* has an important place because it has the highest ethanol tolerance capacity and wide pH range among all bacteria and yeasts [121]. Due to its special character, it reduces the cost of fermentation and distillation by reducing contamination. Therefore, it has become the yeast widely used industrially in the production of bioethanol. Researchers are working on yeasts with high resistance to various stress factors and high ethanol concentration metabolism [10][122][123]. Isolation of new microorganisms may provide new sources for amylase production, as amylases are an enzyme of significant commercial value and sought after in the world market [124]. Interest in enzyme production has increased in recent years due to the needs in the fields of bioenergy, biofuels and textiles [125]. Today, many studies are being carried out on

new microorganisms for the purpose of enzymes with high specific activity and high efficiency.

3. Results

Energy production with biofuels will benefit nature in many ways. For this reason, scientists aim to further advance this issue with their studies. In fact, it is known that there are innovative studies in many areas that will improve bioethanol production. The development of issues affecting bioethanol yield depends on the correct evaluation of the production stages and what the target is. Depending on the rapid development of technology, a wide variety of enzymes or microorganism species are being developed. At the same time, studies at the cellular level have increased the efficiency of these organisms or enzymes. When many plants that have not yet been discovered are included in these studies, or when known plant species are improved by various breeding methods, the production rate will increase in a short time. The main subject of study, which has enabled many countries to become bioethanol producers in a short time, is on breeding. Additionally, it's about different bioethanol production steps. With ongoing genetic engineering studies, it is also possible to develop raw materials with desired properties or to pre-process them easily. Therefore, these studies can gain momentum only with the establishment of innovative and progressive studies.

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