CURRENT STATE AND PROSPECTS OF BIOMASS FERMENTATION

Lappeenranta–Lahti University of Technology LUT

Bachelor's Programme in Energy Technology, Bachelor's thesis

2024

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ABSTRACT

Lappeenranta–Lahti University of Technology LUT

LUT School of Energy Systems

Energy Technology

Double Degree Bachelor's Programme of Lappeenranta University of Technology (LUT) in Cooperation with Hebei University of Technology (HEBUT)

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Current state and prospects of biomass fermentation

Bachelor's thesis

2024

40 pages, 6 figures and 2 tables

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Keywords: Biomass fermentation technology, lignocellulose, bioethanol, pretreatment, hydrolysis, current state and prospects

This work studies the production of bioethanol from lignocellulose material, aiming to discuss the current status and future prospects of biotechnology and fermentation. The key aspects of the biomass fermentation process were discussed, including raw material selection, microbial selection and improvement, pretreatment process, hydrolysis process, fermentation process and ethanol purification. The research focuses on the improvement of various processes, waste treatment and market conditions. Special attention is given to the optimization of fermentation parameters such as temperature, pH, nutrient concentration, oxygen demand, and agitation speed, which are crucial for maximizing yield and efficiency. By analyzing these parameters and their impact on the fermentation process, this study aims to provide a comprehensive analysis of the future prospects of biomass fermentation technology, highlighting potential advancements and their implications for sustainable bioethanol production. The work also acknowledges the significant challenges such as viability of large-scale biomass fermentation, that is often limited by high capital cost, complex logistics associated to biomass supply, and competition for feedstock with food crops. Additionally, technical challenges such as efficient biomass degradation and integration of fermentation process into existing industrial infrastructure.

ACKNOWLEDGEMENTS

Throughout the writing of this dissertation I have received a great deal of support and assistance.

I would first like to thank my first supervisor Clara Mendoza Martinez and my second supervisor Liyao Xie. Their expertise was invaluable in formulating the research questions and methodology. Their insightful feedback pushed me to sharpen my thinking and brought my work to a higher level.

In addition, I would like to thank my parents for their wise counsel and sympathetic ear. You are always there for me.

Finally, I could not have completed this dissertation without the support of my friends, Jiankun Su, who provided stimulating discussions as well as happy distractions to rest my mind outside of my research.

SYMBOLS AND ABBREVIATIONS

Abbreviations

CSLF	Cellulose solvent-based lignocellulose fractionation
SHF	Step-by-step saccharification and fermentation technology
SSF	Simultaneous saccharification and fermentation technology
SSCF	Assimilative saccharification and co-fermentation technology
CBP	Combined biotechnology. Processing Technology
COD	Chemical Oxygen Demand
CO2	Carbon dioxide

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1 Introduction

With the rapid growth of the global economy and the continuous development of industrialization, the world's energy consumption has increased significantly. The consumption of fossil fuels such as coal and oil , the environmental pollution and resource shortages caused by their use have made the development of green renewable alternative energy a hot topic in global energy sustainable development research. According to data from the International Energy Agency (IEA), the total energy usage of global renewable energy reached nearly 90 million TJ in 2021, of which biomass energy contributed more than 55%. On top of this, biofuel demand is expected to increase by 38 billion liters from 2023 to 2028, reaching 200 billion liters, an increase of nearly 30% over the previous five years, with renewable diesel and ethanol accounting for a third of this growth of two. The remainder is biodiesel and biojet fuel. [1]. As a key technology for converting biomass energy, biomass biochemical conversion technology has attracted much attention because of its environmental protection, sustainability and low carbon emissions. By utilizing microorganisms, enzymes or other organisms, wastes from agricultural production and processing can be efficiently converted into liquid or gaseous fuels such as ethanol, biodiesel, and methane. Among them, biochemical conversion technology mainly includes two major application technologies: fermentation and anaerobic digestion technology. [2]

As a key part of biomass biochemical conversion technology, biomass fermentation technology has a wide range of resource adaptability, has high utilization efficiency of waste biological resources, and can maximize the utilization of resources. In addition, through closed-loop recycling of carbon sources, biological fermentation technology helps build a sustainable ecosystem and reduce carbon footprint, thereby mitigating the harm caused by global climate warming. These mainly benefit from the flexibility of microbial metabolism and high conversion efficiency during fermentation [3].

Through pretreatment and hydrolysis (acid, alkali or enzyme), wood residues, municipal solid waste, agricultural waste, energy crops and other organic matter are converted into simple sugars, fermented, and then purified to ultimately produce high-value biofuels and chemicals , such as bioethanol, organic acid, among others. According to statistics in 2023, global bioethanol production is 111.7 billion liters [4], accounting for more than half of all biofuel production. At present, the raw materials for the production of bioethanol mainly come from corn, sugar cane, sugar beet and wheat [5], and the production cost is about 16.2 euros/gallon, which is twice the price of gasoline (7.3 euros/gallon) [6]. In order to reduce production costs, using lignocellulosic waste as raw material to produce fuel ethanol has become the main development direction of ethanol production in the future. It is expected that through the development of technology, the cost of ethanol can be further reduced to 8.6 euros/gallon. It may increase the world's fuel ethanol production while reducing costs, thus promoting the large-scale application of fuel ethanol.

On the other hand, biomass fermentation technology still faces some challenges and limitations. For example, waste treatment during the fermentation process, screening and improvement of microbial strains, and optimization of fermentation process parameters still need to be continuously explored and researched. With the continuous advancement and innovation of science and technology, research on the microbial metabolism mechanism, precise control of fermentation conditions, and improvement of product purity during biomass fermentation will continue to be in-depth [7]. At the same time, the combination of biomass fermentation technology and other clean energy technologies, such as solar energy and wind energy, which is expected to improve the overall utilization efficiency of biomass energy and promote the rapid development of the biomass energy industry.

Combined with the above background, this article will analyze and study the current situation and future development prospects of biomass fermentation. Taking the fermentation of lignocellulose to produce fuel ethanol as an example， investigate the progress made by current biomass fermentation technology in biomass resource

utilization, fermentation process, process optimization, and screening and improvement of microbial strains. And analyze its challenges, opportunities and development prospects in practical applications.

2 Overview of biomass fermentation technology

2.1 Raw materials for biomass fermentation

There are many types of raw materials for biomass fermentation. According to their different sources and properties, they can be divided into lignocellulose-containing raw materials, sugar- and starch-containing crops, algae, and plastics [8]. Lignocellulosic biomass contains on average 43% cellulose, 27% lignin, 20% hemicellulose and 10% other components [8]. Cellulose is a linear polymer composed of D glucose units linked together through β-1,4 glycosidic bonds, with a high degree of polymerization. Hemicellulose is a heteropolysaccharide, mainly composed of pentose sugars (xylose, arabinose), hexose sugars (mannose, glucose, galactose) and sugar acids. It is branched and amorphous and is easily hydrolyzed. Lignin is an aromatic compound with a highly complex structure in which phenylpropane units are linked together through ether bonds. The cell wall has high mechanical strength and is difficult to hydrolyze. [9] Utilizing lignocellulose for biomass fermentation can alleviate agricultural waste treatment management challenges. Reduce air pollution caused by excessive emissions of toxic gases and greenhouse gases caused by agricultural waste burning [10]. Moreover, compared with fossil resources, lignocellulosic biomass has wide sources and low price. This all contributes to the development of circular economy and economic growth. Raw materials containing lignocellulose mainly include crop residues (such as sugarcane bagasse and sweet sorghum bagasse, corn stover, different types of straw, rice husks, olive pits and paper pulp), hardwoods (such as aspen, poplar), softwoods (such as pine , spruce), cellulosic waste (such as waste paper and recycled paper sludge), herbaceous biomass (such as

alfalfa hay, switchgrass and other types of grass) and municipal solid waste [8]. Starch-based crops mainly include cereal crops (e.g. corn, barley, wheat or sorghum) and root/tube crops (e.g. cassava, potato, sweet potato, Jerusalem artichoke, cactus or arrowroot) [8]. Sugar-containing crops mainly include sugar cane and sugar beets [8].

2.2 Selection of fermentation microorganism

To select microbial microorganism,the genetic stability, growth rate, tolerance, metabolic pathways, ethanol production and other factors, are considered[11]. Commonly used strains include *Escherichia coli*， *Zymomonas mobilis*， *Bacillus subtilis*， *S. cerevisiae*, among others.Among them,*Saccharomyces cerevisiae* is the most commonly used strain because its growth conditions are relatively relaxed, it can grow in a wide temperature range, and it has high ethanol tolerance[11]. *Zymomonas mobilis* is a Gram-negative bacterium known for its rapid sugar fermentation rate and high ethanol production efficiency with fewer by-products during the fermentation process. This bacterium is mainly used in the fermentation of glucose, fructose and sucrose[12]. *Escherichia coli* is also a Gram-negative bacterium that grows rapidly and is relatively simple to manipulate genetically. Through genetic engineering, E. coli is able to fermentally utilize a variety of sugar sources, including glucose, xylose, and other biomass-converted sugars[12]. *Bacillus subtilis* is a Gram-positive bacterium. Although it does not directly produce ethanol itself, B. subtilis is able to produce a large number of enzymes. These enzymes help break down cellulose and hemicellulose, providing simple sugars for other ethanol-producing microorganisms such as Saccharomyces cerevisiae[12]

.By improving microbial strains, the efficiency and yield of ethanol fermentation can be improved and production costs can be reduced, thus promoting the further development and application of biomass fermentation ethanol production technology. At present, there are three main strategies for improving microbial strains, namely natural strategy, recombinant strategy and co-culture strategy.

- Natural strategy

The natural strategy refers to modifying some anaerobic microorganisms to adapt to the requirements of fermentation production. There are some microorganisms in nature that can directly convert biomass into ethanol, such as *Candida, Clostridium, Fusarium oxysporum, Neurospora,* among others[13]. The principle is that these strains can work in an aerobic environment and survive in an anaerobic environment. That is, the main activity under aerobic conditions is to produce cellulase to degrade cellulose and then produce soluble sugars, while under anaerobic conditions Metabolic production activities are carried out under these conditions. At present, some fungi and thermophilic microorganisms with high temperature resistance and stronger ability to produce enzymes and ethanol have become research hot spots in recent years. Among them, the *extracellular cellulase* secreted by Trichoderma reesei is a complex cellulase composed of endoglucanase, exoglucanase and β-glucosidase[14]. It has high enzyme activity, With the advantages of good stability and strong adaptability, it is currently the most widely used cellulase.

- Gene recombination strategy

The recombination strategy is to express a series of cellulase genes such as exoglucanase and endoglucanase through genetic recombination, so that microorganisms can use cellulose as a carbon source and convert sugars derived from cellulose into large amounts[13]. The purpose of producing ethanol through partial or complete fermentation is to quickly improve the phenotype of cells. The improved cells have three major advantages: first, they can enhance the ability of microorganisms to synthesize active products; second, they can activate the expression of silent genes, thereby producing new The third is that it can enhance the utilization rate and tolerance of microorganisms to substrates [15]. At present, microorganisms used to express exogenous cellulase and hemicellulase genes to produce ethanol mainly include *Escherichia coli, Pichia pastoris, Saccharomyces cerevisiae.* [16]. According to reports, genes encoding glycoside hydrolases, xylan degrading enzymes and arabinose degrading enzymes encoded by different strains

have been introduced into *Saccharomyces cerevisiae* in order to enable it to utilize cellulose, hemicellulose, cellobiose, Carbon sources such as xylan and arabinose can efficiently utilize pentose and hexose sugars at the same time and produce ethanol[17].

- Co-cultivation strategy

Cellulose saccharification liquid contains a variety of sugars, such as galactose, arabinose, maltose, lactose, xylose and glucose. It is difficult to completely metabolize and utilize it using a single microorganism. However, the use of co-culture method can improve the utilization of substrates. efficiency. The so-called co-culture strategy has two meanings: one refers to the different types of microorganisms present in the fermentation broth that utilize different types of sugar substrates, such as Clostridium thermocellum that can only utilize hexose sugars and microorganisms that can utilize pentose sugars. Co-culture can avoid carbon source competition between different organisms and maximize ethanol production. Second, it means that microorganisms with different characteristics cooperate with each other to enhance the fermentation effect. Shrestha et al. used the white rot fungus Chrysosporium to perform solid-state fermentation with the white rot fungus Chrysosporium and Saccharomyces cerevisiae. The mixed bacteria degraded fiber raw materials at 37°C for 3 days. The results showed that co-culture of Saccharomyces cerevisiae and Chrysanthemum cerevisiae can Ethanol production increased by 3 g per 100 g of substrate [18].

2.3 Biomass fermentation process and key technologies

Taking the production of bioethanol from lignocellulose-containing raw materials as an example, the process of biomass fermentation (see Figure 1) includes raw material collection, pretreatment, removal and recovery of pollutants, material crushing, enzymatic saccharification (enzyme hydrolysis), organic fermentation, biological

Ethanol recovery and purification, gasification of solid residues (providing process heat) and wastewater treatment [19].

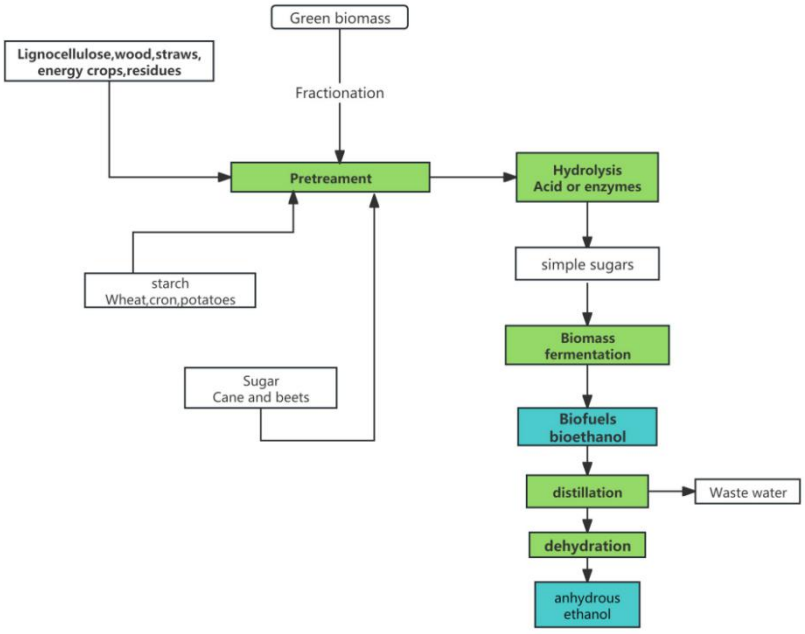

Figure 1.Production of fermentation inputs from cellulosic material and starches

2.3.1 Pretrement

In the process of converting lignocellulose into ethanol, pretreatment is a crucial step. The purpose of the pretreatment process for preparing bioethanol using biomass fermentation technology is to reduce the crystallinity of cellulose, increase the specific surface area of the substrate, break the barrier between hemicellulose and lignin, and make cellulose more susceptible to contact and reaction with hydrolytic enzymes [20].

Traditional pretreatment methods can be divided into four categories (see Figure 2), including physical, chemical, physical and chemical combination and biological methods [22]. Figure 2 shows the pretreatment process of lignocellulose in detail. Physical pretreatment can reduce particle size by disrupting the surface structure of lignocellulosic biomass and using shear forces. Common physical pretreatment

methods include extrusion, grinding, mechanical pretreatment, irradiation and other methods. Among them, grinding is mainly used before other pretreatment methods, as it can destroy the internal structure of biomass and reduce the crystallinity of cellulose, thereby improving the hydrolysis conversion rate of cellulase [23]. Under normal conditions, equipment for physical pretreatment has low pollution and is simple to operate. However, high cost and high energy consumption are the main shortcomings of this technology, so physical pretreatment usually needs to be combined with chemical pretreatment. Compared with physical pretreatment methods, chemical pretreatment is the most widely used method on a commercial scale and is very promising because it can effectively degrade more complex structural substrates. The structure of lignocellulose is disrupted, usually under the action of chemicals, through interactions with intramolecular or intermolecular bonds of cellulose, hemicellulose and lignin. Common chemical pretreatment methods include acid method, alkali method, organic solvent and ionic liquid pretreatment method [23]. In biological pretreatment, natural microorganisms (fungi, bacteria and microbial flora) are used to disrupt the cell walls of lignocellulosic materials. Biological pretreatment mainly refers to the use of degradative enzymes produced by microorganisms such as bacteria, fungi, and actinomycetes to promote the degradation of various components in lignocellulose. Biological pretreatment can degrade lignocellulosic substrates under mild conditions. It has the characteristics of wide range of action, strong adaptability, low investment cost and low energy demand. It has great advantages in the degradation of straw biomass and is a kind of Environmentally friendly pretreatment method. However, biological pretreatment technology is still in the development stage as a whole. There are few types of efficient microorganisms currently discovered, and the pretreatment cycle is long and the hydrolysis rate is low. In addition, the current single strain pretreatment technology has shortcomings, and the complete degradation of lignocellulose depends on the joint action of the microbial community. At present, there are two focuses in the research and development of biological pretreatment technology: one is to screen strains that can produce efficient degradation combination enzymes, and the other is to modify lignocellulose-degrading strains

through genetic modification technology to improve the substrate degradation effect [21].

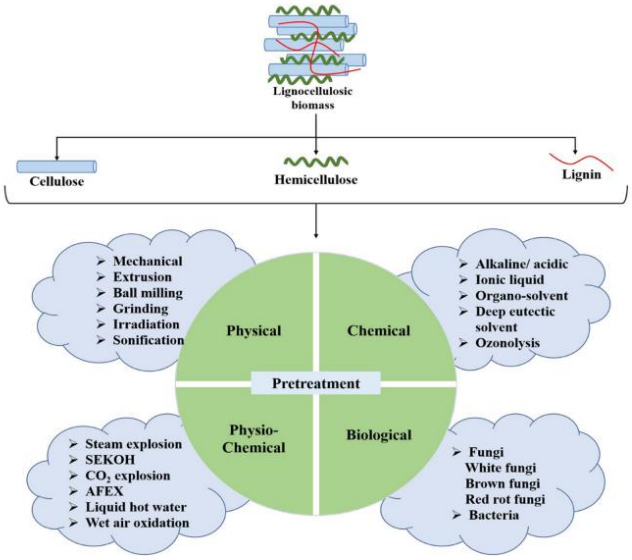

Figure 2.Pretreatment process of lignocellulose[22]

Currently, there is an advanced pretreatment method in the world called Cellulose solvent-based lignocellulose fractionation (CSLF), which usually includes acid-mediatedfractionation and Ionic liquid-based fractionation (ILF). This method is designed to be used under mild operating conditions Fractionating lignocellulose at temperatures (e.g. 50 ℃ and atmospheric pressure) to produce value-added by-products reduces the cost of bioethanol production and helps reduce the amount of enzymes required for subsequent enzymatic hydrolysis. [22]

2.3.2 Enzymatic hydrolysis and fermentation

The pretreatment step changes the structure of the lignocellulosic biomass, making the structural carbohydrates readily available to hydrolytic enzymes in subsequent processes. The pretreated biomass is further hydrolyzed into simple sugars by complex cellulase. Complex cellulase is composed of three enzymes, which work synergistically and are indispensable. First, endoglucanases (endoglucanases) hydrolyze the glycosidic bonds of cellulose substrate molecules to produce small

glucans. Exoglucanases (exoglucanases) then hydrolyze the glucan from its chain ends into cellobiose and other smaller oligosaccharides. Finally, β-glucosidase hydrolyzes cellobiose into glucose. In addition to this, commercial enzyme mixtures may also contain lignin-degrading coenzymes and lignin-modifying enzymes to increase hydrolysis rates and monosaccharide yields [21]. Among them, the hydrolyzate of lignocellulose mainly consists of hexose (glucose) and pentose (xylose and arabinose) [24]. These simple sugars are then fermented under the action of different microorganisms (yeast, fungi or bacteria) to convert into ethanol, acid and gas .Equation 1 shows the conversion of simple sugars into ethanol by *Saccharomyces cerevisiae*.

$$C_6H_{12}O_6 + yeast \rightarrow 2C_2H_5OH + 2CO_2 \quad (1)$$

There are currently 5 main saccharification and fermentation processes in the world (see Figure 3), including direct fermentation technology, step-by-step saccharification and fermentation technology (SHF), simultaneous saccharification and fermentation technology (SSF), assimilative saccharification and co-fermentation technology (SSCF) and combined biotechnology. Processing Technology (CBP)[20].

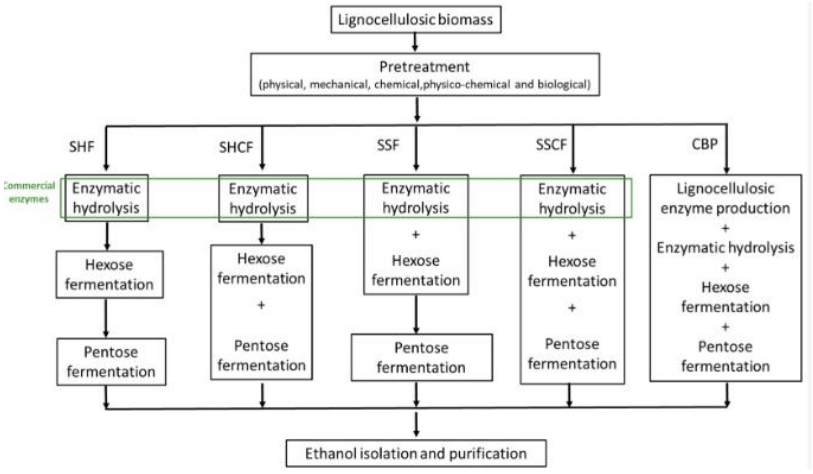

Figure 3.Process configuration for bioethanol production from lignocellulosic biomass[22]

- Direct fermentation technology

Biomass direct fermentation technology is mainly based on fiber-decomposing bacteria to ferment cellulose. Currently, Clostridium thermocellum and Clostridium thermosulfide[7] are used to ferment the pretreated substrate with mixed bacteria, and the ethanol yield can reach 70%.

- Distributed saccharification and fermentation technology (SHF)

The SHF method is also called the two-stage hydrolysis and fermentation method, which is a traditional fiber ethanol production method. In the SHF process, the fiber substrate is first saccharified by cellulase and degraded into fermentable monosaccharides, and then fermented by yeast to convert the monosaccharides into ethanol. The main advantage of the SHF method is that the enzymatic hydrolysis and fermentation processes can be carried out under their respective optimal conditions. The optimal temperature for cellulase hydrolysis is generally 45-50°C, while the optimal growth temperature for most fermenting microorganisms is 30-37°C[7]. The main disadvantage of the SHF method is that the main products of hydrolysis, glucose and cellobiose, feedback inhibit the degradation process of the substrate by cellulase. That is, the accumulation of glucose and cellobiose will inhibit the activity of cellulase, ultimately leading to a reduction in enzymatic fermentation efficiency.According to [25],a study of sludge paper using Saccharomyces cerevisiae with SHF technology was possible to obtain a conversion rate of 34.2% of ethanol and 190 g kg -1 of dry paper sludge. Higher ethanol production of 65% were observed for biomass waste using the SHF technology with Trichoderma viride[26].

- Synchronized saccharification and fermentation technology (SSF)

The SSF method is an upgrade of the SHF method. It allows the pretreatment product to undergo enzymatic hydrolysis and fermentation processes at the same time in the same container, that is, the cellulose enzymatic hydrolysis and saccharification process and the ethanol conversion process are carried out simultaneously. The SSF method allows the glucose obtained by enzymatic hydrolysis to be immediately utilized by fermenting microorganisms and converted into ethanol, effectively

reducing the inhibitory effect of glucose on cellulase products during the enzymatic hydrolysis process, reducing the dosage of cellulase, and shortening the reaction cycle. The reduction in the number of reactors reduces investment costs. Since the glucose produced by enzymatic hydrolysis is promptly metabolized by Saccharomyces cerevisiae and converted into ethanol, the glucose concentration in the reaction system is maintained at a low level. The presence of the product ethanol puts the fermentation process in an anaerobic environment, greatly reducing the chance of bacterial contamination, thus increasing the ethanol yield. . The disadvantage is that the temperatures of enzymatic saccharification and fermentation are not coordinated, and the optimal temperature conditions for the two reactions cannot be met at the same time, so that the two-step reactions of saccharification and fermentation cannot be carried out in the optimal state of the microorganisms.According to [27], a study on the fermentation of sweet sorghum residue using *Trichoderma reesei* and *Saccharomyces cerevisiae* through simultaneous saccharification and fermentation (SSF) technology can obtain 0.33 g bio-ethanol/g substrate. The higher ethanol yield is 0.36 g bio-ethanol/g substrate was observed for tofu waste using the SSF technology with co-culture of mold and yeast[28]

- Assimilation, saccharification and co-fermentation technology (SSCF)

The SSCF method can make full use of the substrate, thereby increasing the ethanol yield. The pentose sugars produced by hemicellulose during the degradation of lignocellulosic raw materials and the six-carbon sugars produced by cellulose are fermented in the same reaction system to produce ethanol. This process requires a fermentation strain that can metabolize pentose sugars. The SSCF process reduces product feedback inhibition during the hydrolysis process, and the technology is integrated into the fermentation process of pentose sugars, improving substrate utilization and ethanol yield. According to [29],a study on batch enzymatic hydrolysis of banana and pineapple waste using *Aspergillus terreus* (fungus) and *Kluyveromyces marxianus* (thermotolerant yeast) via simultaneous saccharification and co-fermentation (SSCF) technology, achieving 0.35 g/L and 0.27 g/L respectively the

maximum ethanol concentration. According to [30], *Escherichia coli KO11*, *Saccharomyces cerevisiae*, cellulase and xylanase were used to simultaneously ferment oil grass pretreated with low moisture and anhydrous ammonia by SSCF technology. The ethanol yield reached 74% of the theoretical yield.

- Combined Bioprocessing Technology (CBP)

The main obstacles in the bioconversion process of lignocellulosic raw materials are that the enzymatic activity of cellulase is not high and the amount of enzyme required for unit cellulose conversion is too high, resulting in low enzymatic hydrolysis efficiency and high cost. [20] In order to reduce production costs during the fermentation process, the CBP process came into being. The CBP process is a bioprocessing process that integrates the production of cellulases and hemicellulases, cellulase hydrolysis and saccharification, and fermentation of pentoses and hexoses to produce ethanol in a single system under the action of a single or combined microbial community [31]. The process flow is simple and easy to operate. The substrate is converted into ethanol in one step under the efficient metabolism of microorganisms, which is beneficial to reducing the cost of the entire bioconversion process.According to[32] a study on the use of combined yeast strains to transform corn stover through CBP technology, produced 1.61 g/L ethanol which achieved 64.7% of the theoretical ethanol yield during 144 h from steam-exploded CS.

2.3.3 Bioethanol recovery and purification

After monosaccharide fermentation, ethanol needs to be recovered from the fermentation broth and purified. First, bioethanol is separated and recovered from the fermentation product through a distillation process. Subsequently, the purity of the ethanol is further improved through distillation, and the difference in boiling points is used to separate the ethanol from impurities. Next, a dehydration process is applied to

reduce the water content in ethanol and increase ethanol concentration and production efficiency. Finally, freeze crystallization technology is used to crystallize and separate ethanol at low temperatures to improve product purity. In addition, membrane separation technologies such as reverse osmosis, pervaporation, and activated carbon adsorption are also commonly used in the purification process of ethanol to remove impurities through selective separation and improve the purity and quality of ethanol. These recovery and purification steps work together to ensure high-quality, high-purity bioethanol

2.4 Comparison of biomass fermentation and anaerobic digestion technologies

Biomass fermentation technology and anaerobic digestion technology are both biomass biochemical conversion technologies, but there are significant differences in conditions, products, microbial types, costs, and applications. Biomass fermentation technology mainly produces organic compounds and is suitable for aerobic or anoxic conditions. The advantage of this technology is that it can utilize a variety of biomass resources to produce diversified products. It is widely used in food, beverages, biofuels and other fields, and has high flexibility and customizability. At the same time, by optimizing the fermentation process and improving microbial strains, production efficiency and product quality are getting higher and higher, and the production process is relatively stable and environmentally friendly. However, biofermentation technology requires a large amount of energy input, and its waste conversion efficiency is affected by factors such as raw material complexity and microbial efficiency. For example, when the raw material contains a large amount of lignin or other components that are difficult to decompose, complex pretreatment is required, which increases energy consumption and cost. Furthermore, biofermentation processes may be affected by the production of inhibitory by-products (such as high ethanol concentrations) that can inhibit microbial activity and thus reduce conversion

efficiency. In contrast, anaerobic digestion technology can use anaerobic microorganisms to directly convert organic waste into methane and digestate under anoxic conditions, with low energy consumption [33]. However, anaerobic digestion technology has relatively low adaptability to substrates, and may be inefficient due to inhibition of microbial activity when processing organic waste with high concentrations or complex components. At the same time, the reaction speed of anaerobic digestion is usually slower than that of biological fermentation, and irritating gases such as hydrogen sulfide and methyl mercaptan are easily produced during the process [34]. From the perspective of future prospects, although anaerobic digestion technology has broad application prospects in processing organic waste and producing renewable energy, biological fermentation technology still plays an important role in the production of specific organic compounds, such as alcohols, organic acids, among others. [31]

3 Environmental conditions and disposal of fermentation processes

3.1 Control and optimization of the fermentation process

In the process of ethanol production through biomass fermentation technology, the control and optimization of the fermentation process are the key to ensuring high yield and high-quality ethanol production. In order to achieve this goal, fermentation conditions need to be controlled, including temperature, pH control, oxygen supply, and nutrient supply control. First of all, it is necessary to maintain an appropriate fermentation temperature. Generally, the fermentation temperature is set between 30-35°C.[35] Too high or too low temperature will affect the activity and growth rate of fermentation microorganisms. Secondly, the pH value of the fermentation broth (about PH4-6,the most suitable is PH5)[35] needs to be adjusted to a suitable range to

ensure the activity of fermentation microorganisms and fermentation efficiency. Thirdly, the fermentation of ethanol is an aerobic process, and sufficient oxygen supply can be ensured through aeration equipment or stirring equipment. Finally, adequate supply of nutrients should be ensured in the fermentation broth to promote the growth of fermenting microorganisms and ethanol production, including carbon sources (such as glucose), nitrogen sources, minerals, and vitamins.

In addition, optimizing the fermentation process is also very important. While selecting the appropriate reaction vessel and stirring equipment and strictly controlling the fermentation time, the ethanol concentration, yeast cell density, oxygen content, pH value, and temperature [36]in the fermentation broth should be detected in real time, and the fermentation conditions should be controlled and adjusted in a timely manner to ensure the fermentation process. stability and maximization of ethanol production. In addition, it is necessary to pay attention to biosafety control and take necessary disinfection measures to prevent contamination of the fermentation system by exogenous microorganisms.

3.2 Fermentation waste recycling

The biological fermentation process will produce a large amount of waste, such as ethanol waste tank liquid, waste solid residues and carbon dioxide emissions. If not handled properly, it will have a very bad impact on the environment. Therefore, fermentation waste needs to be recycled to promote the sustainable development of the fermentation process.

3.2.1 Bioethanol wastewater treatment and recycling technology

According to data, for every 1 ton of ethanol produced by an ethanol plant, approximately 13 to 16 tons[37] of ethanol waste tank liquid is discharged. This kind of organic wastewater is generally acidic, has high concentration, high temperature,

and high viscosity, with COD as high as 50-70g/L[37]. It is the main source of pollution in the ethanol industry. The organic matter in the waste tank liquid is an aerobic substance, and discharging it into the water will cause a lack of dissolved oxygen in the water body. At the same time, organic matter causes corruption through the decomposition of anaerobic bacteria in the water, producing methane, hydrogen sulfide, ammonia and other odorous gases, making the water deteriorate and stink. At present, the ethanol wastewater treatment method with the best treatment effect and the highest economic benefits in the world is wastewater reuse technology. The process is to first remove the coarse residue from the ethanol waste tank liquid through centrifugal separation, and then undergo point catalysis-biological flocculation treatment[11] to remove the harmful effects on yeast growth. and harmful substances from ethanol fermentation, and the effluent is reused for ethanol fermentation. Factual results show that when the effluent treated in this way is used as mixing material and saccharification water, the ethanol fermentation speed and yield are significantly improved, thus improving the economic benefits. Not only does it reduce costs, it also solves the problem of environmental pollution.

3.2.2 Treatment and recycling of solid waste residues

Waste solid residues generated during biological fermentation processes are usually lignin-rich solids and may also include partially hydrolyzed cellulose and hemicellulose [38]. These residues can be processed and utilized in a variety of ways. First, they can be used as feedstock for biomass energy in the production of combined heat and power (CHP) to generate heat and electricity. Secondly, waste solid residues are rich in nutrients and can be used as raw materials for animal feed to increase the nutritional value of the feed. Thirdly, after proper treatment, the residue can also be used as a soil conditioner to improve soil structure and increase fertility. Finally, microorganisms in waste solid residues can be used as starter cultures in the biomass fermentation process to improve fermentation efficiency and yield.

4 Current application status of biomass fermentation

4.1 Application of biomass fermentation in energy production

Biomass fermentation has a wide range of applications, not only in the pharmaceutical industry and food industry, but also in the chemical industry. Among the most noteworthy are its applications in energy production.The most extensive one is to make fermented ethanol into fuel ethanol, which is then mixed with gasoline. As a high-octane fuel, ethanol, with an octane number as high as 115 [39], can replace lead-containing additives that pollute the environment to improve the anti-explosion performance of gasoline. It can also be used as a good gasoline oxygenator and blending agent to improve combustion efficiency, reduce carbon precipitation and the generation of incomplete combustion pollutants such as oxidized carbon in the engine, thereby reducing CO2 and particulate matter emissions in automobile exhaust. Since the ethanol produced by biomass fermentation is not directly suitable for industrial production, in order for the ethanol to be used as fuel, the yeast solids and most of the water must be removed. After fermentation, ethanol can be separated by distillation to form a low waste point water-ethanol azeotrope (also known as aqueous ethanol, with a purity limit of 95-96%[9]. Aqueous ethanol can be used as a fuel alone, but it is not miscible with all proportions of gasoline , so further dehydration is usually required to facilitate mixing and combustion with gasoline in gasoline engines. In the dehydration process, molecular sieves can be used to remove water from fuel ethanol, and the final purity reaches more than 99%. Anhydrous ethanol is available in volume fractions of 5%, 10%, and 15% Mixed with gasoline (represented by E5, E10 and E15 respectively).

At present, the countries that use the most ethanol gasoline in the world are Brazil and the United States. Table 1 shows the world's fuel ethanol production in 2023. The United States ranks first in the world with a production of 15,620 million gallons,

accounting for 53% of the world's fuel ethanol production. Followed by Brazil, accounting for 28% of the world's fuel ethanol production.Brazil uses sugar cane as raw material to develop fuel ethanol technology. As early as 2001, Brazil has fully realized commercial fuel ethanol production.Fuel ethanol supplies about 40% of its domestic vehicle fuel needs [9]. In the past 20 years, the United States has reduced the cost of fuel ethanol production by more than half through continuous development and updating of technology. At present, the cost of fuel ethanol production in the United States has become competitive with gasoline.

Table 1.World Fuel Ethanol Production 2023(Mil.Gal.)[39]

Region	% of World Production	2023
United States	53%	15620
Brazil	28%	8260
European Union	5%	1440
India	5%	1430
China	3%	950
Canada	2%	460
Thailand	1%	370
Argentina	1%	300
Rest of World	3%	760
Total	100%	29590

In addition, the non-fermentable substances and remaining organic waste produced after purification of fermentation products can be used as raw materials for traditional combustion/steam boiler systems to perform cogeneration to generate electricity and heat.

4.2 Application of biomass fermentation in industrial fields

Waste produced by industry can have a harsh impact on the environment. Among them, the most harmful is industrial wastewater. First of all, industrial wastewater contains a large number of pollutants, such as heavy metals, toxic chemicals, suspended solids, organic matter, among others[40]. If these wastewater are directly discharged into natural water bodies without treatment, it will cause water pollution and affect the survival and reproduction of aquatic organisms. It may even pose a threat to human health. Secondly, industrial wastewater may also seep into the ground, pollute groundwater, and destroy groundwater resources. In addition, the discharge of industrial wastewater may also cause soil pollution, affecting the growth of crops and the balance of the soil ecosystem[40]. When treating industrial wastewater, biomass fermentation technology is usually combined with other biological treatment methods, such as biological filters and activated sludge methods.[41][42]. Biomass fermentation can be used in the pretreatment stage of wastewater. Initial decomposition of organic matter in wastewater into substances that are more easily biodegradable. The fermented wastewater is then introduced into the activated sludge system for further degradation[41]. The wastewater and the returned activated sludge enter the exposure tank together to form a mixed liquid. Air enters the sewage through the air diffusion device at the bottom, increasing the dissolved oxygen content and stirring the mixed liquid. Activated sludge absorbs organic pollutants and oxidizes and decomposes them under sufficient oxygen conditions. The sewage after settling in the sedimentation tank is then discharged into the biological filter[42]. The organic matter in the wastewater is adsorbed by the biofilm and undergoes oxidation to complete the final degradation. This helps to improve the degradation rate of organic matter in wastewater.

5 Development prospects of biomass fermentation

5.1 Development Trend of Biomass Fermentation Technology

As a key technology for bioenergy production, biomass fermentation technology can improve production efficiency (improvement of technology and processes), realize diversified products, promote resource recycling, promote green and sustainable development, continue technological innovation, and strengthen industry-university-research cooperation. showing obvious development trends. In particular, there is an obvious development trend for the improvement of fermentation technology.

5.1.1 Optimization and improvement of preptreatment technology

The ideal pretreatment should meet the following requirements. First, it should promote the formation of sugars and avoid excessive degradation of pentoses, which will facilitate subsequent enzymatic saccharification or sugar formation. Secondly, it should avoid the formation of by-products that inhibit subsequent hydrolysis and fermentation. produce. Thirdly, solid residues are reduced and the purity of separated lignin and hemicellulose is improved, allowing full utilization. Finally, economic costs and environmental protection requirements should be considered, and the size of the equipment should not be too large. For this reason, in the future, pretreatment technology will focus on developing in the direction of high raw material adaptability, high raw material separation efficiency, low energy consumption, low cost, less pollution, short treatment time, and simple processes.

5.1.2 Improvements in enzyme production technology

During the enzymatic hydrolysis process, excellent enzyme preparations can not only catalyze the occurrence of relevant reactions, but also greatly improve the conversion efficiency and effectively reduce production costs. However, the enzyme production efficiency in the existing technology is low and the production cost is high. To achieve major breakthroughs in the field of enzyme technology, we first need to find cellulose complex enzymes with high specific activity suitable for industrial production to solve problems such as poor enzyme stability and high production costs. Secondly, genetic engineering methods are used to modify cellulase molecules to achieve directed evolution. Apply microbial enzyme engineering technology to improve enzyme activity and develop cellulose complex enzymes with good thermal stability and high activity.

5.1.3 Development trends of fermentation technology

In recent years, major breakthroughs have been made in the process of fermenting lignocellulosic biomass to produce fuel ethanol. With the continuous development of molecular biology technology, the use of CBP technology to improve the performance of fermentation strains through genetic engineering has become a research hotspot [7]. In the biomass conversion process, the use of a bifunctional single strain that can produce both cellulase and fermentation to produce ethanol can effectively improve the substrate metabolism capacity of the fermentation strain, achieve high ethanol production, and shorten the reaction time. Continuous innovation of this technology is the future development trend of biomass energy. In recent years, researchers have introduced heterologous cellulase systems through genetic engineering into some fungal expression systems or bacterial expression systems that grow faster and are more mature in research, and have developed many genetically improved strains, which not only effectively improve The conversion efficiency of biomass also

effectively saves production time and production raw materials. With the continuous advancement of technology, the development of efficient transformation strains will enable this method to be applied to industrial production on a large scale, showing very broad application prospects.

5.2 Market prospects of biomass fermentation

5.2.1 Global fuel ethanol supply and demand

Global consumption of fuel ethanol has grown significantly over the past few decades. Partly due to policy impetus, coupled with the expansion of the global automobile population and increasing demand for high-octane fuel, the global fuel ethanol market is expected to see considerable growth from 2024 to 2031. It is expected to reach US$120.1 billion by 2030, with a compound growth rate of 3.2% [43], which also indirectly promotes the development of biomass fermentation technology.

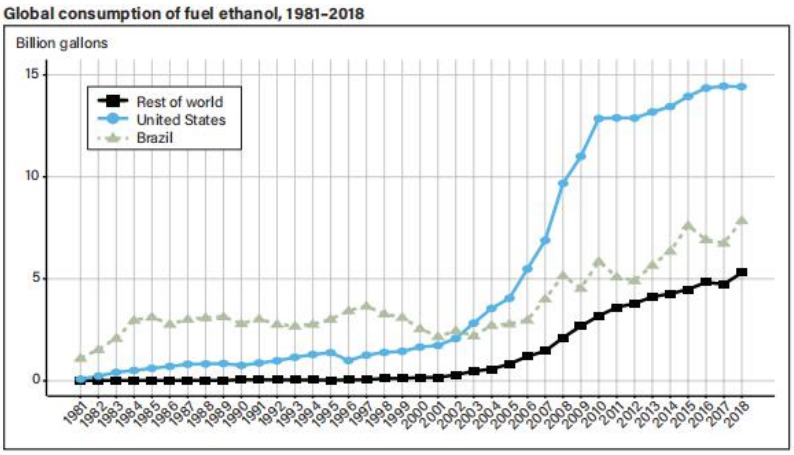

Figure 4.Global consumption of fuel ethanol.1981-2018[44]

Ethanol production in major ethanol-producing regions around the world is rising steadily, especially in North America and Asia. As can be seen from Figure 4. From 1981 to 2018, global fuel ethanol consumption increased from 1.2 billion gallons to 27.6 billion gallons, an increase of 2203%. Fuel ethanol consumption outside the United States grew from 1.1 billion gallons to 13.2 billion gallons in 2018[43].

AEO 2022 predicts the U.S. fuel ethanol market in the next ten years. Forecasts indicate that domestic consumption of fuel ethanol will continue to increase in the United States over the next decade. Total domestic consumption of fuel ethanol in motor gasoline and E85 will increase from approximately 13.9 billion gallons in 2021 to 14.7 billion gallons in 2030. Compared with 2018, consumption in 2030 will increase by approximately 1.7%. At the same time, net exports are expected to reach 2.1 billion gallons in 2030 (see figure 5). That's an increase of 27%, 65% and 78% respectively from 2018, 2019 and 2021 levels. U.S. fuel ethanol production is expected to be 16.3 billion gallons in 2030, an increase of 1.4%, 3.4%, and 8.7% from 2018, 2019, and 2021, respectively.[45] In the future, both supply and demand are expected to grow steadily.

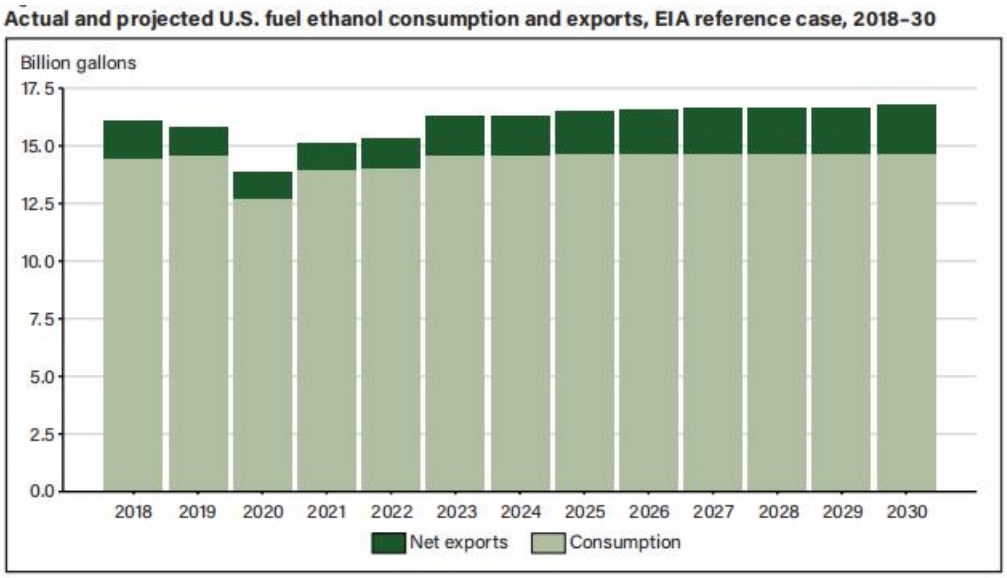

Figure 5.Actual and projected U.S. fuel ethanol consumption and exports, EIA reference cases,2018-2030.[45]

The size of the increase is related to future global economic development, oil prices and oil and gas supply. Different results may occur under different scenarios. Under the future scenario of rapid global economic growth, fuel ethanol consumption may have the largest increase (see figure 6). From 2021 to 2030, the increase is expected to increase from 188 million gallons under the "low economic growth" scenario. to 1.4 billion gallons under "high economic growth."

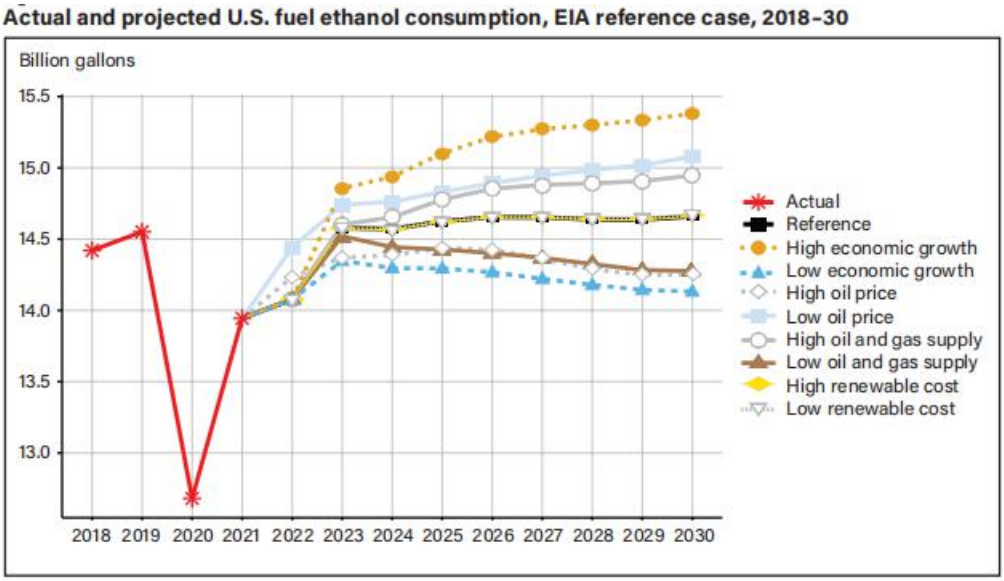

Figure 6.Actual and projected U.S.fuel ethanol consumption,EIA reference case,2018-2030[45]

5.2.2 The development potential of cellulosic ethanol

From an economic perspective, the use of low- or zero-cost lignocellulose-containing wastes to produce fuel ethanol is most attractive. Not only can the feedstock be cost-effective, but the non-fermentable biomass and remaining organic waste (such as sewage sludge) produced after purification of fermentation products can be used as feedstock for traditional combustion/steam boiler systems, thereby converting into electricity and heat.The generated heat energy and 75% of the electrical energy are used for ethanol production[46], which is expected to meet the total energy demand of the ethanol production process. While achieving energy self-sufficiency, reducing energy consumption and costs, the remaining 25% of electricity can also be exported to the public grid, about 3,000 GWH, accounting for 2.5% of the Netherlands' annual electricity generation (the total Dutch electricity generation in 2023 will be 120,000GWh[47]). Help the Netherlands raise revenue in disguise. In this regard, the Netherlands, as a leading country in environmental policy and sustainable technologies, can provide feasibility analyses and success stories to other countries.

Table 2 The potential scale of ethanol production from some biomass waste in the Netherlands[46]

Waste stream			Bioethanol production		Heat and electricity production	
Type	[kt/yr]	[kt dry/yr]	[PJ_{th}/yr]	[ton/yr]	Electricity [GWh_e]	Heat[PJ_{th}]
organic domestic waste	3340	1340	3.2	122000	1400	6.2
straw	700	600	4.9	186000	270	1.2
verge grass	460	180	0.9	32900	170	0.7
forestry residue	2690	1350	12	458400	660	3
food industry residues	50000	10000	49.5	1887500	9500	42.8
Total	57190	13470	70.5	2686800	12000	53.9

Table 2 shows the potential scale of ethanol production in the Netherlands from some biomass wastes. The ethanol production as well as the thermoelectricity production are estimated based on the lignocellulosic content of each waste stream. It can be found that there are, in principle, large enough waste streams to support large-scale ethanol production. If the total amount of potential biomass waste available in the Netherlands is 120PJ_{th}/year, and the annual fuel consumption of Dutch automobiles is 400 PJ, then replacing 1% of the Dutch automobile fuel consumption with fuel ethanol would require using approximately 10% of the available potential biomass waste in the Netherlands[46]

5.2.3 The impact of policy support on the future market of fuel ethanol

Policies on fuel ethanol in various countries around the world focus on multiple considerations such as environmental protection and energy security, and encourage the production and use of fuel ethanol through legislation, subsidies, tax incentives and other means to reduce dependence on fossil fuels and reduce greenhouse gas emissions. Purpose. As the world's largest producer and consumer of fuel ethanol, the development of fuel ethanol in the United States cannot be separated from the promotion of policies. In 1978, the United States enacted the "Energy Tax Act" to reduce the personal income tax of fuel ethanol users and open up the application market. In 1979, the government formulated the "Fuel Ethanol Development Plan" and began to vigorously promote mixed fuels containing 10% ethanol. Since then, the maximum addition ratio has reached 15%.The "Clean Air Act" Amendment passed in 1990 requires that starting from the winter of 1992, 39 areas in the United States with excessive carbon monoxide must use oxygenated gasoline with an oxygen content of no more than 2.7m% (equivalent to adding 7.7v% ethanol)). The 2005 New Energy Act encourages the use of ethanol fuel. The U.S. government also implements income tax exemptions and financial subsidies for ethanol fuel production companies, and supervises companies and stakeholders including manufacturers, gas stations, and corn growers [48]. At the same time, the United States has increased its efforts to support technological innovation and develop cellulosic fuel ethanol. Similarly, Brazil is actively developing its ethanol industry by promoting Flex Fuel vehicles, setting legal mixing ratios, and providing production subsidies. In the EU, the government has promoted the production and use of ethanol and promoted the application of renewable energy in the transportation field through measures such as production subsidies, tax incentives and the Renewable Energy Directive. In the future, as countries attach importance to sustainable development, more policies will be implemented to support the sustainable and healthy development of the biomass fermentation industry and promote the growth of the fuel ethanol market.

6 Conclusion

As a green and clean technology, biomass fermentation technology has greatly promoted the transformation of the energy industry and promoted global sustainable development. This article uses lignocellulose as raw material to conduct biomass fermentation and produce bioethanol as an example. Analyze the current status and prospects of biomass fermentation technology. As a source of biomass fermentation, lignocellulose not only helps reduce waste emissions and consumption of limited fossil fuels, but also helps reduce production costs. At the same time, fuel ethanol, as the main product of biomass fermentation technology, has become an alternative solution to current fuel problems and has potential uses in transportation and industrial processes. It offers huge advantages over traditional liquid fuels. In recent years, achievements have been made in renewable biomass pretreatment, development of efficient cellulase, optimization of enzymatic hydrolysis process, upgrading of fermentation process, construction of fermentation strains that simultaneously efficiently utilize pentose and hexose sugars, and separation and purification. Bioethanol. Significant progress has been made. At the same time, it also faces many challenges and opportunities. The biggest challenge remains cost. In the future, bioethanol production costs could be reduced by utilizing, for example, biorefineries to more fully utilize renewable materials and produce more value-added by-products.

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