# Research progress in the resource reuse of distiller's grains

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Abstract: Distiller's grains are the main by-products produced in the process of brewing and ethanol production. Although they are rich in nutrients, they are highly susceptible to spoilage. Their unscientific utilization not only causes environmental pollution, but also leads to huge waste of resources. Therefore, it is urgent to carry out research on the resource utilization of distiller's grains. In recent years, various studies have been conducted on the resource utilization of distiller's grains both domestically and internationally. According to the utilization methods, it can be mainly divided into four categories: energy production, agricultural products, food, and chemical raw materials, including the production of biogas, animal feed, flour products, and succinic acid, to achieve the resource utilization of distiller's grains. This article reviews the nutritional composition, production and utilization status of distiller's grains, as well as the challenges and future development directions faced by the resource utilization of distiller's grains, in order to provide reference for the treatment and technical research of distiller's grains.

Keywords: Distillers' grains; Resource utilization; Nutritional composition; Technical study

#### 1. Introduction

Distiller's grains are solid by-products produced during the brewing process, with complex components, huge yield, high moisture content, high acidity, and easy decay. Improper treatment can seriously pollute the ecological environment. According to statistics, the annual output of Baijiu distillers' grains of Chinese Baijiu enterprises has reached more than 20 million tons<sup>[1]</sup>. However, currently, most enterprises still find it difficult to handle and utilize distiller's grains resources reasonably. Due to various limitations in transportation, treatment, and preservation, it is highly likely to cause serious environmental pollution and significant resource waste. Promoting the resource utilization of distiller's grains to turn waste into treasure and achieve green circular development will effectively solve the long-term environmental problems caused by brewing waste and bring considerable economic benefits to the entire society.

Except for water, most of the other components in distiller's grains are organic compounds, rich in a large number of nutrients such as cellulose, protein, starch, lipids, etc [2-4]. which have high utilization value and huge resource potential. If not utilized, it will pollute the environment. If it can be utilized, it can instead be used as a resource. Therefore, the vast majority of distiller's grains processing technologies are based on the resource reuse of distiller's grains. A large number of studies have reported different resource utilization pathways of distiller's grains, as shown in Figure 1, including measures such as producing fuel, feed, and organic fertilizer from distiller's grains<sup>[1-6]</sup>.

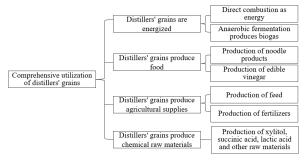


Figure 1: Approaches of comprehensive utilization of distillers' grains

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This article aims to summarize the current application status and development potential of distiller's grains in energy, agriculture, and food industries, as well as the opportunities and challenges for their application and development.

## 2. Composition Analysis of Distiller's Grains

Distiller's grains, also known as distiller's grains or distiller's grains, are one of the by-products produced during the brewing process. It is composed of the solid parts remaining during the fermentation and brewing process of grains (usually rice, wheat, corn, or grapes, etc.). As shown in Table 1, the main components of different types of distiller's grains are different. It is composed of organic substances such as water, rice husk, starch, sugar, protein, and organic acids, with a moisture content of about 60%. In addition, it also contains a small amount of inorganic components such as calcium, phosphorus, and potassium<sup>[4]</sup>. Distiller's grains are rich in carbohydrates, including starch, sugars, and cellulose. These carbohydrates endow distiller's grains with energy value and play an important role in food and feed production. Distiller's grains also contain abundant phenolic compounds, such as phenolic acids and flavonoids, which are related to antioxidant effects and are believed to be a source of antioxidant active substances<sup>[5-8]</sup>. Phosphorus and potassium in distiller's grains are important components of inorganic fertilizers, while organic substances such as lignin and cellulose starch are combustible and can be used as combustion energy<sup>[9,10]</sup>.

Fresh distiller's grains mainly have two parts: solid and liquid. The solid part contains proteins, insoluble carbohydrates, organic acids, yeast, inorganic salts, and phenolic molecules, while the liquid part is mainly composed of ethanol, acetic acid, and lactic acid. In addition to the main components mentioned above, its polyphenols are diverse and rich in content, possessing certain antioxidant capabilities, making it an ideal raw material for obtaining phenolic compounds<sup>[7-9]</sup>. In our previous research, it was found that the water content of distiller's grains was around 65%, and the total phenolic content reached 2.5mg/g. After solid-state fermentation, the phenolic content increased by 2-3 times, indicating good antioxidant activity<sup>[9]</sup>, as shown in Table 1.

Constituent	White	sake	Wine lees	Yellow wine	Brewer's
	lees			lees	Spent Grains
Dry matter(%)	88.47		89.72	86.8	91.12
Crude protein (%)	9.91		25.13	26.3	26.98
Crude fat(%)	4.83		6.7	4.51	10.2
Fiber(%)	28.57		18.72	27.2	17.36
Starch(%)	13.68		8.2	14.8	3.31
Neutral detergent fiber (%)	45.93		41.4	15.6	27.58
Acid detergent fiber (%)	53.43		12.2	36.4	46.16

*Table 1: Typical chemical composition (all constituents on a dry matter basis)* 

## 3. Research progress in the resource utilization of distiller's grains

## 3.1 Energy production from distiller's grains

Except for water, the vast majority of distiller's grains are organic compounds. Therefore, distiller's grains can be utilized as energy. There are various technologies for utilizing energy from distiller's grains. It can be directly burned, fermented as a raw material to produce biogas energy, used as a raw material to produce fuel ethanol, and gasified to produce gas<sup>[9-11]</sup>. Distiller's grains contain a large amount of easily degradable organic matter such as starch, protein, and organic acids. Under anaerobic conditions, through anaerobic microbial fermentation, easily degradable organic matter can be decomposed into biogas (a mixture of methane and carbon dioxide) and water. The main component of biogas is methane, which is the main component of natural gas.

The biogas production during anaerobic fermentation process includes two stages, namely the initial hydrolysis of raw materials to improve the availability of components, and the methane production stage where acidogenic microorganisms metabolize the substances released during the hydrolysis process into short chain fatty acids such as acetic acid, butyric acid, and propionic acid. Then these acids are converted into methane by methane bacteria. By utilizing this feature, anaerobic fermentation of distiller's grains can be carried out to produce biogas energy, while biogas residues can be used as fertilizers<sup>[12-16]</sup>. In

addition, research has shown that supplementing with Mg, Co, K, and a small amount of Ni and Fe can improve the stability of methane production<sup>[13]</sup>.

Distiller's grains can be directly used for anaerobic fermentation to produce biogas. The anaerobic fermentation of distiller's grains to produce biogas usually adopts a medium temperature process, which is easy to control. The biogas production of different types of distillers' grains is different, from high to low, it is corn fuel ethanol distillers' grains, soy sauce flavor Baijiu distillers' grains, Luzhou flavor Baijiu distillers' grains, cassava fuel ethanol distillers' grains. The research results of Mussatto et al. indicate that after 15 days of anaerobic fermentation of beer lees under laboratory conditions, approximately 100 grams of beer lees can be produced to produce 3476 cubic centimeters of biogas<sup>[14]</sup>. By pre-treatment of distiller's grains raw materials, the yield can be increased. Cater et al. studied the addition of anaerobic hydrolysis bacteria to distiller's grains and their impact on biogas production. Pure cultures or microbial communities are used to increase the hydrolysis of lignocellulosic compounds in waste grains and generate biogas. The increase in methane production is mainly achieved through single cultivation of Vibrio lignosum (+17.8%), followed by Vibrio lignosum and Fibrobacter succinate (+6.9%), as well as Clostridium cellulosum and Fibrobacter succinate<sup>[15]</sup>. And distiller's grains and molasses can be used in a 3:1 ratio under fungal fermentation conditions to produce biodiesel, and can be combined with distiller's grains to produce fuel ethanol equipment<sup>[16]</sup>.

Bioethanol is a substitute for gasoline, with high production efficiency and safety for the natural environment. Allowing the production of bioethanol from lignocellulosic waste has led to the consideration of using distiller's grains rich in these substances as raw materials for this production<sup>[17]</sup>. These raw materials are mainly composed of cellulose glucose polymer and hemicellulose polysaccharide mixture composed of glucose, mannose, xylose, arabinose, and lignin. In addition to producing biogas, distiller's grains can be used for solid-state fermentation to produce fuel ethanol, with a fuel ethanol yield of up to 4.18%. Using mold to decompose cellulose and then utilizing excellent yeast for fermentation can decompose some of the cellulose in distiller's grains and improve fuel ethanol yield<sup>[18]</sup>. Microwave assisted pretreatment of distiller's grains can also increase the yield of fuel ethanol<sup>[19]</sup>. In subsequent studies, compared to other microbial groups, the combination of Aspergillus oryzae and Saccharomyces cerevisiae achieved the highest bioethanol production from waste grains (ethanol production of 37 g/L after 1024 hours of fermentation)<sup>[20]</sup>.

## 3.2 Production of food from distiller's grains

## 3.2.1 Production of edible vinegar from distiller's grains

Distiller's grains contain relatively rich crude protein and fat, and the composition of amino acids in their proteins is relatively balanced, basically full valent. In addition, they also contain a large amount of flavor substances such as acids, esters, alcohols, carbonyl compounds<sup>[21]</sup>. Distiller's grains can be used as raw materials for the production of edible vinegar. Using distiller's grains as raw material to produce vinegar base, followed by acetic acid fermentation to produce edible vinegar, the product has a soft taste and rich aroma. Adding Ganoderma lucidum residue and other ingredients to distiller's grains can also produce edible vinegar with health benefits. Distiller's grains are also a good substitute material for producing edible mushrooms. Edible mushroom production usually uses natural wooden raw materials and uses distiller's grains for mushroom cultivation. Not only can it achieve waste utilization, but it can also effectively reduce the cost of edible mushrooms<sup>[22]</sup>.

Traditional vinegar is mainly made from yellow wine, bran, and rice husk, and is completed through solid-state fermentation. It not only has a unique flavor, but also contains rich nutrients. According to the traditional process of fragrant vinegar, using yellow wine lees as the main raw material for fermentation, the vinegar obtained is rich in protein, further enhancing its flavor and nutrition, greatly enhancing the value of fragrant vinegar. The fragrant vinegar obtained by Wan Jizhi and others using yellow wine lees contains rich aroma enhancing substances and amino acids, and the content is more than that of traditional fragrant vinegar. The ethanol content increases by 1.21%, the ester content increases by 1.03%, and the amino acid content increases by 1.51%<sup>[23]</sup>. Using distiller's grains to brew vinegar can realize the recycling and efficient utilization of liquor making waste, which is one of the effective ways to reasonably solve the by-products of Baijiu<sup>[24]</sup>.

#### 3.2.2 Using Distiller's Grains to Produce Noodle Products

Distiller's grains are rich in phenolic substances, which can prevent chronic cardiovascular and neurogenic diseases. Their high fiber content helps eliminate cholesterol and fat, and improves ulcerative colitis<sup>[25-28]</sup>. Therefore, using distiller's grains as raw materials for food production is very attractive

because it can increase the content of protein, fiber, vitamins, and minerals, while reducing the starch and calorie content in grain products. At present, there have been many studies on the use of flour made from beer lees to apply to the noodle industry, such as bread and biscuits. Nocente et al. found that compared to wheat flour, adding brewer's grains increased the fiber content of hard grain flour by 135%,  $\beta$ - The glucan content increased by 85%, and the total antioxidant capacity increased by 19%<sup>[26]</sup>.

Research has shown that adding 10% beer lees is the best for the sensory and processing characteristics of concentrated pasta<sup>[27]</sup>. The amount of added lees should be controlled at 10-15%<sup>[24-27]</sup>. With the increase of lees addition, not only does the fiber and protein content increase with the increase of lees content, but it also affects the rheological and gelatinization properties of the dough, significantly increasing biaxial tensile viscosity and reducing uniaxial ductility. On the other hand, the storage modulus will also increase. These characteristics have a negative impact on the baking quality of dough, leading to small volume and dense structure of bread, affecting taste and aroma, and altering rheological properties<sup>[29]</sup>. Bread rich in this type of beer lees flour has a high fiber content, which can enhance immunity, including increasing digestion and preventing some gastrointestinal diseases. Just like bread, the addition of beer lees flour can affect the appearance, hardness, chewiness, odor, and taste of biscuits.

Utilizing distiller's grains can also produce noodle snacks, and snacks containing distiller's grains contain a large amount of fiber and protein. However, the large amount of insoluble fiber lignin and cellulose content increases the hardness of snacks, directly leading to poor taste. This impact can be alleviated by adding corn starch and whey protein isolate. In the production of crisps, a 10% content of distiller's grains flour does not affect the taste and consistency of the crisps, and helps to increase the fiber content in the snacks produced<sup>[31]</sup>. Stojceska et al. reported that in order to obtain products similar to commercially available snacks, up to 20% of distiller's grains may be added to the snack extrusion, and a maximum addition of 30% can also achieve acceptable physical and chemical properties<sup>[32]</sup>.

## 3.2.3 Application of distiller's grains in other food industries

In recent years, the food industry has been committed to producing natural pigments from plant and microbial sources to overcome the use of synthetic pigments that may be harmful to human health and the environment. Silbird et al. studied the production of natural red pigment by Monascus strain CMU001 in a deep fermentation system using distiller's grains as the raw material substrate. Monascus pigments can be used as natural colorants, natural food additives, and as drugs, because it has been reported that they have anti mutagenic, anti-cancer, anti obesity, anti-inflammatory, anti diabetes and cholesterol lowering activities<sup>[33,34]</sup>. In the study conducted by Naibaho et al, waste grain flour and three different waste grain protein extracts were added to plant-based yogurt substitutes to maintain the texture and gel formation of yogurt<sup>[35]</sup>, while increasing shear stress and viscosity Utilize<sup>[36]</sup>.

#### 3.3 Production of feed and fertilizer from distiller's grains

## 3.3.1 Using distiller's grains to produce feed

As animal feed, distiller's grains are rich in amino acids, vitamins, various organic acids, and fats, and have rich nutritional value [37-40]. Direct feeding of fresh distiller's grains to animals is one of the most commonly used methods of distiller's grains feed. Low levels of distiller's grains have little impact on growth performance, but the high crude fiber content of fresh distiller's grains has poor palatability, leading to a decrease in the utilization rate of distiller's grains and even animal malnutrition and low immunity<sup>[38]</sup>. Moreover, fresh distiller's grains have a high water content, are prone to spoilage, and are not easy to store. They are suitable for feeding animals with high fiber ratio requirements and low nutritional needs and high food intake<sup>[39]</sup>. Alexandros et al. added wine lees to feed and found that the content of polyphenols in wine lees was high. After consumption, the activity of superoxide dismutase in the blood of broilers increased, and their antioxidant capacity improved<sup>[41]</sup>. Adding distiller's grains to the daily feeding of cows can improve the tenderness of beef, increase the fat and protein content in milk, and reduce dry matter intake. Using distiller's grains as a protein source can reduce nitrogen excretion and reduce the environmental impact of milk production<sup>[42,43]</sup>. S. Katsumata et al. added liquefied distiller's grains to the feed of Japanese black calves and found that feeding 100 g/d of liquefied distiller's grains can promote rumen development and increase blood levels in Japanese black calves β- The concentration of hydroxybutyric acid<sup>[44]</sup>. Obeida et al. used corn distiller's grains as a substitute for part of the diet to feed lambs, and the results showed that adding distiller's grains had no significant impact on the health of lambs, but could increase the intake of dry matter and protein<sup>[45]</sup>.

Due to the fact that the husks in distiller's grains can easily puncture the digestive tract of animals, have poor palatability, high acidity, and require the addition of baking soda during the feeding process,

the proportion of unfermented distiller's grains in the diet cannot exceed 75%[46-53]. Research has found that enzymes produced by microorganisms can degrade cellulose and improve the problem of high crude fiber content in distiller's grains, which is more conducive to animal digestion and improves the palatability of direct feeding of distiller's grains<sup>[47]</sup>. Fan Fangyong et al. used Lactobacillus casei, Candida albicans, Trichoderma viride, Aspergillus niger, and Rhizopus to ferment distiller's grains and found that the content of crude protein and true protein in the fermented distiller's grains increased, while the content of crude fiber decreased<sup>[37]</sup>. Zhang et al. used Bacillus subtilis and Enterococcus faecalis to ferment distiller's grains and added them to the pig diet. Compared with unfermented distiller's grains, growth performance, gut microbiota related to fiber degradation, meat quality, and immune status were improved<sup>[48]</sup>. They also promoted the synthesis of short chain fatty acids and growth<sup>[49]</sup>. The probiotics in fermented distiller's grains can alter the diversity of gut microbiota, help improve gut conditions, and prevent infections<sup>[50]</sup>. Feeding animal microorganisms to ferment distiller's grains can increase animal weight and improve meat quality, but improper proportion and composition of microorganisms can also affect animal growth performance and immunity<sup>[51,52]</sup>.

The yield of distiller's grains is large, the cost is low, and the nutritional content is rich. The feed utilization of distiller's grains is a reliable way to turn waste into treasure, which can not only alleviate the pressure on feed yield, but also save ingredients and reduce the waste of distiller's grains. As a new type of feed raw material, there are no relevant standards for the production of distiller's grains, and there are still many unresolved problems. Therefore, its industrialization has not yet been widely applied, and more in-depth and comprehensive research is needed to promote the development of convenient distiller's grains feed preparation technology.

## 3.3.2 Utilizing Distiller's Grains to Produce Fertilizer

Distiller's grains contain nitrogen, phosphorus, potassium, etc., and can be used as raw materials for organic fertilizer production. The nitrogen content, phosphorus content, and organic matter content of organic fertilizer produced from distiller's grains can reach 3%, 1%, and 70%, respectively. Distiller's grains can also be added to other organic matter to produce organic fertilizer, and the burning ash of distiller's grains can also be used as fertilizer<sup>[52]</sup>. Research has shown that organic fertilizer produced from distiller's grains, when combined with other microbial agents, has a higher fertilizer efficiency than compound fertilizer and ordinary organic fertilizer<sup>[53-56]</sup>. The study by Agata Bartkowiak et al. showed that treating soil with an appropriate amount of distiller's grains as fertilizer can significantly increase the content of phosphorus, potassium, and magnesium in the soil, as well as increase the activities of alkaline phosphatase, acid phosphatase, and dehydrogenase. However, its acidity has no significant effect on soil pH value<sup>[53]</sup>. Huang et al. used Baijiu distiller's grains as the main raw material to join a variety of microbial strains for composting, fermentation and decomposition to produce organic fertilizer. The results showed that after the sorghum fertilization test, the organic matter, total nitrogen, total phosphorus and total potassium contents of the tested soil increased by about 30% respectively, and the sorghum yield exceeded the control group. Moreover, it can also promote the increase of soil microbial community diversity[54].

However, frequent irrigation of soil with fertilizers containing more than 50% distiller's grains can increase the content of K<sup>+</sup>, Na<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>, and available phosphorus in the soil, as well as reduce the number, richness, and diversity of arbuscular mycorrhizal fungal spores<sup>[55]</sup>. In addition to traditional composting for fertilizer, Andrade et al. used distiller's grains liquefaction as hydroponic fertilizer and found a good synergistic effect between distiller's grains hydroponic fertilizer and plants<sup>[56]</sup>.

Distiller's grains fertilizer increases the content of soil organic matter, the stability of aggregates, water retention, and effective moisture, and also reduces the carbon nitrogen ratio, which is conducive to mineralization. The low pH and high organic carbon content of distiller's grains are related to the abundance of bacterial groups involved in cellulose degradation, and have a high assimilation effect on ammonia and nitrate. When applied to bioremediation of soil contaminated with engine oil, distiller's grains can promote plant growth and promote microbial degradation of hydrocarbons.

#### 3.4 Chemical raw materials for distiller's grains production

Distiller's grains are acidic and have a high content of organic acids. Through fermentation, chemical raw materials such as succinic acid, xylitol, and lactic acid can be produced. The production of xylitol generally includes extraction, chemical synthesis, biosynthesis, etc. Distiller's grains can be used as raw materials for xylitol production. The production of xylitol usually utilizes bacteria, molds, yeast, and other bacteria that can biosynthetically synthesize xylitol, among which yeast is the most commonly

used<sup>[60]</sup>. Distiller's grains contain about 12% starch and can be used as raw materials for succinic acid production. Succinic acid production includes chemical synthesis method and biological fermentation method. Succinic acid production from distiller's grains generally adopts biological fermentation method, which should be used before fermentation. Cellulase or saccharifying enzyme can hydrolyze white distiller's grains respectively, and the yield can reach 130mg/g<sup>[61]</sup>. Adding distiller's grains to a certain amount of dilute acid solution for acid leaching, and then undergoing a series of post-treatment to obtain phytic acid products, but the cost is relatively high. Under the conditions of adding nitrogen and salts, distiller's grains can be used to produce 3-hydroxybutyrate, which is widely used in the pharmaceutical, pesticide, and food industries<sup>[62]</sup>.

Distiller's grains can also be used as raw materials for preparing activated carbon. Activated carbon is usually made from coal through a series of processes such as carbonization and activation. Replacing coal with distiller's grains to produce activated carbon can not only solve the problem of distiller's grains pollution, but also reduce coal consumption, making it a promising utilization method. Waste ash can be used as a cement additive.

#### 3.5 The impact of microorganisms on the utilization of distiller's grains

#### 3.5.1 Selection of microorganisms

The crude fiber content of distiller's grains is relatively high. In early research on the utilization of distiller's grains, due to the lack of cellulose degradation pathways, the utilization rate was low and the economic efficiency was poor. Biodegradable lignocellulose in distiller's grains has lower costs compared to physical and chemical treatment methods, making it a promising method for reducing environmental pollution and resource waste in distiller's grains<sup>[63-65]</sup>.

There has been extensive research on the pre-treatment of resource utilization of distiller's grains using microbial fermentation. Li et al. analyzed the genome of Bacillus subtilis E1 and found that there were 372 genes associated with carbohydrate active enzymes (CAZymes), of which 98 were associated with NSPs degrading cellulose, hemicellulose, and pectinase, with strong non starch polysaccharide degradation ability. Using Bacillus subtilis E1 solid-state fermentation to degrade distiller's grains, the degradation rate of hemicellulose after fermentation was 11.86%, cellulose degradation rate was 11.53%, and lignin degradation rate was 8.78%, The crude protein content increased from 26.59% to 30.59%[42]. Yang et al. identified a heat-resistant cellulase producing Bacillus subtilis strain, which produced endoglucosidase with an activity of 82% at 80 °C. After co culturing with Bacillus subtilis, the degradation rate of distiller's grains by the two strains was increased by 70% compared to the cultivation of Bacillus subtilis alone, and the reducing sugar content was increased to 16.6 mg/mL<sup>[40]</sup>. This provides a reference for using distiller's grains as raw materials for protein production. Ana et al. found that Bacillus cereus, Enterococcus faecalis, and Enterobacter aerogenes have good potential for hydrogen production and volatile fatty acid production in the co fermentation of sugarcane distiller's grains<sup>[60]</sup>. In our previous research, we found that using Bacillus subtilis, Aspergillus niger, and Aspergillus oryzae to ferment distiller's grains. The total phenol content has increased by 2-3 times<sup>[9]</sup>.

Distiller's grains can be used as a raw material for fertilizer production, so it is particularly important to screen strains with efficient nitrogen fixation in distiller's grains. Zeng et al. screened two strains of Bacillus subtilis from distiller's grains, which have a nitrogen fixation capacity of 20 mg/L and strong nitrogen fixation capacity. However, large-scale nitrogen fixation production experiments have not yet been conducted, and further research is needed for practical application in production [63].

The impact of microorganisms on the utilization of animal feed from distiller's grains is particularly significant. Traditionally, when fresh or dry distiller's grains are used as feed, due to their high content of crude fiber, organic acids, and ethanol, their high proportion in feed can easily lead to animal distiller's grains poisoning. The use of microbial fermentation can effectively improve this situation. And the microorganisms used are mainly probiotics, which can also improve the diversity of intestinal microorganisms, promote the absorption of nutrients such as proteins, and improve intestinal villi<sup>[19]</sup>.

Banjo et al. used brewer's grains to produce ascorbic acid through deep fermentation of Aspergillus flavus and Aspergillus tamarix, obtaining 6.25 and 7.25 g/L ascorbic acid, respectively. brewer's grains has been used as a culture medium for Cordyceps militaris to produce cordycepin, a nucleoside analogue with anti-tumor, anti proliferative, anti metastatic, insecticidal, and antibacterial activities. In addition, the polysaccharide from Umbrella militaris showed significant anti-tumor activity against cervical cancer and liver cancer cells in vitro. Its fruiting body extract showed antioxidant, antibacterial, antifungal, and anti-tumor activities against human cell lines, as well as anti-inflammatory, anti fibrotic, anti obesity, and

anti angiogenic and insulin secretion activities. Therefore, the use of brewer's grains by Bacillus militaris to produce cordycepin has been proven to be a very effective technique for producing high-value food and feed additives<sup>[64]</sup>.

The utilization of distiller's grains has shown a diversified trend, with different emphasis on the functional requirements of microorganisms in the production of energy, chemical raw materials, and animal feed. Cassman et al. found that when distiller's grains were co applied with inorganic nitrogen fertilizer, an increase in N2O emissions was observed. Therefore, a metagenomic analysis was conducted on distiller's grains, and it was found that there were denitrification genes in the genome, which was unfavorable for distiller's grains fertilizer<sup>[65]</sup>. By analyzing the microbial diversity of distiller's grains through metagenomics and metabolomics, it is possible to understand the relevant functional genes of the main metabolic pathways of distiller's grains, screen dominant bacteria, improve the expression of important enzyme genes, and provide reference for screening functional bacteria.

#### 3.5.2 Safety issues

Since distiller's grains have been studied as raw materials for food, animal feed, and plant feed production, it is necessary to consider some microbial parameters that affect the quality of the raw materials, such as the presence of pathogenic microorganisms and fungal toxins.

Mycotoxins are secondary metabolites produced by fungi, which can affect human and animal health due to their neurotoxic, immunosuppressive, teratogenic, and carcinogenic effects. Mycotoxins are a serious and recurring problem worldwide, causing economic losses and health problems<sup>[66]</sup>. Several studies emphasize the need to monitor and determine the exact types and quantities of fungal toxins in distiller's grains, their final products, and by-products to ensure food and feed safety. Distiller's grains are prone to fungal growth, and during utilization, some fungi degrade cellulose in distiller's grains. Although most of the utilized bacteria are probiotics, pollution is inevitable during use, so it is necessary to monitor and control the changes in the content of fungal toxins during the production process.

#### 4. Potential applications and future directions

The aforementioned techniques for treating distiller's grains are all based on the composition of the distiller's grains and adopt a resource based approach to treat the distiller's grains. The production of feed from distiller's grains has been widely used due to its simple and feasible technology, and its scale can be large or small. But it is obvious that its economic benefits are not very high. In addition, due to the impact of transportation radius, the product market area is limited, and the impact of rice husks on feed quality limits its application<sup>[1-8]</sup>. Especially when the amount of distiller's grains is large, this technology is not applicable. The anaerobic treatment and fermentation of distiller's grains to produce biogas have been widely used due to the principle of organic wastewater anaerobic fermentation, which has good maturity, low operating costs, and the ability to generate clean energy biogas<sup>[11-13]</sup>. But its disadvantage is that it occupies a large area and the rice husks in the distiller's grains cannot be fermented, so this technology cannot be used alone and needs to be applied in collaboration with other technologies. The combustion and utilization technology of distiller's grains is simple, thoroughly treated, and can be used as energy, but it is necessary to solve the secondary pollution problem caused by distiller's grains combustion. Most of the other technologies are only in the laboratory stage and have not been widely applied or have poor economic benefits, which are not recognized by the market.

The research on the processing technology of distiller's grains should be based on the aforementioned research, comprehensively developing different functional components of distiller's grains, producing different products, and utilizing various components of distiller's grains as resources to improve the economic efficiency of distiller's grains processing. If the protein in the distiller's grains can be extracted first, or further fermented, the protein can be extracted as feed protein<sup>[9]</sup>, rice husks can be used as fuel to produce heat or cogeneration, and rice husk ash can be used as raw material for organic fertilizer production<sup>[12]</sup>. In this way, the added value of protein is high, and higher value active proteins can be separated from the protein, which will greatly increase the value of distiller's grains utilization; Low value-added fiber substances, on the other hand, not only achieve resource utilization but also greatly reduce the waste of distiller's grains through combustion and energy utilization; Finally, utilizing distiller's grains ash to produce organic fertilizer<sup>[46]</sup> can achieve complete harmless treatment of distiller's grains. In the research on the utilization of distiller's grains, most reuse methods require fermentation. Therefore, in the subsequent process optimization research, based on the previous research, comprehensive development of different functional components of distiller's grains should be carried out to produce different products, so that various components in distiller's grains can be utilized as resources

to improve the economic efficiency of distiller's grains treatment. And metagenomics and metabolomics analysis can be introduced<sup>[60-61]</sup> to screen corresponding functional bacteria for fermentation utilization, thereby increasing yield, profitability, and reducing their negative impact on the environment. In addition, before producing such products based on distiller's grains on an industrial scale, it is necessary to establish relevant agricultural, food, and industrial product standards and regulations, improve resource utilization, and achieve diversified recycling of distiller's grains resources.

#### 5. Summary

Waste of distiller's grains is an inevitable major issue for large and medium-sized brewing enterprises. The comprehensive utilization of distiller's grains resources will contribute to the sustainable development of the liquor industry. However, the yield of distiller's grains is large and the components are complex. Different brewing and ethanol production raw materials also produce significant differences in the composition of distiller's grains, making it difficult to find widely applicable and large-scale transformation methods. Exploring the ways of its comprehensive utilization still requires more in-depth and extensive research.

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#### Academic Journal of Materials & Chemistry

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