

# The Pretreatment Methods and Influence Factors During Anaerobic Fermentation of Straw: A Review

Haoxiang Yuan\*

Leicester International Institute, Dalian University of Technology, Panjin, Liaoning, 124221, China

**Abstract.** Straw is a common agricultural waste; its quantity is huge. In 2018, China produced 110 million tons of waste straw. The traditional way for farmers to treat straw is to burn it. However, burning straw will cause serious air pollution. At the same time, straw can be used to produce biogas via anaerobic fermentation, which is a clean and renewable energy source. Therefore, the research on anaerobic fermentation of straw is widely concerned. Straw is difficult to ferment directly because of its composite structure of lignin and cellulose. Therefore, straw needs to be pretreated. This review summarizes the previous research results in recent years on main straw pretreatment methods and factors affecting the efficiency of anaerobic fermentation of straw. In addition, the solutions to the problems existing in anaerobic fermentation of straw were put forward. This review could provide guidance for the follow-up research on anaerobic fermentation of straw.

## 1. Introduction

Straw is a kind of major side-products of agriculture. Primary agriculture workers in China tend to directly burn straw generated by crops which have chances to be recycled [1]. Heilongjiang Province has made a major contribution to China's agriculture, which incorporates about 10% of agricultural acreage of China [2]. In recent 10 years, the theoretical resources of straw in Heilongjiang Province have increased year by year, and at 2018, the amount of straw which can be recycled was over 110 million tons. There are more than 700 million tons of agricultural wastes in China every year, and more than 20% of them are directly burned [3], which has certain pollution to the environment.

At the same time, natural gases can be produced by anaerobic fermentation from straw, which is an effective method for straw resource utilization. The main components of straw include lignin, cellulose and hemicellulose. It is difficult to produce methane by straw fermentation because lignin and hemicellulose cover cellulose tightly and prevent cellulose from being zymolytic by anaerobic bacteria [3]. The pretreatment of straw can solve this problem and increase yield of methane. At present, physical method, chemical method and biological method are three main paths for pretreatment.

Physical pretreatments generally can destroy the fiber

structure of straw, separate cellulose, hemicellulose and lignin from straw and then increase the contact area between cellulose and anaerobic bacteria, including mechanical crushing, steam explosion, hydrothermal, and microwave treatment etc. Chemical pretreatment can always be used to change the structure of straw cellulose and lignin to make them more prone to fermentation reaction. the dilute acid H<sub>2</sub>SO<sub>4</sub> and base NaOH are always used to do chemical pretreatments. The processes of biological pretreatment are to make the composite structure of lignin and cellulose becomes loose by fungi or enzymes.

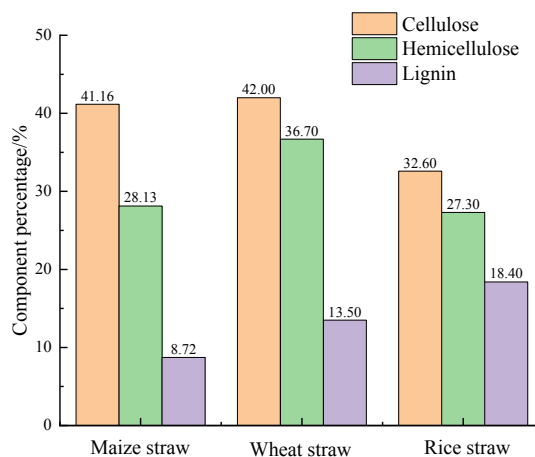
At present, there are many researches on straw pretreatment and fermentation in China, but there is a lack of comprehensive summary and comparison. In this research area recently. In this review, the current pretreatment methods of straw are compared and analyzed, and different straw fermentation methods are also introduced and compared. This review can provide reference for the future research work of straw anaerobic fermentation.

## 2. Composition of straw

Straw used for anaerobic fermentation mainly includes the following three kinds: maize straw, wheat straw and rice straw. The figure below shows the compositions of each kind of straw [4,5,6].

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Email: [yhx0107@mail.dlut.edu.cn](mailto:yhx0107@mail.dlut.edu.cn)



**Fig.1** The components of each straw

The cellulose content in straw is the highest, and followed by hemicellulose content, the lignin content is the lowest. Among these above three kinds of straws, the cellulose content in rice straw is the lowest, and that in wheat straw is the highest. The lignin content in rice straw is the highest, followed by wheat straw, and the lignin content in maize straw is the lowest. Because of this characteristic and the wide geographical distribution of corn planting, maize straw is often used in the research of anaerobic fermentation of straw.

Hydroxyl groups on cellulose molecules in straw components form intermolecular and intramolecular hydrogen bonds, which leads to the stable composite structure of cellulose and lignin in corn straw, which is not easy to be destroyed. In addition, this structure is also insoluble in water [7]. The pretreatments can break intermolecular and intramolecular hydrogen bonds, Therefore, the pretreatments can promote the process of anaerobic fermentation of straw.

Recently, the pretreatment method of straw can be mainly divided into 3 categories: physical methods, chemical methods and biological methods. The common purpose of these methods is to increase the expose area between cellulose and anaerobic bacteria. For biological methods, they also change the structure of cellulose and convert it into the structure which is more easily to react with anaerobic bacteria [8].

### 3.1 Physical methods

Physical pretreatments include mechanical crushing, hydrothermal, steam explosion, microwave treatment and high-energy radiation treatment [8]. The basic principle of the physical pretreatment is to grind the straw into small particles, and then break the cell wall and the composite structure formed by cellulose and lignin. Then, cellulose can approach to bacteria more efficiently.

**Table 1** below lists the basic methods and more detailed information of the physical pretreatment.

## 3. Pretreatment methods

**Table 1.** Treat conditions and results of physical pretreatment

Approaches	Basic operations	Results	Reference
Mechanical crushing	Separately enzymatic hydrolysis straw with different meshes (18, 34, 50, 100, 120, 150) and comparing reaction times.	The smaller the particles, the higher the gas production rate.	[9]
Steam explosion	Steam explosion the straw under the condition of 200°C, 120 s.	The gas production increase to 51% compared with non-treatment groups.	[10]
Hydrothermal	Under 160°C and heat for 30min by water	The best gas production volume and gas production after hydrothermal pretreatment increase to 2.06 times of the blank group.	[11]

Microwave treatment	Treat the straw under 680W microwave for 24mins	Cellulose saccharification, hemicellulose saccharification and total saccharification separately rose to 31%, 43% and 30%	[12]
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Mechanical crushing is the most direct method that can break the crystallization between cellulose and lignin therefore increases the approach area between bacteria and cellulose. Steam explosion can make the fibers and cell cytoplasm inside the straw gasify and expand under high temperature and high pressure, which makes cellulose hemicellulose and lignin dispersed. This is beneficial to full contact between cellulose and anaerobic bacteria. Hydrothermal is to increase the boiling point temperature of water by increase the pressure. Therefore, water with high temperature and pressure can infiltrate into the straw structure and break the structure of fiber. Microwave radiation can break intermolecular hydrogen bonds between cellulose molecules; hence, the structures of straws can be loose and easy to ferment [13].

### 3.2 Chemical methods

The principle of chemical pretreatment is the chemical compositions of straws are changed by chemical reaction, and cellulose, hemicellulose and etc. are hydrolyzed, so it is easy to react with anaerobic bacteria to produce gas. The commonly used methods are mainly acid pretreatment and base pretreatment.

#### 3.2.1 Pretreatment by acid

Some inorganic acid, such as H<sub>3</sub>PO<sub>4</sub>, H<sub>2</sub>SO<sub>4</sub>, and some organic acid including CH<sub>3</sub>COOH, HCOOH are always used to do acid pretreatment. Wang Xin and his colleagues used H<sub>3</sub>PO<sub>4</sub> with different concentrations to pretreat the rice straws [15]. In their experiment, the crushed straw samples were separately pretreated by 0%, 2%, 4%, 6%, 8% H<sub>3</sub>PO<sub>4</sub> and treated in biochemical incubator for 7 days under 25°C. Then, straw and cow dung with a rate of 1:1 were mixed and fermented at 35°C.

The straws pretreated by 6% H<sub>3</sub>PO<sub>4</sub> had the highest gas production and methane production. The methane production and methane production rate increased as the concentration of H<sub>3</sub>PO<sub>4</sub> increased when the concentration of H<sub>3</sub>PO<sub>4</sub> is under 6%. When the concentration of H<sub>3</sub>PO<sub>4</sub> was over 6%, the gas production and methane production decreased with the increase of H<sub>3</sub>PO<sub>4</sub> concentration.

Table 2. The conditions of two groups in Cui’s study [17].

Group 1 immersed group	Group 2 non-immersed group
Blank and only add 100mL water, then immersing for 24h	Blank and only 100mL water.
Separately adding 100mL 1%, 2.5%, 5%, 7.5% of NaOH solution and immersing for 24h.	Separately adding 100mL 1%, 2.5%, 5%, 7.5% of NaOH

Cui Fengjie and his colleagues found that the gas production is the highest during the first 24h under 2.5% NaOH pretreatment, which is 73.11L/kg VS. However, if the concentration used to do pretreatment reaches 7.5%, the amount of gas production decreases because excessive

Guo Yan has studied the effects of different concentrations of sulfuric acid, hydrochloric acid and acetic acid on anaerobic fermentation of straws [16]. He pretreated corn straws with 1%, 2% and 3% sulfuric acid, hydrochloric acid and acetic acid respectively, and fermented them anaerobically at 35°C for 60 days. Then, the gas production of straw fermentation every day was detected. During the whole experiment, the total solid proportion was 8%.

The experimental results showed that when the concentration of dilute sulfuric acid was 1%, the overall gas production was the largest, and when the concentration of sulfuric acid was 3%, the fermentation process was inhibited and the fermentation recurrence started normally [16]. The peak of daily gas production occurred between the 10th and 25th days. For the pretreatment of straw with hydrochloric acid, when the concentration of hydrochloric acid was about 1%, the effect of anaerobic fermentation of straw is the best, but when the concentration of hydrochloric acid was 2% and 3%, the efficiency of anaerobic fermentation of straw was significantly reduced. Although the acidity of hydrochloric acid was beneficial to the dissolution of volatile fatty acids from straw, when the acidity was too high, it was not conducive to the growth of methanogens, resulting in the inability to consume volatile fatty acids in time [16]. For the pretreatment of straw with acetic acid, the optimal concentration was 1%. The increase of acetic acid concentration has no obvious inhibitory effect on anaerobic fermentation of straw, and the daily gas production had a continuous peak within 60 days, with little weakening effect. This might be because acetic acid was more selective to lignin and can consume more lignin.

#### 3.2.2 Pretreatment by base

NaOH is the most commonly used method to do base pretreatment. NaOH solution can decompose lignin completely, which can increase the contact areas between cellulose and hemicellulose and anaerobic bacteria. Cui et al had set up 2 groups of experiments [17]. The pretreatment methods are as below.

alkali concentration will reduce the activity of cellulose hydrolysis bacteria and acidification bacteria, which can lead to decrease in gas production.

Ammonia soaking is another base pretreatment method. Alkaline substances can be used to dissolve lignin,

thus leads to the structure containing cellulose and hemicellulose expand to increase the contact area between cellulose and enzyme [18]. Li et al carried out a series of orthogonal experiments and determined that the most optimal condition to implement ammonia pretreatment was 15% ammonia with the pretreatment time of 39h, at 40°C and the solid straw content was 162.5g/L. Under this condition, the removal rate of lignin was 61.20% [18].

In addition, Kim et al. also used the method of pretreatment with calcium hydroxide [19]. They found that pretreated straw at 55°C for 28 days, the removal rate of lignin could reach 87.5%. Furthermore, the average use of Ca(OH)<sub>2</sub> for each gram of straw was only 0.073g.

The condition for base pretreatment is relatively moderate and very economical, especially for the using of NH<sub>3</sub> and Ca(OH)<sub>2</sub>. Additionally, ammonia can react with organic contents in the straw and form ammonium ion which can be nutritional ingredient for anaerobic bacteria [19].

### 3.2.3 Biological method

The main way of biological pretreatment is to use fungi. There are some natural fungi that can directly decompose lignin in straw. The most commonly used fungi are white rot fungi, brown rot fungi and Trichoderma reesei.

White rot fungi have the ability of breaking down the mineralizing lignin. Cellulase and hemicellulose hydrolysis system and oxidative ligninolytic system play the most important role in breaking down the lignin. The key enzymes of oxidative ligninolytic system are lignin peroxidase and manganese peroxidase and their function is to break the phenyl rings in lignin [20].

In straw, lignin, cellulose and hemicellulose are closely polymerized to form a mutually wrapped structure [20]. Brown rot fungi can degrade straw without changing

lignin content because it changes the degree of polymerization of cellulose and makes the approach areas between anaerobe bacteria and cellulose increase [21]. The principle that how brown rot fungi break down cellulose has not been fully mastered yet. However, Murmanis et al found that brown rot fungi can not break down cell walls of straws directly but work through Mechanism of degradation of cellulose by HO radical oxidation [21].

In addition, Trichoderma reesei can directly degrade straw by producing cellulase [22].

## 4. Influencing factors of anaerobic fermentation

Besides pretreatment, there are totally five factors that influence the processes of anaerobic fermentation greatly. They are separately listed in the table below.

Li and his colleagues had researched the relationship between gas production and fermentation time and fermentation temperature [23]. Every 5°C was set to be a group of the experiment between 25 to 40°C under the condition of TS=8%. In each temperature, the ratios of pig manure, cow dung and corn straw are separately 1:1, 2:1, 3:1 and collecting data of gas production rate, gas production, and fermentation time. Wang and his colleagues has studied the effect of fermentation gas production of different inoculum which were separately sludge, pig manure and their mixture under different inoculum quantities on cotton straws [24]. The optimal pH and TS conditions were found in their experiments. Gao et al. has studied the effect of anaerobic fermentation effect on corn stalks with different particle sizes (100-200, 20-30, 0.6-0.8mm) at room temperature 25°C and medium temperature 35°C [25].

Table 3. The main influencing factors during anaerobic fermentation

Factors	Descriptions	Optimal conditions	Reference
Temperatures	Temperature can affect the activity of methanogens in anaerobic fermentation.	When pig manure, cow dung and straw are mixed for anaerobic fermentation, the optimum temperature is 34°C and the fermentation time is 58 days.	[23]
pH values	Methanobacteria are very sensitive to pH, and too low pH will lead to the death of methanogens.	When original pH=7.0, rice straw has the highest gas production.	[24]
Total solid (g/L) (TS)	If TS values are too low, the amount of gas production tends to be too low. If TS values are too high, fatty acid tends to accumulate.	When the total solid is 45g/L, the growth rate of gas production efficiency is the largest.	[24]
Straw diameter	A method of physical pretreatment.	The fermentation effect is the best when the straw diameter is about 20-30mm.	[25]
	If C/N is too high, the bacterial nitrogen source tends to be deficiency.	When C/N=27:1, cumulative gas production and carbon	

C/N	If C/N is too low, ammonium salts are toxic to methanogens.	conversion efficiency is the highest.	[25]
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## 5. Conclusion and prospect

The pretreatments of straw are mainly divided into three types: physical pretreatment, chemical pretreatment and biological pretreatment. Although the gas production rate of straw pretreated by physical and chemical methods is significantly improved and the gas production cycle is shorter, the cost is higher, and it is easy to cause the change of acidity and alkalinity of the system, which will affect the activity of anaerobic bacteria. Biological pretreatment is mild and will not produce secondary pollution. Therefore, the integrative pretreatment of physical-chemical-biological methods could be investigated in the future.

Besides pretreatment, the yield of anaerobic fermentation of straw is also related to fermentation conditions. The factors which can greatly change the fermentation condition are separately temperatures, pH values, total solid mass concentrations, straw diameters and C/N values. Although there are many studies on the optimum conditions of anaerobic fermentation of straw, the optimum conditions required by different regions, different straws and different loads on straws (pig manure-straw, cow dung-straw and etc.) are different, so further research in this field is necessary.

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