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Pretreatment Methods for Straw Feed Utilization and the Mechanism of Action of Fermentation Inhibitory Compounds: Postprint

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Abstract

Crop straw, as an abundant and widely sourced biomass resource, holds tremendous application potential in animal husbandry and bioenergy industries. However, its complex chemical composition and recalcitrant structure prevent direct and efficient utilization through bioconversion. Pretreatment can reduce the crystallinity of straw cellulose and enhance its utilization efficiency. Nevertheless, the pretreatment process inevitably leads to excessive degradation of straw under high temperature or chemical catalysis, accompanied by the generation of by-products that inhibit subsequent microbial fermentation. This paper reviews the research progress of dilute acid, alkaline, steam explosion, and biological pretreatment technologies for straw, and provides a comprehensive overview of the generation and inhibition mechanisms of pretreatment by-products, including furan derivatives, weak acids, and phenolic compounds.

Full Text

Pretreatment Methods for Straw Feed and the Mechanism of Action of Fermentation Inhibitors

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Abstract: Crop straw represents a biomass resource with abundant reserves and diverse origins, holding tremendous potential for applications in animal hus-

bandry and bioenergy industries. However, its complex chemical composition and recalcitrant structure prevent direct and efficient utilization through biological conversion. Pretreatment can reduce the crystallinity of cellulose in straw and improve its utilization efficiency. Nevertheless, the pretreatment process inevitably leads to excessive degradation of straw components under high temperature or chemical catalysis, accompanied by the generation of by-products that inhibit subsequent microbial fermentation. This review summarizes research progress on dilute acid, alkali, steam explosion, and biological pretreatment technologies for straw, and provides an overview of the generation and inhibition mechanisms of pretreatment by-products, including furan derivatives, weak acids, and phenolic compounds.

Keywords: crop straw; pretreatment; inhibitors

1. Straw Pretreatment Methods

Widely applied pretreatment methods for straw include physical, chemical, and biological approaches. Physical methods mainly comprise grinding, ball milling, and steam explosion; chemical methods include alkali and acid treatments; and biological methods involve enzymatic hydrolysis and microbial fermentation. While these pretreatment methods improve straw characteristics and enhance degradation rates, each has its limitations. Traditional mechanical and chemical treatments suffer from high energy consumption and environmental pollution issues, making them unsuitable for modern industrial production and application. Biological treatment offers cost advantages but is limited by long pretreatment times and production cycles, restricting its industrial-scale application. Steam explosion treatment demonstrates clear advantages in energy consumption, environmental friendliness, and treatment efficacy compared to other methods, representing a promising direction for pretreatment technology development. compares several common straw pretreatment methods.

Table 1. Comparison of Common Pretreatment Methods for Straw

Methods	Mechanism	Advantages	Disadvantages	Scope
Dilute acid pretreatment	Disrupts cellulose crystalline structure, breaks lignin-cellulose linkages, and dissolves hemicellulose	High pretreatment efficiency, low solvent cost	High equipment corrosion resistance requirements; produces fermentation inhibitors such as acetic acid and furfural	Cellulosic ethanol production [5-6]

Methods	Mechanism	Advantages	Disadvantages	Scope
Alkali pretreatment	OH^- cleaves lignin ether bonds, weakens hemicellulose-cellulose hydrogen bonds, saponifies hemicellulose-lignin ester bonds	Low reagent usage and cost; effective pretreatment	Environmental pollution, biomass loss, produces phenolic inhibitors	Straw feed processing [7-9]; cellulosic ethanol production [10]; biogas production [11]
Steam explosion	Under high temperature/pressure, hemicellulose partially degrades and lignin softens; rapid decompression weakens fiber crystallinity and destroys cell wall structure	Short treatment time, minimal chemical usage, pollution-free	Incomplete lignin separation, partial xylose destruction, produces acetic acid, furfural and other fermentation inhibitors	Straw feed processing [12-13]; cellulosic ethanol production [14]; biogas production [15]
Biological pretreatment	Utilizes microorganisms or enzymes (cellulases, xylanases, feruloyl esterases, etc.) that degrade lignin, cellulose, and hemicellulose	Low energy consumption, pollution-free, mild processing conditions	Limited available microbial strains, low enzymatic efficiency, long processing cycles	Straw feed processing [16-17]; biogas production [18]

1.1 Dilute Acid Pretreatment Dilute acid pretreatment is one of the earliest studied and most mature pretreatment methods. It disrupts cellulose crystalline structure, breaks linkages between cellulose and lignin, and simultaneously dissolves hemicellulose. Acids used for lignocellulosic pretreatment include

dilute sulfuric acid, nitric acid, hydrochloric acid, phosphoric acid, and oxalic acid. Among these, dilute sulfuric acid is most suitable for industrial application due to its low cost, high efficiency, and minimal environmental pollution. Chen et al. [19] reported that corn straw pretreated with 0.75% dilute sulfuric acid at 150°C for 80 minutes achieved a hemicellulose degradation rate of 98.02% and a cellulose enzymatic hydrolysis yield of 66.95% from the pretreated residue. Kootstra et al. [20] found that compared to dilute sulfuric acid, organic acids such as maleic acid and fumaric acid produced fewer furfural inhibitors in pretreatment by-products.

The addition of appropriate surfactants during dilute acid pretreatment of straw materials can enhance cellulose enzymatic hydrolysis efficiency. Qing et al. [21] investigated the effects of Tween 80, sodium dodecylbenzenesulfonate, and polyethylene glycol 4000 as additives in corn straw dilute acid pretreatment. The results showed these surfactants effectively removed lignin components from straw and improved cellulose enzymatic hydrolysis efficiency by increasing biomass hydrophobicity. Qi et al. [5] obtained similar results when adding 0-1% Tween 20 during dilute sulfuric acid pretreatment of wheat straw. Therefore, adding surfactants during dilute acid pretreatment of straw materials yields better results.

Combining dilute acid treatment with other pretreatment methods proves more effective in practical production. Zhang et al. [6] found that combined acid-alkali treatment could remove most non-fibrous materials from corn cobs, achieving an ethanol concentration of 69.2 g/L and a high ethanol yield of 81.2% through simultaneous saccharification and fermentation. Pan et al. [22] studied enzymatic hydrolysis of oxalic acid-preimpregnated steam-exploded corn cobs, reporting glucose yields increased by 32.3% and 214.87% compared to neutral steam-exploded and untreated corn cobs, respectively. While dilute acid pretreatment offers advantages of low cost and simple process, it requires highly corrosion-resistant equipment and produces fermentation inhibitors such as acetic acid and furfural.

1.2 Alkali Pretreatment Alkali pretreatment primarily utilizes OH^- to cleave lignin ether bonds, weaken hydrogen bonds between hemicellulose and cellulose, and saponify ester bonds between hemicellulose and lignin, thereby reducing the crystallinity of lignocellulosic materials and making them more susceptible to hydrolysis. Straw alkali treatment typically employs NaOH, $Ca(OH)_2$, and ammonia solutions. Varga et al. [10] reported that treating corn straw with 10% NaOH at 120°C for 60 minutes removed 95% of lignin. NaOH demonstrates excellent delignification and swelling capacity for cellulose, increasing the accessible surface area for cellulase, reducing cellulose polymerization degree, and is essential for achieving efficient biomass conversion. However, NaOH pretreatment also presents challenges such as difficult reagent recovery and environmental pollution. Compared to NaOH, $Ca(OH)_2$ offers lower cost, reduced environmental impact, and can be recovered from hydrolysates through reaction with CO_2.

Gu et al. [11] investigated the effects of $Ca(OH)_2$ pretreatment on anaerobic digestion of rice straw, finding that it significantly increased biogas production and enzymatic hydrolysis efficiency. Fourier transform infrared spectroscopy (FTIR) analysis confirmed that $Ca(OH)_2$ pretreatment caused delignification and reduced crystallinity.

Anaerobic alkali storage technology for straw feed has been extensively studied both domestically and internationally. Shi et al. [7] used CaO-treated corn straw combined with dried distillers grains with solubles (DDGS) to partially replace wild ryegrass, corn silage, and corn grain in diets for mid-to-late lactation dairy cows. The results showed no significant effects on lactose content, 4% fat-corrected milk yield, or milk fat and protein production, while increasing profitability. Wanapat et al. [8] found that feeding urea-treated rice straw to buffalo increased the populations of *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, and *Ruminococcus albus* in rumen fluid, indicating that urea-treated straw better facilitates adhesion and colonization of rumen fiber-degrading bacteria. Additionally, Polyorach et al. [9] demonstrated that combined urea and $Ca(OH)_2$ treatment improved the nutritional value of rice straw, promoted rumen fermentation in beef cattle, and was more economical than urea treatment alone. While alkali treatment effectively delignifies and reduces crystallinity, it simultaneously generates compounds such as phenol, p-cresol, and vanillyl alcohol that inhibit microbial fermentation and causes partial biomass dissolution, resulting in raw material loss.

1.3 Steam Explosion Steam explosion is currently the most widely used physical pretreatment method due to its potential to disrupt cellulose and lignin crystalline structures and hydrolyze hemicellulose. In this process, biomass feedstock is heated with high-pressure saturated steam for a specific duration before rapid pressure release. Under high temperature and pressure, hemicellulose partially degrades and lignin softens; the instantaneous pressure release generates enormous shear forces that weaken inter-fiber crystallinity, destroy cell wall structure, partially degrade lignin, and expose cellulose. Wang et al. [23] investigated the effects of steam explosion pretreatment on rice straw fiber structure. FTIR results showed minimal impact on cellulose structure but significant reduction in hemicellulose content. Scanning electron microscopy and X-ray diffraction analysis revealed substantial surface morphology changes, with crystallinity increasing as pressure and residence time increased.

The effectiveness of steam explosion primarily depends on temperature, residence time, particle size, and moisture content. Maximum hemicellulose degradation and hydrolysis efficiency can be achieved either at high temperature with short residence time (270°C, 1 min) or at low temperature with long residence time (190°C, 10 min) [15]. López-Linares et al. [14] optimized conditions for steam explosion pretreatment of rapeseed straw for bioethanol production using response surface methodology (RSM), achieving maximum bioethanol yield at 215°C and 7.5 minutes.

Currently, steam explosion technology is widely applied in industrial papermaking and biomass energy production, though its adoption in straw feed processing has been relatively slow. Viola et al. [12] found that steam explosion treatment of corn, barley, and oat straws increased dry matter digestibility by 25% in sheep, with an additional 9% improvement when followed by alkali treatment. Chang et al. [13] conducted a 3-day metabolic trial using steam-exploded and *Aspergillus oryzae*-fermented corn straw to replace 4%, 8%, and 12% of corn meal in broiler diets. Compared to the control group, the 4% and 8% replacement groups showed no significant effects on dry matter, organic matter, energy, crude protein, or crude fat digestibility, while neutral and acid detergent fiber digestibility significantly increased; however, the 12% replacement group showed significantly reduced energy digestibility. Steam explosion pretreatment offers short processing time, minimal chemical usage, and pollution-free operation, but suffers from incomplete lignin separation, partial xylose destruction, and generation of fermentation inhibitors such as acetic acid and furfural.

1.4 Biological Pretreatment Biological pretreatment primarily utilizes microorganisms that degrade cellulose, hemicellulose, and lignin, or the enzymes they produce during cultivation—including exoglucanases, endoglucanases, β-glucosidases, β-xylanases, feruloyl esterases, and acetyl esterases—to degrade lignin, cellulose, and hemicellulose in straw, thereby improving fiber degradation rates. White-rot fungi show promising application potential due to their ability to secrete extracellular oxidative enzymes for efficient lignin degradation without producing pigments. Lalak et al. [18] reported that the white-rot fungus *Flammulina velutipes* achieved hemicellulose and lignin degradation rates of 29.1% and 35.4%, respectively, in tall wheatgrass. Shrivastava et al. [16] used white-rot fungi for solid-state fermentation of wheat straw, significantly increasing crude protein content, improving organic matter digestibility in beef cattle, and reducing the carbon-to-nitrogen ratio. Feruloyl esterase pretreatment of crop straw can promote further degradation of cell wall cellulose and hemicellulose by rumen microorganisms. Yang et al. [17] investigated the fibrolytic activity of *Neocallimastix* sp. YQ1 on corn straw using different combinations of glucose levels and nitrogen sources, finding that feruloyl esterase and acetyl esterase activities were highest in media containing 1.0 g/L glucose, 2.8 g/L yeast extract, and 0.5 g/L $(NH_4)_2SO_4$, and 1.0 g/L glucose, 1.7 g/L yeast extract, and 1.4 g/L tryptone, respectively. Biological pretreatment offers mild conditions, low energy consumption, and minimal environmental pollution, but is limited by few available microbial strains, low enzymatic efficiency, and long processing cycles. Additionally, microbial introduction inevitably results in sugar consumption by pretreatment microorganisms, reducing substrate concentration during subsequent fermentation.

2. Types and Inhibition Mechanisms of Fermentation Inhibitory Compounds

The formation of by-products during steam explosion and chemical pretreatment is well established, with excessive by-products inhibiting microbial fermentation. Based on their origin, these by-products are typically classified into three categories: furan derivatives, weak acids, and phenolic compounds. Understanding the types and inhibition mechanisms of these fermentation inhibitors is crucial for selecting appropriate pretreatment methods and optimizing pretreatment conditions in practical production. [Figure 1: see original paper] illustrates the formation of various inhibitors during pretreatment.

Figure 1. Formation of inhibitors during straw pretreatment [24]

2.1 Furan Derivatives Furfural and 5-hydroxymethylfurfural (5-HMF) are common pretreatment by-products of lignocellulose, generated through dehydration of pentoses and hexoses derived from hemicellulose hydrolysis. Furan aldehydes affect *Saccharomyces cerevisiae* primarily by inhibiting yeast growth, prolonging the lag phase, and consequently reducing ethanol yield and productivity. Furfural concentrations below 0.5 g/L can promote growth of *Pichia stipitis*, while 2.0 g/L completely inhibits yeast cell growth, and 1.5 g/L reduces ethanol yield by 90.4% [25]. Furfural inhibits aldehyde oxidases involved in converting reactive aldehydes, leading to accumulation of reactive oxygen species (ROS) that damage mitochondria, vacuolar membranes, actin cytoskeleton, and nuclear chromatin. Under anaerobic conditions, *S. cerevisiae* can reduce furfural and 5-HMF to less toxic furfuryl alcohol and 2,5-bis-hydroxymethylfuran, respectively, using NADPH-dependent reductases [26]. Li et al. [27] used yeast whole-genome expression profiling to study the transcriptional response to furfural, revealing that furfural addition downregulated genes involved in folate metabolism, spermidine and spermine synthesis, while upregulating oxidative stress-related genes. Therefore, cell survival in the presence of furan aldehydes requires not only conversion of aldehydes to less toxic alcohols but also self-protection mechanisms to repair damage caused by furan compounds and develop tolerance to their inhibitory effects.

2.2 Weak Acids Weak acids in lignocellulose pretreatment by-products mainly include acetic acid, formic acid, and levulinic acid. Acetic acid is produced from the extensive acetylation of hemicellulose and lignin in cellulosic feedstock, with acetyl groups being converted during pretreatment. Formic acid is a degradation product of furfural and 5-HMF, while levulinic acid is formed from 5-HMF degradation.

Weak acids inhibit microorganisms through two mechanisms: intracellular acidification and uncoupling [24]. Undissociated weak acids can diffuse across cell walls and membranes; upon entering the neutral pH environment of the cytoplasm, they dissociate, causing intracellular pH reduction. Maintaining constant cytoplasmic pH is critical for normal cellular functions, affecting signal trans-

duction and optimal enzyme conditions. To maintain intracellular pH, active transport can partially remove dissociated weak acids, and ATPases can pump free H^+ out of the cell. Both active transport and ATPase activity consume substantial ATP. Under this mechanism, weak acid concentrations below 100 mmol/L can stimulate metabolism, while concentrations above 100 mmol/L cause sluggish microbial metabolism and impair normal fermentation [28]. As the most abundant weak acid by-product, acetic acid has been extensively studied. Schüller et al. [29] found that acetic acid causes mitochondrial damage with cytochrome C leakage into the cytoplasm, exhibiting typical apoptosis features such as chromatin condensation and nuclear collapse. Almeida et al. [30] used proteomics to demonstrate that acetic acid-induced apoptosis occurs through oxidative stress and subsequent signaling via the target of rapamycin (TOR) pathway.

Some aromatic hydroxy acids in pretreatment by-products can act as uncouplers. Norman et al. [31] found that salicylic acid uncouples plant cells by increasing mitochondrial inner membrane permeability to H^+, eliminating the proton gradient and preventing phosphorylation and ATP generation, thereby inhibiting cell growth and fermentation. In contrast, another common by-product, aromatic carboxylic acid *p*-hydroxybenzoic acid, does not exhibit uncoupling effects like salicylic acid, possibly due to differences in membrane permeability among various weak acids.

2.3 Phenolic Compounds Phenolic compounds are primarily formed from lignin degradation. Though produced in low quantities, they exhibit high toxicity, particularly low-molecular-weight monophenols. Phenolic inhibition mainly involves membrane damage by disrupting membrane integrity, affecting selective permeability and the intracellular enzymatic reaction environment. Fitzgerald et al. [32] found that phenol, *p*-cresol, and other potential phenolic compounds increase membrane fluidity in *Escherichia coli* and *Lactobacillus plantarum*, allowing greater diffusion and causing intracellular K^+ efflux. Keweloh et al. [33] studied the effects of phenol on protein and lipid composition in *E. coli* membranes, finding that phenol alters membrane function by changing the protein-to-lipid ratio and shifting fatty acid composition toward greater saturation. Feron et al. [34] investigated phenol' s DNA damage mechanism, attributing potential damage to intracellular ROS formation and the strong positive charge potential of aldehyde groups (particularly when the adjacent carbon bond is unsaturated). Zhang et al. [35] examined the inhibitory effects of four phenolic pretreatment by-products, including phenol and vanillyl alcohol, on the xylitol-producing yeast *Candida athensensis* SB18, finding these compounds inhibited intracellular xylose reductase activity without affecting xylitol dehydrogenase activity. These findings demonstrate the strong toxic effects of phenolics, which consequently affect microbial fermentation efficiency of pretreated straw.

Detoxification methods for inhibitors include physical approaches such as acti-

vated carbon adsorption [24] and vacuum evaporation [36] to remove most acetic acid and furfural; chemical methods like slaked lime [37] to precipitate certain inhibitors; and biological methods such as laccase [38] to reduce phenolic toxicity. Detoxification inevitably increases production costs, and some methods lack practicality. The slaked lime method is simple, economical, and effective, while biological detoxification should be prioritized in future research. summarizes the inhibition mechanisms and common detoxification methods for various inhibitors.

Table 2. Inhibition Mechanisms and Common Detoxification Methods for Inhibitors

Inhibitors	Inhibition Mechanism	Detoxification Techniques
Furan derivatives	ROS accumulation	Activated carbon adsorption [24]; vacuum evaporation [36]
Weak acids	Intracellular acidification and uncoupling [24]	Neutralization with $Ca(OH)_2$, NaOH, KOH [37]; ethyl acetate extraction; ion exchange resins
Phenolic compounds	Membrane integrity disruption [32-33]	Laccase or peroxidase treatment [38]; ion exchange resins

Pretreatment technology will be key to the rational and effective utilization of crop straw. While these methods improve straw characteristics and degradation rates, each has limitations. In practical production, appropriate pretreatment methods should be selected based on local conditions, straw type, and specific requirements. Understanding the inhibition mechanisms of fermentation inhibitors provides important reference value for method selection and condition optimization. Currently, straw feed processing alone cannot solve all feeding issues, and optimal feeding effects can only be achieved through scientific supplementation or dietary formulation techniques.

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