مجله دنیای میکروب‌ها
سال دوازدهم شماره دوم (پیاپی ۳۸) تابستان ۱۳۹۸
صفحات ۲۰۲-۱۸۶

Review Article

# تولید بوتانل زیستی با استفاده از کاه برنج: مروری بر پیش تیمار، هیدرولیز و تخمیر

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## چکیده

کاه برنج به دلیل فراوانی و ارزش پایین اقتصادی، به عنوان یکی از محصولات مازاد کشاورزی مناسب برای تولید بوتانل زیستی مطرح می‌باشد. کاه برنج دارای مقادیر زیادی قند می‌باشد که می‌توانند به قندهای قابل تخمیر تبدیل شوند. با این حال، تولید بوتانل زیستی با استفاده از کاه برنج بر اساس تکنولوژی فعلی به دلیل چالش‌ها و محدودیت‌های فرایندهای زیستی تبدیل کاه برنج به بوتانل اقتصادی نیست. برای این‌که این فرآیند به لحاظ اقتصادی امکان پذیر باشد، استفاده از یک روش پیش تیمار مناسب از اهمیت بسیار زیادی برخوردار است، زیرا به کارگیری یک روش پیش تیمار مطلوب منجر به فرایند هیدرولیز کارآمد می‌شود. در این مقاله، در ابتدا با استفاده از واژگان کلیدی تعداد ٦٦ مقاله در فاصله سال‌های ۱۹۸۰ تا ۲۰۱۸ در پایگاه‌های اطلاعاتی جستجو و سپس ارزیابی شد. این مقاله رویکردهای فعلی و پیشرفت‌های موجود در زمینه تولید بوتانل زیستی را با استفاده از کاه برنج بررسی می‌کند. همچنین، چالش‌های موجود در فرآیند کلی تبدیل زیستی کاه برنج به بوتانل مورد بحث قرار گرفته است. اگرچه با هدف توسعه تکنیک مناسب پیش تیمار کاه برنج پژوهش‌های زیادی انجام شده است، انجام تحقیقات بیشتر برای توسعه یک فرایند پیش تیمار کارآمد و اقتصادی ضرورت دارد. استفاده از سویه‌های اصلاح شده ژنتیکی مقاوم به بوتانل منجر به تولید بوتانل در غلظت‌های بالاتر شده است. بنابراین سیستم‌های برداشت محصول در محل، مقرون به صرفه گردیده واثر مهاری سوبسترا و سمیت بوتانل به میکروارگانیسم به شدت کاهش می‌یابد.

*واژگان کلیدی:* کاه برنج، بوتانل زیستی، پیش تیمار، هیدرولیز، تخمیر.

دریافت مقاله: شهریور ماه ۹۷ پذیرش برای چاپ: مهر ماه ۹۷

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Journal of Microbial World
Volume 12, No. 2, July 2019

**Review Article**

# Production of biobutanol from rice straw: An overview on pretreatment, hydrolysis, and fermentation

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## Abstract

Rice straw is a promising agricultural residue for the production of biobutanol due to its plentitude and low commercial value. Rice straw has high potential sugars that can be converted into fermentable sugars. However, biobutanol production from rice straw-based on the current technology is not economically viable due to the challenges and restrictions throughout the overall process of rice straw-biobutanol conversion. For the process to be economically viable, the use of an appropriate pretreatment technique is of great significance since an effective pretreatment can result in efficient hydrolysis. In this article, a total number of 66 articles from 1980-2018 were first searched on databases using keywords and then were reviewed. This paper reviews the current approaches and advances available for the production of biobutanol from rice straw. The challenges encountered throughout the overall process of bioconversion of rice straw into biobutanol are discussed as well. Even though much attempt has been made to develop a proper technique for pretreatment of rice straw, efforts are still required to make the process more efficient and economically feasible. The use of genetically modified butanol tolerant strains has resulted in butanol production at higher concentrations. Therefore, in situ product-recovery systems have become more economical as substrate inhibition and butanol toxicity to the culture are drastically reduced.

*Keywords*: Rice straw, Biobutanol, Pretreatment, Hydrolysis, Fermentation.
Received: August 2018 Accepted: September 2018

## Introduction

Biofuels are renewable resources-based and environmentally friendly. In response to the world unstable oil market, limited supply of conventional fossil fuels and increasing concerns over global warming, research has been guided towards the production of biofuels such as biobutanol from agricultural residues and wastes (1-3).

Rice straw is an abundant agricultural residue and a potential substrate utilized for biofuel production (4). The distribution of rice straw in the world is depicted in Table 1.

Cellulose, hemicellulose, and lignin are the three main components of rice straw.

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Journal of Microbial World 2019, 12(2): 186-202.

Table 1. Worldwide quantities of rice straw potentially

| Region | Rice straw availability (million tons) |
|---|---|
| Africa | 20.9 |
| Asia | 667.6 |
| Europe | 3.9 |
| America and Oceania | 38.6 |
| Total | 731 |

Pretreatment of rice straw is carried out aiming at lignin removal and a rise in cellulose content of rice straw. Pretreated rice straw then undergoes hydrolysis resulting in the production of fermentable sugars. Fermentable sugars are then converted into butanol through the fermentation process (6). The general overview of butanol production from rice straw is depicted in Figure 1.

*Types of pretreatment*

There are different strategies for the pretreatment of rice straw. Here, the technologies used to pretreat rice straw have been described.

*Physical pretreatment*

Physical pretreatment involves the breakdown of the lignocellulosic substrates into smaller particles and this reduction in particle size makes the biomass feedstock more amenable to subsequent enzymatic hydrolysis (7). Physical pretreatment can improve the biodegradability of lignocellulosic substrates. Physical pretreatment causes a rise in the accessible surface area as well as the pore size and a decrease in cellulose crystallinity and the degree of polymerization (8). Chipping, grinding, milling, and irradiation are among different types of physical pretreatment (8-10). After chipping the materials are usually reduced in size to a size reduction of 10-30 mm, whereas after milling or grinding there is a size reduction to 0.2-2 mm (9,10).

Physical pretreatment is insensitive to the physical and chemical characteristics of the biomass and this is considered as an advantage of physical pretreatment. However, not being able to remove lignin and being energy demanding is known as the major disadvantage of this method (7).

As reported by Jin & Chen (2006), superfine ground steam-exploded rice straw proved a better substrate as compared to the ground residue when enzymatically hydrolyzed (11). However, the high energy demand required for the process in industrial applications has to be considered as well. Hideno et al. (2009) tried wet disk milling and ball milling of rice straw and wet disk milling of rice straw proved advantageous over ball milling since more glucose was recovered and less energy was consumed (12). As reported by Hideno et al. (2009) hot-compressed water-pretreated rice straw showed better as the substrate when hydrolyzed, as compared to rice straw pretreated with wet disk milling. However, wet disk milling was reported as a practical method of pretreatment of biomass such as rice straw due to its lower energy consumption (12).

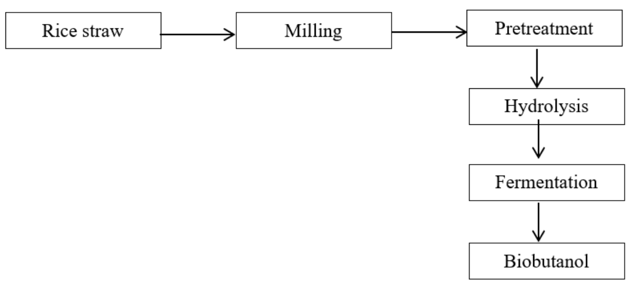


Figure 1. General concept of biobutanol production from rice straw

Milled dry rice straw was subjected to physical pretreatment by Bak et al. (2009), followed by electron beam irradiation. As a result of enzymatic hydrolysis of rice straw pretreated by electron beam irradiation, after 132 h, a glucose yield of 52.1% was obtained, as compared to that of 22.6% when rice straw was not pretreated. This result could be due to the larger loss in lignin and hemicellulose content after pretreatment of rice straw by beam irradiation that may be due to the greater chance of lignin and xylan to be hit by electrons during irradiation.

Irradiation techniques are superior to chemical pretreatment methods since the former do not require extreme temperatures. Hence, unlike acid and alkali pretreatment methods, almost no inhibitory and/or toxic compounds are generated. However, such techniques are costly, require high energy and have difficulties while going for commercial applications (13).

The use of microwave irradiation for pretreatment of rice straw could improve rice straw enzymatic hydrolysis in presence of water (14) as well as in the presence of glycerin and less amount of water (15).

According to Zhu et al. (2005), hydrolysis rate and reducing sugar yield obtained was almost the same when rice straw pretreated by microwave irradiation alone was used and when raw rice straw was applied (4). Ma et al. (2009) carried out microwave irradiation for pretreatment of rice straw and reported a 30.3% increase in saccharification rate as compared to raw rice straw.

According to Ma et al. (2009), microwave irradiation could successfully improve enzymatic digestibility of rice straw as it could break down the silicified waxy surface, disrupt the lignin–hemicellulose complex followed by partial removal of silicon and lignin (16).

*Chemical pretreatment*

Dilute NaOH pretreatment is known as an an effective method for the hydrolysis of straws primarily low in lignin content (10-18%) (9). As compared to acid pretreatment and oxidative reagents, alkali pretreatment is a promising method that can efficiently break ester bonds present between the three major components of lignocellulosic substrates. In alkali pretreatment, fragmentation of the hemicellulose polymer is avoided as well. However, this method is not environmentally friendly and some alkali is consumed by the biomass itself (17).

Alkali pretreatment of rice straw by (1% (w/v) NaOH] caused the cellulose content of rice straw to increase to 65.4%. Whereas, a reduction in lignin and hemicelluloses content of the substrate occurred to 6.0% and 14.3%, respectively (4). Jeya et al. (2009) used (2% (w/v) NaOH] to pretreat rice straw and reported a decrease in lignin and hemicelluloses content of rice straw from 15.8% and 25.8% to 9.8% and 18%, respectively.

An increase in cellulose content of alkali-pretreated rice straw from 36.8% to 52.2% was noted by Jeya et al. (2009) as well (18). According to Zhang and Cai (2008), the chemical composition of rice straw differed when rice straw was pretreated by (2% (w/v) NaOH].

Alkali pretreatment of rice straw increased cellulose by 54% and decreased hemicellulose and lignin by 61% and 36%, respectively (19). Dilute acids do not dissolve lignin, but alter the structure of lignin, and hydrolyze hemicelluloses out of the solid biomass.

Hemicellulose is hydrolyzed to xylose and other sugars and almost 100% hemicellulose removal is possible (8,9). Hydrolysis of cellulose can be significantly improved using dilute acid pretreatment. However, the cost of dilute acid pretreatment is much higher, as compared to physiochemical pretreatments such as steam explosion or ammonia fiber explosion (AFEX), since pH should be neutralized before fermentation processes and toxic substrates are formed (9).

Hsu et al. (2010) investigated the effect of dilute acid pretreatment of rice straw on structural properties. The removal of hemicellulose may increase in porosity and pore volume of the pretreated solid residue and maximum sugar yield of 83% was obtained after rice straw was pretreated by 1% (w/w) dilute sulfuric acid (20).

Alkaline/oxidative pretreatment is more effective in improving enzyme digestibility of lignocellulosic residues, as compared to NaOH pretreatment alone.

Alkaline peroxide can improve enzymatically hydrolysis through the delignification of the biomass (8). As a result of this method of pretreatment, lignin, and hemicellulose are removed and cellulose becomes more accessible to hydrolytic enzymes. pH proved to be a key and major parameter in delignification of agricultural residues and a pH of 11.5-11.6 was reported as pH optimum for the process. The delignification probably occurs because of the formation of hydroxyl ion (OH), produced as a result of the degradation of hydrogen peroxide (21). Wei & Cheng (1985) pretreated rice straw by using hydrogen peroxide.

Pretreatment was conducted at 60◦C for 5 h and the solution contained 1% (w/w) H2O2 and sodium hydroxide. As a result, pretreated rice straw was delignified to 60% and a 40% weight loss was noted. The accessibility of Cadoxen and the water-holding capacity was increased to fivefold and one time, respectively. However, the crystallinity of the pretreated rice straw was decreased very slightly. The use of NaOH at a higher initial concentration and a rise in the pretreatment temperature could result in a more efficient pretreatment (22).

Taniguchi et al. (1982) reported on the use of peracetic acid for the pretreatment of rice straw. As a result of peracetic acid pretreatment of rice straw cellulose and hemicellulose content of rice, straw was slightly lost even though the crystalline structure of cellulose was not disrupted (23).

Rice straw pulping was conducted by Mohammadi-Rovshandeh et al. (2005) and using organosolvent pretreatment, rice straw was successfully delignified (24). The use of acetic acid and formic acid for rice straw pretreatment was reported by Jahan et al. (2006). The highest dissolution of pentoses occurred in 80% acetic acid with 0.6% $H_2SO_4$ catalyst at 80◦C for 120 min. Formic acid could dissolve pentoses more quickly, as compared to acetic acid (25).

Surfactant-assisted ionic liquid pretreatment of rice straw was studied by Chang et al. (2017) and rice straw exhibited 26.14% reduction in lignin content (26).

*Physiochemical pretreatment*

These pretreatments focus mainly on dissolving hemicelluloses and alteration of lignin structure. As a result, cellulose is more accessible to hydrolytic enzymes (27,28).

The steam explosion was reported as a very efficient method for delignification of rice straw and almost all the polysaccharides (cellulose and hemicellulose) present in rice straw were efficiently and rapidly hydrolyzed to glucose and xylose (29).

Moniruzzaman et al. (1996) studied the changes occurring in the physical and chemical characteristics of rice straw as a result of a steam explosion. As reported by Moniruzzaman et al. (1996), a steam explosion could efficiently pretreat rice straw and resulted in a dramatic rise in fiber porosity. Enzymatic hydrolysis of steam-exploded rice straw resulted in the production of a maximum 83% glucose yield (30).

Ammonia fiber explosion (AFEX) is an effective method for pretreatment of rice straw and it only resulted in 3% sugar loss (31). Yu et al. (2010) carried out hot-compressed water for pretreatment of rice straw. According to Yu et al. (2010), considering sugar recovery, degradation product formation, and process severity, a temperature of 180◦C for 30 min should be applied for pretreatment of rice straw by hot-compressed water (32). Hot-compressed water pretreatment of rice straw was carried out by Hideno et al. (2009) and a glucose and xylose yield of 89.4% and 88.6% were achieved, respectively (12).

Chang et al. (2011) indicated that freeze pretreatment was highly effective for pretreatment of rice straw and its subsequent enzymatic hydrolysis. Water is among the few materials that expand when they freeze. Pre-incubation of the rice straw in acetate buffer for 1 h caused the buffer to penetrate the pores of rice straw. The buffer expanded when frozen adding accessible surface area and pore volume while disrupting the structure followed by being thawed at room temperature. Freeze pretreatment of rice straw significantly increased the enzyme digestibility of the fiber from 48% to 84% (33).

*Biological pretreatment*

Four white-rot fungi (Phanerochaete *chrysosporium, Trametes Versicolor, Ceriporiopsis subvermispora*, and *Pleurotus ostreatus*) were evaluated for pretreatment of rice straw in a study performed by Taniguchi et al. (2005). The structural changes in the components of the pretreated rice straw were evaluated as well as the susceptibility of the pretreated rice straw to enzymatic hydrolysis.

Of the fungi tried, *Pleurotus ostreatus* selectively degraded lignin rather than the holocellulose component. According to Scanning Electron Microscopic (SEM) observations, pretreatment with *Pleurotus ostreatus* could increase enzymatic digestibility of rice straw since as a result of pretreatment lignin was partially degraded making it easier for cellulase to penetrate in rice straw (34).

A wood-rot fungus, Dichomitus squalene was used for biological pretreatment of rice straw. Fermentation of rice straw by *Dichomitus squalene* resulted in a rise in susceptibility of rice straw to enzymatic hydrolysis and digestibility of the fungal-fermented rice straw was reported as 58.1%. X-ray diffraction analysis of fermented rice straw indicated changes in the crystalline structure of cellulose and Scanning Electron Microscopic (SEM) images revealed microstructural changes in the fermented rice straw (35). Patel et al. (2007) conducted a preliminary study on the microbial and fermentation of rice straw and as a result of screening, five different fungi were obtained.

Among the selected fungi, fermentation with *Aspergillus niger* and *Aspergillus awamori* yielded the highest concentration of total sugars and consequently resulted in the highest yield of ethanol (36).

*Combined pretreatment*

Niu et al. (2009) used a combination of alkali assisted by photocatalysis in order to pretreat rice straw. As a result of this combined pretreatment, the physical properties and microstructure of rice straw were efficiently altered. A combination of alkali assisted by photocatalysis appears to be an effective method for pretreatment of rice straw resulting in a hydrolysis rate of 73.96% that is 2.56-fold higher than that of rice straw pretreated only with alkali. The higher rice straw enzymatic degradability could be due to the removal of a

Table 2. Pretreatment techniques and corresponding conditions applied for pretreatment of rice straw.

| Pretreatment technology | Procedure/ Chemicals | References |
|---|---|---|
| Freeze pretreatment | Mixed with acetate buffer 1 h; Frozen at -20 ºC 2 h; thawed at room temperature 1 h | (33) |
| Dry ball milling | Air dried rice straw added to a pod having 90 balls; milled for 2, 5, 10, 15, 30, 60, 120, 180 min at 1700 rpm | (12) |
| Hot-compressed water | Mixture of rice straw and water in autoclave reactor; $N_2$ at initial pressure 2 MPa; autoclave heated to 160-180 ºC at a heating rate of 2-3 ºC /min, stirred 30 min at ±3 ºC | (12) |
| Electron beam irradiation | Milled dry rice straw irradiation with accelerated electrons; at the current range of 0.03-0.24 Ma for a duration of 4-36 m | (13) |
| Electron beam irradiation | Radiation in the presence of NaOH | (41) |
| Alkali pretreatment | 2% NaOH | (18,19) |
| Alkali assisted by photocatalysis | 1.5% NaOH assisted by 2 g/L nano-$TiO_2$, irradiation by UV for 1 h | (37) |
| Ammonia fiber/freeze explosion/ expansion (AFEX) | 140 ºC, 30 min, 1:1 g ammonia/g dry biomass | (31) |
| Aqueous ammonia pretreatment | 21% (w/w) aqueous ammonia solution, 69 ºC,10 h, S: L ratio of 1:6 | (42) |
| Dilute acid pretreatment | 1%H 1% $H_2SO_4$, 1-5 min, 160-180 ºC | (20) |
| Oxidizing agents | Dried straw suspended in peracetic acid solution, heated at 80-90 ºC, 1 h | (23) |
| Microwave pretreatment | Micro Microwave/acid (2% $H_2SO_4$) /alkali (1%NaOH) / $H_2O_2$ (1% NaOH containing $H_2O_2$) | (40) |
| Biological pretreatment | *Pleurotus ostreatus* | (34) |
| Steam explosion combined with superfine grinding | Steam exploded rice straw, 220ºC/4 min combined with superfine grinding using a fluidized bed opposed mill | (11) |
| Organosolv pretreatment | 75% (v/v) aqueous ethanol containing 1% (w/w) $H_2SO_4$, 150 ºC, 60 min | (43) |
| Ionic-liquid | Renewable cholinium lysine ionic liquid-water mixture | (44) |
| Iionic-liquid | 1-ethyl-3-methylimidazolium acetate, 120 ºC/ 12 h | (45) |
| Organosolv pretreatment | N-methylmorpholine-N-oxide, atmospheric pressure, 120 ºC, 5 h | (46) |

larger amount of alkali-soluble lignin and hemicellulose that could probably make cellulose more accessible to hydrolytic enzymes (37). Alkali pretreatment of rice straw in the absence of $H_2O_2$ favored solubilization of hemicelluloses with a small molecular size that is rich in glucose, probably originating from α-glucan. However, the addition of alkaline peroxidase enhanced solubilization of hemicelluloses that are rich in xylose and have a larger molecular size (38).

According to Chen et al. (2011), an integrated process of dilute-acid/steam explosion was more effective than an acid-catalyzed steam explosion in the pretreatment of rice straw since the former had a higher xylose yield, a lower level of inhibitor in the hydrolysate and a greater degree of enzymatic hydrolysis (39).

Microwave is found to be effective in the pretreatment of lignocellulosic materials when combined with other pretreatment methods. According to Zhu et al. (2006), microwave/acid/alkali/H2O2 resulted in the highest total weight loss, the highest rise in the amount of cellulose, and the lowest moisture, ash, lignin, and hemicellulose content of rice straw. This pretreatment resulted in the highest cellulose content due to the efficient removal of lignin and hemicellulose, and the lowest loss of cellulose. The lowest moisture probably was obtained due to the pretreatment, where the pore size of cellulose fiber was enlarged, leading to a decrease of water bound to rice straw. Microwave/alkali removed more lignin and hemicellulose, as compared to the alkali alone (40).

Using an electron beam accelerator, Xin and Kumakura, (1993), studied the effect of radiation on pretreatment of rice straw in the presence of alkali (NaOH) solutions. Irradiation causes NaOH to penetrate the lignocellulosic structure easily. As a result of irradiation, cellulose and hemicellulose are scissored to smaller size molecules and lignin removal occurs easier. Consequently, cellulase penetrates the cell structure easier and cellulose is enzymatically digested. With the rise in irradiation dose, an increase in enzymatic hydrolysis of rice straw pretreated with a combination of irradiation and NaOH solution was observed (41).

Steam explosion of rice straw in combination with superfine grinding of the substrate was carried out and the pretreated rice straw was subsequently enzymatically hydrolyzed. The superfine ground steam-exploded rice straw product was different from the ground steam-exploded rice straw residue in terms of chemical composition, fiber characteristics, and enzymatic hydrolysis. The difference in

Table 3. Comparison of enzymatic hydrolysis of rice straw by various enzyme sources and the saccharification yield.

| Source | Substrate | Reducing sugar (mg/g-substrate) | Hydrolysis (%) | Reference |
|---|---|---|---|---|
| *T. reesei* A1 | Rice straw | 374 | 70.50 | (55) |
| *Penicillium* sp. B1 | Rice straw | 316 | 57.90 | (55) |
| *T. reesei* ZM4F3 | Rice straw | 733 | - | (19) |
| Celluclast | Rice straw | 84 | - | (56) |
| *Trametes hirsuta* | Rice straw | 685 | 88 | (18) |
| Celluclast, Periconia sp. bcc2871 | Rice straw | 132 | - | (56) |
| *A.heteromorphus, T. reesei* | Rice straw | - | 84 | (50) |

enzymatic hydrolysis and structural properties indicates that superfine grinding is a good way to fractionate superfine ground steam-exploded rice straw into an easily bio-Convertable part and the part which is not easily hydrolyzed. Superfine ground steam-exploded rice straw product was subjected to enzymatic hydrolysis. As a result, the highest hydrolytic rate and reducing sugar were obtained. However, enzymatic hydrolysis of the superfine ground residue resulted in the production of reducing sugar at a lower concentration of that of untreated rice straw (11). Table 2 illustrates several pretreatment methods and the conditions applied for the substrate pretreatment.

*Enzymatic hydrolysis*

In the bioconversion of lignocellulosic substrates into value-added products such as biofuel, enzymatic hydrolysis occurs as the second step and only after lignocellulosic substrates are pretreated (47). Enzymatic hydrolysis is conducted under mild conditions. The majority of cellulases are active optimally at acidic pH ranges (4.0-5.0) and are most active at temperatures 50±5 ºC (48).

To improve the efficiency of enzymatic hydrolysis in terms of both the yield and the rate, research has focused on the optimization of the hydrolysis process as well as enhancing cellulase activity. Table 3 indicates a comparison of enzymatic hydrolysis of rice straw by various enzyme sources and the saccharification yield.

The use of organosolv-pretreated rice straw as the substrate for enzymatic hydrolysis resulted in the production of 31 g/L total sugar (43). Ionic liquid pretreatment of rice straw proved to be promising and a yield of 81% and 48% of glucose and xylose was obtained, respectively (44).

Sun et al. (2013) used rice straw pretreated with the ionic liquid as the substrate for enzymatic hydrolysis and reducing sugar yield of 70.5% was achieved at 48 h hydrolysis that was 7-fold higher than that of untreated rice straw. The pretreated rice straw exhibited lower crystallinity and the crystalline form of rice straw was partly transformed from cellulose I structure to amorphous or cellulose II after the pretreatment (45).

Enzymatic hydrolysis of rice straw pretreated with organic solvent resulted in a sugar yield of 90% at 72 h (46). As a result of enzymatic hydrolysis of rice straw pretreated with alkali assisted by photocatalysis, 73.96% hydrolysis rate was achieved that is 2.56-fold higher, as compared to when alkali was used alone (37). The use of ammonia-pretreated rice straw as the substrate for enzymatic hydrolysis, increased the production of monomeric sugars from 11% in the untreated substrate to 61% (79.2% reducing sugars) (49).

Singh & Bishnoi (2012) reported 84% enzymatic hydrolysis of microwave alkali-pretreated rice straw (50).

Ultrasonic-pretreated rice straw was enzymatically hydrolyzed and glucose concentration of the pretreated rice straw was significantly greater than that of the untreated substrate. This could occur due to the effect of ultrasound on the plant cell wall. Ultrasound can break the plant cell wall and open up the cell structure, thus making cellulose more accessible to the enzymes (51). It is well known that the efficiency of hydrolysis of lignocellulosic biomass increases when cellulase is supplemented with other enzymes such as xylanases and pectinases (31). However, the rise in the process cost is a major drawback.

Alkali-assisted acid pretreated rice straw was subjected to hydrolysis with the cellulases from *Aspergillus niger* BK0 and a maximum yield of 23.78% sugars and 35.96% saccharification value was obtained (52). Alkaline peroxide-assisted wet air oxidation pretreated rice straw was enzymatically hydrolyzed and resulted in enhanced glucose yields due to significant delignification (53). Enzymatic hydrolysis of rice straw pretreated by oxidants assisted with photocatalysis ($TiO_2$/UV/ $H_2O_2$) was conducted and yielded reducing sugar concentration of 8.88 ± 0.10 mg/mL as compared to 5.47 ± 0.03 mg/mL when the untreated substrate was applied (54).

*Fermentation*

Substrate cost is an important factor affecting the overall cost of biobutanol production from lignocellulosic biomass. Hence, various agricultural by-products including rice straw have been applied. Since agricultural residues are used as fermentation substrates, the ability to consume mixed sugars is significant to economize the production process. Unlike yeasts, hexose and pentose sugars obtained as a result of pretreatment and hydrolysis of agricultural wastes can be utilized by butanol-producing cultures (2).

The use of Clostridium acetobutylicum MTCC 481 and rice straw hydrolysate under the optimum process conditions resulted in the production of total ABE and butanol of 15.84 g/L and 12.17 g/L in a 2 L bioreactor and 16.91 g/L and 12.22 g/L in a 5-L bioreactor, respectively (57).

At high initial cell concentrations of Clostridium saccharoperbutylacetonicum N1-4, butanol production from non-pretreated rice straw under non-sterile environmental conditions was comparable with that under sterile conditions. At high cell concentrations, interference from other microbes is minimized and butanol production is comparable to sterile biobutanol production which makes the process more cost-effective (58).

Butanol production was carried out from rice straw by a non-acetone forming Clostridium

Table 4. Fermentation of rice straw hydrolysate by various microorganisms.

| Microorganism | ABE (g/L) | Butanol (g/L) | ABE Productivity (g/L/h) | Reference |
|---|---|---|---|---|
| *C.acetobutylicum* MTCC 481 | □15.84 | 12.17 | - | (57) |
| *C. acetobutylicum* MTCC 481 | ⊙16.91 | 12.22 | - | (57) |
| *C. sporogenes* BE0 | 5.52 | 3.43 | - | (59) |
| *C. acetobutylicum* NRRL B-591 | 10.5 | - | 0.20 | (43) |
| *C. acetobutylicum* NCIM 2337 | 20.56 | 13.5 | - | (61) |
| *C. acetobutylicum* ATCC 824 | 2.73 | 1.62 | 0.04 | (62) |

♯ 2-L bioreacter; ⊙5-L bioreacter

sporogenes BE0. The strain gave a maximum butanol yield of 3.43 g/L and 5.52 g/L total solvents were produced (59).

Non-detoxified alkali and phosphoric acid pretreated rice straw hydrolysates were used for biobutanol production by *C. acetobutylicum* NRRL B-591 and above 44 g and 17 g butanol and acetone were produced from each kg of rice straw (60).

Rice straw was pretreated with ethanol organosolv followed by enzymatic hydrolysis. Rice straw hydrolysate was then fermented by C. acetobutylicum NRRL B-591 and a yield of 123.9 g ABE was obtained from each kg of rice straw. The highest ABE concentration and productivity were reported as 10.5 g/L and 0.20 g/L/h, respectively (43).

Anaerobic fermentation of rice straw hydrolysate using *C. acetobutylicum* NCIM 2337 resulted in the production of 6.24 g/L acetone, 13.5 g/L butanol and only 0.82 g/L ethanol (61).

Fermentation of NaOH-pretreated rice straw by *C. acetobutylicum* ATCC 824 resulted in an ABE yield, ABE productivity, and biobutanol yield of 0.27 g/g glucose, 0.04 g/L/h and 0.16 g/g glucose, respectively (62).

Kiyoshi et al. (2015) applied the co-culture of cellulolytic Clostridium thermocellum NBRC 103400 and butanol-producing *C. Saccharoperbutylacetonicum* strain N1-4 for butanol production from rice straw pretreated with 1% (w/v) NaOH. They showed that butanol production was significantly increased by the addition of cellulase attributed to the enhancement of exoglucanase activity (63).

Cellulose hydrolysate of rice straw was consumed by an *Escherichia coli* co-culture system and the system showed to be promising for the production of cellulosic biobutanol (64).

Naturally, butanol production by bacteria is an exclusive characteristic of the members of the genus *Clostridia*.

However, not all *Clostridium* species are high butanol producers. *C. acetobutilycum* is the most common species for butanol production (65). Solvent toxicity is known as a major obstacle in ABE fermentation. At a concentration of approximately 16 g/L, butanol can inhibit cell growth resulting in the termination of fermentation. To address the problem with butanol toxicity the possible ways suggested are to develop more butanol tolerant strains using genetic engineering approaches and simultaneous fermentation and product removal through engineering techniques (66). Aiming at improving butanol production, much attempt has been made in order to make mutants of *C. acetobutylicum* which are resistant to degeneration, are butanol tolerant, and have regulated sporulation (65). Applying genetic engineering techniques and by using *E. coli* and *Saccharomyces cerevisiae* as hosts, researchers have produced butanol by transforming the genes responsible for the expression of the key enzymes in butanol metabolic pathway of *Clostridium* (65).

Apart from genetically developed *E. coli* and *S. cerevisiae*, engineered *Bacillus subtilis* are also reported as a butanol-synthesizing microorganism (1).

Among genetically modified microbes with the potential capability of butanol production *Zymomonas, Enterococcus, Rhodococcus, Pichia, Candida, Pseudomonas*, and *Klebsiella* are also listed (65).

However, these microbes are not as well studied as *E. coli, S. cerevisiae*, and *B. subtilis*. Therefore, more attempt is required so that the above-mentioned potential butanol producers could be explored widely. Separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) are two different strategies for enzymatic hydrolysis and fermentation to be accomplished. SSF is more favored due to its low potential costs and problems such as enzyme inhibition, sugar accumulation and contamination can be avoided. However, a major drawback of SSF is the difference between the optimum temperature for enzymatic hydrolysis and fermentation (48). Recovery of fermentation by-products such as CO2 and H2 would favor the biobutanol I ndustry as well and is suggested. H2 gas can be recovered and could serve as an excellent source of energy. A comparison of the fermentation of rice straw hydrolysate by various microorganisms is depicted in Table 4.

## Conclusion

The use of rice straw as a substrate for biobutanol fermentation has great potential. However, industrial utilization of rice straw for biobutanol production is not feasible unless cost-effective technology is available. Biological conversion of rice straw into fermentable sugars through enzymatic hydrolysis has proved to be the most promising method since it is carried out under mild conditions and is not hazardous to the environment. So far, much attempt has been made to develop a proper technique for pretreatment of rice straw and much progress has been made to date. Yet, efforts are still required to make the process more efficient and economically feasible. The performance of the strain is a crucial factor in the overall competitiveness of the bioprocess. Through genetic engineering, strains that are more butanol tolerant have been developed. However, more attempts are still required so that strains that are more butanol tolerant and resistant to degeneration could be developed. By using genetically modified strains, butanol can be produced at a higher concentration and solvent toxicity problems will be reduced. Moreover, production of butanol at higher concentrations could reduce operating costs of in situ recovery systems that in turn favor process economics. Fermentation and recovery technologies have been well developed. However, further, improvement is required. By employing in situ product-recovery systems, substrate inhibition and butanol toxicity to the culture are drastically reduced. Given that biobutanol production from lignocellulosic substrates is moving forward at a rapid pace and considering rice straw as a potent candidate for biobutanol production, production of biobutanol from rice straw appears to have a bright future and is likely to become an economically feasible process in the coming years.

## Acknowledgment

The author would like to acknowledge the researchers all over the world whose publications helped prepare this manuscript.

## Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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