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# Comparison of various pretreatments for ethanol production enhancement from solid residue after rumen fluid digestion of rice straw

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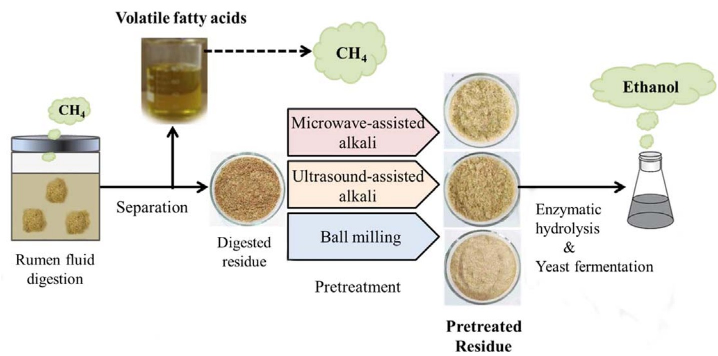
## GRAPHICAL ABSTRACT

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## ABSTRACT

The rumen digested residue of rice straw contains high residual carbohydrates, which makes it a potential cellulosic ethanol feedstock. This study evaluated the feasibility and effectiveness of applying microwave assisted alkali (MAP), ultrasound assisted alkali (UAP), and ball milling pretreatment (BMP) to enhance ethanol production from two digested residues (2.5%-DR and 10%-DR) after rumen fluid digestion of rice straw at 2.5% and 10.0% solid content. Results revealed that 2.5%-DR and 10%-DR had a cellulose content of 36.4% and 41.7%, respectively. MAP and UAP improved enzymatic hydrolysis of digested residue by removing the lignin and hemicellulose, while BMP by decreasing the particle size and crystallinity. BMP was concluded as the suitable pretreatment, resulting in an ethanol yield of 116.65 and 147.42 mg $g^{-1}$ for 2.5%-DR and 10%-DR, respectively. The integrated system including BMP for digested residue at 2.5% solid content achieved a maximum energy output of 7010 kJ $kg^{-1}$.

## 1. Introduction

Rumen microorganisms, with high cellulolytic activity, have been successfully employed to digest various lignocellulosic biomass, such as agricultural residues, organic fraction of municipal solid wastes and aquatic plants (Yue et al., 2013). Compared with conventional anaerobic digestion, the system inoculated with rumen microbes showed a higher hydrolysis and acidification efficiency for both lignocellulosic and high-cell-soluble wastes (Yue et al., 2013). As the major intermediate products of hydrolysis and acidification, volatile fatty acids

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(VFAs) can be used to produce methane, hydrogen, electricity, biodiesel, and bioplastic polyhydroxyalkanoates (Motte et al., 2015). Although high degradation efficiency and valuable products can be achieved in a rumen microbes dominated anaerobic digestion process, large amounts of undigested residue remains, which still contains a high content of residual carbohydrates (Yue et al., 2013). For example, Zang et al. (2010) studied the anaerobic digestion of *Canna indica* by rumen microorganisms and found that the digested residue had a higher cellulose content of 24% and a lower hemicellulose content of 26% than the raw feedstock (21% cellulose and 31% hemicellulose). Yue et al. (2007) reported that the digested residue of *Canna indica* contained 23.2% cellulose, 9.9% hemicellulose and 14.8% lignin after 120 h anaerobic digestion by rumen cultures. High content of carbohydrates remained in the digested residue suggested that these carbohydrates can be recovered and further reused to produce bioenergy. Global energy demand is growing rapidly, and about 80% of energy is still based on fossil fuels, which causes serious environmental damage, such as global warming, acid rain, urban haze, etc (International Energy Agency, 2016). The production of biofuel from lignocellulosic biomass is considered to be a viable alternative for fossil-derived fuel, because the biofuel is clean and renewable (Zhu et al., 2015). Thus, an integrated process of rumen microorganisms dominated anaerobic digestion and cellulosic ethanol refining should be a promising approach for biofuel production. Moreover, proper handling of the digested residue can reduce environmental problems. However, the investigation on ethanol refining of digested residue by rumen microorganisms as feedstock has little been reported.

However, lignin-carbohydrate complex ester/ether linkages and relatively high content of lignin still remains in the digested residue, which mainly contributes to its recalcitrance for biofuel conversion (Zhao et al., 2017, 2014). Furthermore, natural properties of plant body also contribute to the recalcitrance of biomass, such as the plant tissue arrangement, cellulose crystallinity, degree of polymerization and so on (Himmel et al., 2007). Therefore, suitable pretreatment is needed to break down this recalcitrant structure for efficient conversion of digested residue to cellulosic ethanol. The goal of pretreatment is to remove the barrier of lignin and hemicellulose around the cellulose, to decrease the degree of polymerization and crystallinity of cellulose, and to increase the internal surface of biomass, thus improving its saccharification efficiency (Zhu et al., 2015). Chemical, physical pretreatment or a combined strategy of them can be considered (Kapoor et al., 2015; Yuan et al., 2015).

Alkali can break the ester/ether bonds cross-linking lignin and xylan, remove lignin, swell cellulose and partially decrystallize cellulose, making the cellulose more accessible to enzymatic attack (Wang et al., 2016). The main limitations of alkaline pretreatment of lignocellulosic biomass include relatively long pretreatment time and high alkali consumption. To improve the efficiency of alkali pretreatment, microwave and ultrasound has been applied to assist the alkali pretreatment of various lignocellulosic biomass. Zhu et al. (2006a,b,c) pretreated wheat straw with microwave-assisted alkali pretreatment (MAP) and presented a higher hydrolysis efficiency than that with conventional heating. Jin et al. (2016) studied enzymatic saccharification of catalpa sawdust by microwave-assisted alkali pretreatment, and concluded that the maximum reducing sugar yield under optimal conditions reached 402.7 mg $g^{-1}$, which increased by 682.1% compared with the control. Silva et al. (2016) reported that ultrasound-assisted alkali pretreatment (UAP) led to a total glucose recover of 95.8% from sugarcane bagasse. In addition, mechanical pulverization of biomass by ball milling improved enzymatic digestion of biomass (Yuan et al., 2015). The ball milling efficiently reduced the particle size and crystallinity of cellulose, and loosened the inner structure of biomass, which resulted in a glucose yield as high as 78.7% for sugarcane bagasse (Silva et al., 2010) and 89.4% for rice straw (Hideno et al., 2009). Moreover, no weight was lost and no hydrolysis or fermentation inhibitors were produced with ball milling pretreatment (BMP). These studies have shown that the MAP, UAP and BMP are promising for glucose production from biomass, which might be applied for effective ethanol production from digested residues.

Preliminary experiments showed that complete rumen fluid digestion of rice straw occurred at a solid content of 2.5% after 72 h, resulting in nearly complete conversion of cellulose and hemicellulose that could be used by ruminal microbes. While sharp pH decrease caused by accumulation of VFAs at 10.0% solid content led to incomplete rumen fluid digestion due to some ruminal microbes (eg. cellulolytic bacteria, protozoa, and fungi) could not tolerate low pH (Jiao et al., 2016).

This study evaluated the feasibility and effectiveness of MAP, UAP, and BMP to enhance ethanol production from digested residues after rumen fluid digestion of rice straw. It determined the properties of solid residue from complete (2.5% solid content) and incomplete (10.0% solid content) rumen fluid digestion of rice straw, and evaluated the effectiveness of MAP, UAP and BMP for enzymatic hydrolysis and ethanol fermentation of 2.5%-FR and 10%-FR. The chemical composition and physical structure of pretreated residue were also investigated to explain the mechanisms of different pretreatments. Finally, the integrated rumen microorganisms dominated anaerobic digestion with ethanol refining process was proposed and evaluated, and total energy output were calculated.

## 2. Materials and methods

### 2.1. Materials

Rice straw (about 90 d from sowing to harvesting) was harvested in October 2015 from Wangcheng County, Hunan Province, China. The whole aboveground plant was initially air-dried at room temperature for 3 month and chopped to 2–3 cm using a paper knife. The air-dried rice straw was then dried at 60 °C for at least 24 h to a constant weight in an oven and grounded to a size of 30 mesh with a grinder (HC-700. Huangcheng, China). The processed rice straw was then sealed in plastic bags and stored at room temperature for further use. The total solid (TS) and volatile solid (VS) of rice straw were 91.0% and 78.2%, respectively. The rice straw was composed of 39.1% cellulose, 28.3% hemicellulose, and 7.6% lignin.

Rumen fluid was obtained from fresh stomach of cattle from one slaughterhouse in Changsha of China, and transferred to the laboratory in a sealed bottle. The rumen fluid was filtered through four layer of gauze with $N_2$ protection, and used in experiments within 5 h.

### 2.2. Integration of rumen fluid digestion and ethanol refining process

Fig. 1 shows the experimental strategy used for the bioconversion of rice straw into biofuel. This integrated rumen fluid digestion and ethanol refining process included rumen fluid digestion, residue pretreatment, enzymatic hydrolysis and ethanol fermentation. The biofuel produced in the integrated process included methane and ethanol. The generated liquid and solid waste should be further reused, treated or disposed. There was no liquid waste generation with the BMP compared to the MAP and UAP.

#### 2.2.1. Rumen fluid digestion of rice straw

The rice straw with solid content of 2.5% and 10.0% were adopted in the anaerobic rumen fluid digestion. The batch digestion experiments were implemented using 1.0 L reactors. Six reactors were at a solid content of 2.5% and three reactors at a solid content of 10.0%. The rice straw of 20 or 80 g were respectively added and inoculated with 300 ml rumen fluid; In addition, 300 ml artificial saliva was added to provide pH buffering capacity of the liquid medium as well as nutrients for ruminal microbes growth. The artificial saliva contained 9.8 g $L^{-1}$ $NaHCO_3$, 3.71 g $L^{-1}$ $NaHPO_4$, 0.57 g $L^{-1}$ KCl, 0.12 g $L^{-1}$ $MgSO_4 \cdot 7H_2O$, 0.05 g $L^{-1}$ $CaCl_2 \cdot 2H_2O$ and 0.47 g $L^{-1}$ NaCl (McDougall, 1948). The