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# Pretreating wheat straw by phosphoric acid plus hydrogen peroxide for enzymatic saccharification and ethanol production at high solid loading

Jingwen Qiu [a,b], Lunjie Ma [a,b], Fei Shen [a,b,*], Gang Yang [a,b], Yanzong Zhang [b], Shihuai Deng [a,b], Jing Zhang [a,b], Yongmei Zeng [a,b], Yaodong Hu [a,b]

[a] Institute of Ecological and Environmental Sciences, Sichuan Agricultural University, Chengdu, Sichuan 611130, PR China
[b] Rural Environment Protection Engineering & Technology Center of Sichuan Province, Sichuan Agricultural University, Chengdu, Sichuan 611130, PR China

## HIGHLIGHTS

- PHP-pretreatment can promote solid loading to higher than 20% for hydrolysis.
- 73% cellulose was hydrolyzed with lower enzyme input of 20 mg protein/g cellulose.
- Decrease on glucose yield was observed after the pretreated substrate being dried.
- Dryness shrank the large-size pores resulting in decreasing hydrolysis efficiency.
- 1 kg wheat straw yielded 112 g ethanol by SSF at 20% solid loading and 20 mg/g CTec2.

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## ABSTRACT

Wheat straw was pretreated by phosphoric acid plus hydrogen peroxide (PHP) for enzymatic hydrolysis and ethanol fermentation at high solid loadings. Results indicated solid loading could reach 20% with 77.4% cellulose-glucose conversion and glucose concentration of 164.9 g/L in hydrolysate, it even was promoted to 25% with only 3.4% decrease on cellulose-glucose conversion as the pretreated-wheat straw was dewatered by air-drying. 72.9% cellulose-glucose conversion still was achieved as the minimized enzyme input of 20 mg protein/g cellulose was employed for hydrolysis at 20% solid loading. In the corresponding conditions, 100 g wheat straw can yield 11.2 g ethanol with concentration of 71.2 g/L by simultaneous saccharification and fermentation. Thus, PHP-pretreatment benefitted the glucose or ethanol yield at high solid loadings with lower enzyme input. Additionally, decreases on the maximal cellulase adsorption and the direct-orange/direct-blue indicated drying the PHP-pretreated substrates negatively affected the hydrolysis due to the shrinkage of cellulase-size-accommodable pores.



## 1. Introduction

Concerns about the environmental deterioration and the limited future supply as much as higher cost of crude oil motivated the development of processes based on alternative energy sources (Chu and Majumdar, 2012). Biofuel, such as ethanol from lignocellulosic biomass, typically represents an alternative solution for the sector of transportation and its introduction into the current fuel distribution is being promoted by governmental mandatory quotas (Alvira et al., 2016). Among the different lignocellulosic materials available for ethanol production, agricultural residues like wheat straw appear as one of the main candidates for large-scale ethanol production due to their abundance and low cost (Zhang et al., 2015). According to the statistics, wheat straw is one of the most abundant agricultural residues in the world represented by a total production of approximately $5.59 \times 10^{11}$ kg in 2014 (Bhattarai et al., 2015). However, most of wheat straw has been used for animal feeds or directly burnt for living fuels or without any utilizations, of which the latter leads to not only a great waste on resource but also environmental pollutions. Therefore, wheat straw is considered as an appropriate and attractive lignocellulosic feedstock for biofuels.

* Corresponding author at: 211 Huimin Road, Wenjiang District, Chengdu, Sichuan 611130, PR China.
*E-mail addresses:* fishen@sicau.edu.cn, fishensjtu@gmail.com (F. Shen).

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Generally, ethanol production from lignocellulosic feedstocks involves 3 key steps, namely pretreatment, enzymatic saccharification, and fermentation. The pretreatment is crucial to overcome biomass recalcitrance, renders the accessible lignocellulose structure, and exposes cellulose to enzymatic digestion. State-of-the-art pretreatment methods developed for obtaining fermentable sugars and ethanol (Talebnia et al., 2010) involve mechanical milling, grinding, chipping, hot water, steam explosion with or without chemical supplementation (Chen and Liu, 2007; Petersen et al., 2009), acid, alkali, oxidant and fungal degradation (Davinia et al., 2011). In contrast, the recently developed pretreatment, the concentrated phosphoric acid plus hydrogen peroxide (PHP), offers some outstanding features, such as a wide adaptability to various raw materials (such as agricultural residues, grassy biomass, softwood, hardwood, bamboo residues and their mixtures), mild conditions (40–50 °C), and high enzymatic hydrolysis efficiency (>90%) (Wang et al., 2014). PHP pretreatment can efficiently remove hemicellulose and lignin, and recovery most of cellulose from lignocellulosic feedstock besides the destruction of the 3-D structure of lignocellulose and the crystalline structure of cellulose (Wang et al., 2016). In addition, Qui et al. optimized the PHP pretreatment conditions as 40.2 °C, 2.9 h, $H_3PO_4$ + $H_2O_2$ of 79.6% + 1.9%, by which 282 mg/g glucose yielded from wheat straw with 100% cellulose-glucose conversion (Qiu et al., 2017). These features mainly contribute to the high efficient conversion of lignocellulosic feedstock into fermentable sugar.

Enzymatic hydrolysis at high solid loading is considered to be a potential solution to reduce the process cost as water consumption can be decreased whereas the sugar concentration in hydrolysate will be increased greatly. Consequently, the cost of ethanol distillation can be considerably reduced after fermentation. However, large amounts of substrate will definitely decrease the hydrolysis due to much lower mixing efficiency, lower contact between substrate and enzyme, nonspecific adsorption on non-cellulosic components such as lignin, and loss of the catalytic activity by shearing effects (Ramachandriya et al., 2013). Facing these issues, high amount enzyme is always input for hydrolysis or fermentation at high solid loading, which, however, involves additional problems, such as an increased competition for the substrate sites available for hydrolysis and enzyme jamming (Várnai et al., 2013). Moreover, the high cost of enzyme and the slow hydrolysis rate are 2 important obstacles for ethanol production with high solid loadings (Newman et al., 2013). Besides, most of the pretreatment methods are water-involved in practice, and the high content of free water greatly limits the increase of substrate loading in enzymatic hydrolysis. However, reducing moisture in the pretreated substrate will potentially increase the difficulties of liquefaction and enzymatic saccharification. On the basis of the mentioned advantages of PHP pretreatment, further investigations on enzymatic saccharification at high solid loading are required, moreover, dewatering the PHP-pretreated substrate deserve to be investigated to clarify its effects on enzymatic hydrolysis at high solid loading.

In this context, wheat straw was PHP-pretreated using the optimized conditions obtained from the previous work (Qiu et al., 2017). The increased solid loadings of the pretreated wheat straw were performed to assess the potential of PHP pretreatment on promoting the solid loading. The decreased enzyme inputs in hydrolysis at high solid loading were also attempted to seek a relative lower one. In addition, the pretreated wheat straw was dewatered by air- and oven-drying for enzymatic saccharification to evaluate their effects on sugar conversion. The simultaneous saccharification and fermentation (SSF) was performed to evaluate the ethanol yield from the PHP-pretreated wheat straw at high solid loading.

## 2. Materials and methods

### 2.1. Wheat straw, enzyme, and yeast

Wheat straw was harvested from the farm of Sichuan Agricultural University in 2015. The collected wheat-straw was air-dried, chopped by a knife-mill through a 20-mesh, and stored in a tightly-locked plastic bag at ambient temperature. The main composition of wheat straw was displayed in Table 1.

Enzyme of Cellic CTec2 was obtained from Novozymes in Beijing of China, and the protein concentration was determined as 228.72 mg protein/mL. The active dry yeast (*Saccharomyces cerevisiae*) was purchased from Angel Yeast Co., Ltd. (Yichang, China) for ethanol fermentation. It is characterized by high-speed fermentation, high resistance to ethanol, especially, it can work well at higher temperature of 38–40 °C.

### 2.2. PHP pretreatment

Concentrated $H_3PO_4$ (85%, w/w) and $H_2O_2$ (30%, w/w) were employed for preparing the PHP solution. According to the optimized results (Qiu et al., 2017), the actual concentration of $H_3PO_4$ and $H_2O_2$ in the prepared PHP solution was 79.6% and 1.9%, respectively. Pretreatment was carried out in a 250-mL kettle coated with a polytetrafluoroethylene lining. Wheat straw (10.0 g, dry basis) was supplemented to 100.0 mL of the prepared PHP solution. The reactor was shaken at 180 rpm, and the optimized temperature and duration were controlled at 40.2 °C and 2.9 h, respectively. After pretreatment, the monomeric sugars can be dissolved in ethanol, however, the oligomeric sugars with DP (degree of polymerization) $\geq$ 3 could not be dissolved, and ethanol (220 mL, 95% v/v) was thereby added to cease the pretreatment by a rapid dilution, and achieved the cellulose precipitation (Vejdovszky et al., 2015). The precipitated solid was filtrated and washed with ethanol for 3–5 times. Afterwards, the solid was washed with tap water to pH higher than 5.0 and stored at −20 °C for composition analysis and enzymatic hydrolysis. The recovered liquor, containing a mixture of the ethanol and $H_3PO_4$, was distilled for recycling ethanol, and the lignin in the separated $H_3PO_4$ can be recovered and $H_3PO_4$ can be recycled for pretreatment (See Supplementary Fig. 1).

In order to investigate the effects of dewatering on enzymatic hydrolysis at high solid loading, the washed substrates were air-dried for 3 h (AD3), 6 h (AD6), and 12 h (AD12) with moisture content of 53.5%, 28.0%, and 13.0%, respectively. The oven-drying was conducted for 12 h at 30 °C (OD30), 50 °C (OD50), 70 °C (OD70), and 105 °C (OD105) and the dried solids presented final moistures of 6.0%, 3.8%, 2.5%, and 0.0%, respectively.

### 2.3. Enzymatic hydrolysis

The solid loadings were increased as 2%, 10%, 15%, and 20% (w/w, dry basis) for hydrolysis with enough enzyme input of 80 mg protein/g cellulose. In order to clarify the effect of dewatering on enzymatic hydrolysis, the solid loading of dried substrates was fixed at 20% with enzyme input was 80 mg protein/g cellulose. CTec2 loading was reduced stepwise from 80 to 10 mg protein/g cellulose for enzymatic hydrolysis at 20% solid loading to minimize enzyme input.

Enzymatic hydrolysis was carried out in 10 mL centrifuge tubes allowing a total working volume of 2.0 mL, and 16 tubes were employed for each run. The enzymatic hydrolysis was carried out at 50 °C in acetate buffer (0.05 M, pH 5.0) and incubated at 170 rpm with the designed solid loadings. 10 μL tetracycline solution (250 mg/L) was added prior to the hydrolysis to inhibit the