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# Correlation of porous structure, mass transfer and enzymatic hydrolysis of steam exploded corn stover

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## HIGHLIGHTS

- Reveal porous property of stover by characterizing its structure and mass transport.
- Find that porous property directly affects enzymatic hydrolysis of stover.
- Threshold pressure can reflect effect of porous property on enzymatic hydrolysis.
- Propose the concept of seepage recalcitrance to describe mass transport resistance.
- Import successfully porous media theory into the research of plant biomass refining.

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## ABSTRACT

Establishing the relationships between intrinsic structure and enzymatic hydrolysis of plant biomass is critical for understanding the process of enzymatic hydrolysis. This manuscript aims to explore the relationships between porous structure and enzymatic hydrolysis by examining the pore size distribution and the parameters of mass transfer in steam exploded corn stover. Results indicated that the pore size distribution and other parameters of porous structure of corn stover were altered by steam explosion pretreatment. These structural changes enhanced the percolation probability and permeability, reduced the threshold pressure, and subsequently improved the enzymatic hydrolysis yield of steam exploded corn stover. Multiple factors regression analysis demonstrated that threshold pressure was a significant factor of enzymatic hydrolysis with a correlation coefficient of −0.885. It revealed that the changes of porous structure could reduce threshold pressure and subsequently improve enzymatic hydrolysis. Based on these results, we have proposed the concept of seepage recalcitrance which provides a key index for pretreatment technology.



## 1. Introduction

Understanding and overcoming the natural resistance of plant biomass to the enzymatic hydrolysis is one of the most important problems for the biofuels production (Chen and Qiu, 2010). The unknown intrinsic structural factors of plant biomass have been hypothesized to be a direct resistance to enzymatic hydrolysis (Ding et al., 2012). To characterize the structural resistance, a significant number of researches have adopted particle size (Silva et al., 2012), specific surface area or accessibility (Wiman et al., 2012). However, the particle size is mainly responsible for specific surface area which contributes to enzymatic hydrolysis through promoting cellulose accessibility. Accessibility represents mainly the surface property of plant biomass. Although being accessible means that cellulase can touch substrate, it does not ensure that cellulase and hydrolysate (reducing sugar) can transfer effectively during the whole process of enzymatic hydrolysis due to capillary effects. On the one hand, if cellulase stays there after hydrolysis is finished (it is hypothesized that there is lignin and some polysaccharides left after enzymatic hydrolysis), it cannot move to other active sites to continue to hydrolyze cellulose. On the other hand if hydrolysates cannot move out of the capillary in time, this will inhibit the activity of cellulase. Therefore, the transfer of cellulase and hydrolysates is crucial to promote enzymatic hydrolysis yield and reduce hydrolysate inhibition.

From the insight into the process of biomass conversion, including pretreatment, enzymatic hydrolysis and fermentation (Chen and Qiu, 2010; Duan et al., 2012), the authors have discovered that plant biomass is essentially a porous medium. The porous structure formed by highly ordered and tight structure at different levels

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has been summarized (Table 1), including tissue level, cell level and cell wall level. (1) Pores in tissue level (Liu, 2006) consist mainly of different cell lumen and intercellular spaces (1–130 μm). (2) Pores in cell level point to pit in cell wall, especially vessel cell wall (30 nm–50 μm). Liquid diffusion throughout plant tissues and cells is controlled by the arrangement of vascular bundles, as well as pit between connecting cells. (3) Pores in cell wall level consist of space among polymers, including lignin, hemicellulose, cellulose as well as microfiber. Atomic force microscope images have shown that pores in the primary wall and the secondary wall are among 1–30 nm (Ding et al., 2012).

Porous structure of cell wall resists the transfer of catalyst (Himmel et al., 2007). Therefore, pore size distribution is largely responsible for the high cost of enzymatic hydrolysis (Arantes and Saddler, 2010). It has been hypothesized there is a relation between enzymatic hydrolysis and pores bigger than cellulase (Divne et al., 1994). Although the critical pore diameter for enzymatic hydrolysis has been proved to be 5.1 nm (Stone et al., 1969), the size of cellulase was reported to be 3–18 nm (Ladisch et al., 1992; Luterbacher et al., 2013; Wong et al., 1988). However a pore-hindered diffusion and reaction model has also been proposed on the basis of a critical pore size of 5.1 nm (Zeng et al., 2007). All the results above reveal that the effect of porous structure, especially pore size on enzymatic hydrolysis is crucial. Hence what are the concrete relationships between porous structure parameters and enzymatic hydrolysis? How does porous structure resist the enzymatic hydrolysis, especially in terms of mass transfer? It would be desirable to seek answers to these critical questions.

This paper aims to establish the relationships between porous structure and enzymatic hydrolysis in terms of mass transfer. Materials with different porous structures were obtained by pretreating corn stover with steam explosion (Zhang and Chen, 2012) which had been proved to be an effective pretreatment technology for plant biomass (Chen and Liu, 2007; Grous et al., 1986). Considering the heterogeneity of corn stover (Chen et al., 2011; Zeng et al., 2012), porous structure of different organs before and after steam explosion were compared separately. The effects of porous structure on mass transfer parameters were examined. Finally, multiple linear regression method was adopted to determine the significant factors of mass transfer for enzymatic hydrolysis.

## 2. Materials and methods

The schematic of experimental procedure is shown in Fig. 1.

### 2.1. Steam explosion pretreatment

Corn stover was obtained from the experimental plot of Chinese Academy of Agricultural Sciences in Beijing, China, in October 2011. It was air-dried naturally at room temperature after collecting from the field, and then separated manually into leaf, node, pith and rind. Each organ was pretreated separately by steam explosion in a batch steam explosion tank at 1.5 MPa for 5 min (leaf and pith) or 7 min (node and rind). Finally, the steam exploded materials were dried naturally.

### 2.2. Enzymatic hydrolysis

Each organ of corn stover, before and after steam explosion pretreatment, was hydrolyzed by cellulase (Ningxia Xiasheng Business Co. Ltd., China). Cellulase (filter paper unit, FPU, 110 IU/mL) was added as 16.5 FPU/g substrate. The weight ratio of substrate to buffer (0.2 M acetic acid-sodium acetate, pH 4.8) was 1:30. The reaction was carried out in a 250 mL flask filled with 2.0 g substrate, 60 mL buffer, and 0.3 mL cellulase, at 50 °C, 150 rpm for 48 h. All the experiments were carried out in duplicate.

### 2.3. Reducing sugar content analysis

Reducing sugar was tested with the 3,5-dinitrosalicylic acid (DNS) method. A standard curve was prepared with the glucose concentration of 0.048, 0.064, 0.080, 0.096, 0.112, 0.128, and 0.144 mg/mL. The yield of enzymatic hydrolysis was calculated as the following:

$$\text{Yield} = \text{reducing sugar weight} \times 0.9/\text{total substrate weight} \times 100\% \quad (1)$$

### 2.4. Imaging

The morpha of leaf, node, pith and rind before and after steam explosion was characterized by scanning electron microscope (SEM) (JSM6700F, JEOL, Tokyo, Japan).

### 2.5. Porous structure and mass transfer parameter analysis

Mercury intrusion porosimetry (Auto Pore IV 9500, Micromeritics instrument, Norcross, USA) was adopted to characterize the porous structure and the mass transfer parameters. Steam exploded organs

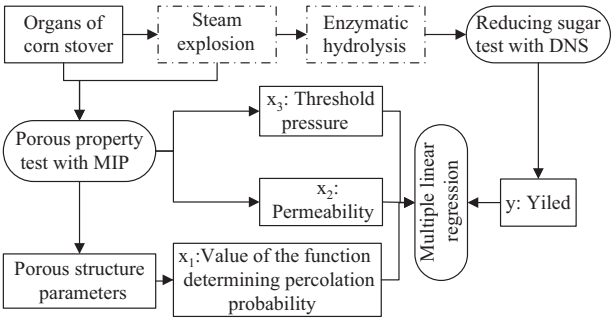


**Fig. 1.** Overall experimental procedure. Leaf, node, pith and rind were steam exploded first, and then hydrolyzed by cellullase. Reducing sugar was tested with the 3,5-dinitrosalicylic acid (DNS) method. The parameters of porous property were tested using mercury intrusion porosimetry (MIP) for samples both before and after steam explosion. Finally, the correlation of enzymatic hydrolysis yield with threshold pressure, permeability and the value of the function determining percolation probability was analyzed with the multiple linear regression method.

**Table 1**
Pore distribution in corn stover.

| Level | Origin | Width (μm) | Level | Origin | Width (μm) |
|---|---|---|---|---|---|
| Tissue | Rectangular cell | 20–35 | cell | pit | 0.5–50.0 |
| | Stomata | 2–10 | | plasmodesma | 0.03–0.06 |
| | Vessel cell | 30–130 | cell wall | space among macrofibrils | 0.001–0.100 |
| | Sieve tube | 5–50 | | | |
| | Sieve cell | 5–50 | | space among micorofibrils | 0.001–0.030 |
| | Fiber cell | 13 | | | |
| | Intercellular space | $<1$ | | lamellar gaps among polyphenols | 0.001–0.030 |
| | Cell corner | $\approx 1$ | | | |