

Analysis of particle size reduction on overall surface area and enzymatic hydrolysis yield of corn stover

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Abstract Particle size of lignocellulose materials is an important factor for enzymatic hydrolysis efficiency. In this study, corn stover was milled and sieved into different size fractions from 1.42, 0.69, 0.34, to 0.21 mm, and the corresponding enzymatic hydrolysis yields were 24.69, 23.96, 25.34, and 26.97 %, respectively. The results indicate that the hydrolysis yield is approximately constant with changing corn stover particle sizes in the experimental range. The overall surface area and the inner pore size measurement show that the overall specific surface area was less than 2 % with the half reduction of particle size due to the greater inner pore surface area. The scanning electron microscope photographs gave direct evidence of the much greater inner pore surface area of corn stover particles. This result provided a reference when a proper size reduction of lignocellulose materials is considered in biorefining operations.

Keywords Corn stover · Particle size · Enzymatic hydrolysis · Inner surface area · SEM photograph

Introduction

Conversion of lignocellulose biomass into ethanol includes feedstock handling (size reduction), pretreatment, hydrolysis, and fermentation. Particle size of lignocellulose feedstock is considered as an important factor for the overall conversion efficiency: the smaller, the better [1–3]. Therefore, lignocellulose generally is milled into small size before it is pretreated and enzymatically hydrolyzed. However, grinding of lignocellulose into small pieces is an energy-intensive process, and undoubtedly increases the cost of conversion process. Thus, it is important to determine a proper size of lignocellulose feedstock for biorefinery processes.

Many studies support that cellulose conversion efficiency increases with decreasing particle size. Desari and Berson [4] found that the cellulose conversion yield of sawdust increased 50 % in the enzymatic hydrolysis when the particle size was reduced to 1/10 of the original size in the range 0.033–0.850 mm. Pedersen and Meyer [5] found that the cellulose conversion yield of the pretreated wheat straw reached 90 % for the fine particles in the range 0.053–0.149 mm, while the yield was only 65 % for the large particles in the range 2–4 cm. Yeh et al. [6] observed similar results on microcrystalline cotton cellulose when it was converted to cellobiose and glucose.

On the other hand, the concept of “the smaller, the better” has also been frequently challenged. Rivers and Emert [7] found that cellulose conversion yield of Avicel and other six lignocellulose materials (bagasse, rice straw, pulp sludge, newspaper, corrugated card board, and sawdust) in the enzymatic hydrolysis was almost the same following ball milling into small particles that ranged from 0.25 to 0.47 mm. Ballesteros et al. [8] studied the enzymatic hydrolysis of the steam-explosion pretreated

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agriculture residue (*Brassica carinata*) in the range 2–12 mm, and they found that the largest particles (8–12 mm) showed the highest conversion yield. Nahzad et al. [9] found that although the cellulose conversion yield of pulp slurry increased with decreasing particle size, the reason is the fiber swelling and internal structure disruption due to Kraft mechanical treatment, instead of particle size reduction. The information indicates that the relevance of hydrolysis yield with particle size is complicated.

It is generally accepted that the increase of the specific surface area enhances the effective contact area with cellulase enzyme and the hydrolysis yield [2, 3, 10], but the decrease of particle size may not directly relate to the increase of the specific surface area [1]. In this study, a trial was made to clarify the relevance between the overall surface area and hydrolysis yield with the particle size reduction. Corn stover particles were milled and sieved into different size fractions, but the internal structure was not changed by any pretreatment steps before enzymatic hydrolysis. The results show that the enzymatic hydrolysis yield is approximately the same for the particles with different size in the experimental range, and the unchanged overall specific surface area of the corn stover particles may play an important role. The results may give help on industrial milling operations for a proper particle size with reasonable power consumption.

Materials and methods

Raw materials

The virgin corn stover (CS) was grown in Dancheng, Henan, China, and harvested in fall 2012. The corn stover was washed to remove the dirt and stones, and then dried at 105 °C until the weight was constant. The materials were milled into the fine pieces using a pulverizer (HR 2094, Philips, the Netherlands), then divided into four size fractions of 10, 20, 40, and 60 mesh per square inch by using norm sample sieves, corresponding to the equivalent size fractions of 1.42, 0.63, 0.34, and 0.21 mm in the mean diameter.

The cellulose and xylan contents of corn stover were determined according to the method by Van Soest et al. [11] using a fiber analyzer ANKOM 2000 (ANKOM Technology, Macedon, NY). Cellulose and hemicellulose contents were 43.79 and 33.11 %, respectively.

Enzymatic hydrolysis of corn stover

The commercial cellulase enzyme Youtell #6 was provided by Hunan Youtell Biochemical Co. (Yueyang, Hunan, China). The filter paper activity was 135 filter paper units (FPU) per gram enzyme powder and the beta-glucosidase

unit is 344 units per gram (CBU/g) using the protocol of NREL LAP-006 [12]. The enzymatic hydrolysis was handled in a 100 ml flask containing 20 ml citric buffer medium. The corn stover solid content was 5 % (w/v), the enzyme dosage was 15 FPU/g dry matter (DM). The pH was kept at 4.8 and 50 °C, stirred at 150 rpm for 72 h.

Analysis of pore size and specific surface area

The inner pore size distribution of the corn stover particles was determined using a mercury porosimeter PoreMaster33 (Quantachrome, Boynton Beach, FL, USA). The overall specific surface area of the corn stover particles was determined using a physisorption analyzer ASAP2020 (Micromeritics, Dublin, Ireland).

Scanning electron microscope (SEM)

The surfaces and pores of corn stover were observed using a Hitachi S-3500 N scanning electron microscope (SEM) (Hitachi Science Systems, Ibaraki, Japan). Specimens were dried at 105 °C until the weights were constant, then sputter coated with gold palladium, and observed using a voltage of 15 kV.

HPLC analysis

Glucose and xylose were analyzed using HPLC (LC-20AD, refractive index detector RID-10A, Shimadzu, Kyoto, Japan) with a Bio-Rad Aminex HPX-87H column at the column temperature 65 °C. The mobile phase was 5 mM H₂SO₄ at the rate of 0.6 mL/min. The samples was diluted with proper ratio, then filtered through a 0.22 mm filter before analysis.

Results and discussion

Determination of enzymatic hydrolysis yield of corn stover with different particle size

The enzymatic hydrolysis of the milled corn stover particles was carried out and the conversion yields of glucose and xylose are shown in Fig. 1. The results clearly indicate that both glucose and xylose yields were essentially the same for the four corn stover samples with the particle size from 1.42, 0.69, 0.34, and 0.21 mm. In another word, the cellulose hydrolysis yield of corn stover did not increase with decreasing particle size.

To give further verification, two control experiments were carried out: (1) the enzymatic hydrolysis at different time periods, and (2) the enzymatic hydrolysis using different cellulase enzyme dosage. Figure 2 shows that both

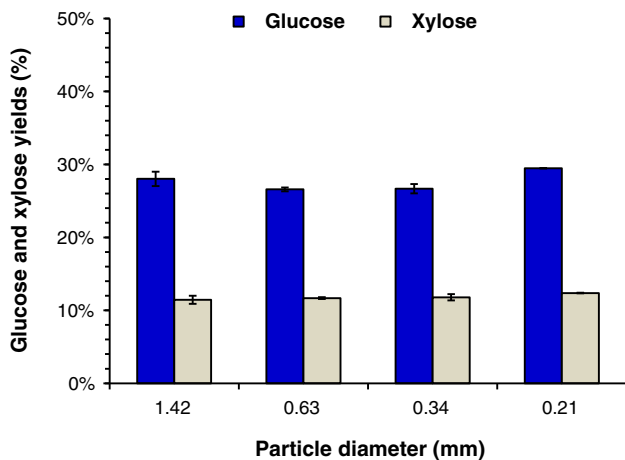


Fig. 1 Enzymatic hydrolysis of corn stover particles at different particles size. Conditions: solids content was 5 % (w/w), cellulase enzyme dosage was 15 FPU/g DM, 50 °C, 72 h at 150 rotation rate

the glucose (Fig. 2a) and xylose (Fig. 2b) yields were approximately the same at each detected hydrolysis time point (2, 6, 12, 24, 48, and 72 h) for the four corn stover samples, respectively, indicating that the hydrolysis yields with different particle size was still unchanged with changing particle size in the experimental range at different hydrolysis time. Figure 3 shows that although the glucose and xylose yields increased with the increasing enzyme dosage (7, 15, or 30 FPU/g DM), however, both the glucose and xylose yields were approximately same with changing particle size at each enzyme dosage addition. There still no difference was found that the hydrolysis yields of the samples with different particle size.

Analysis of particle size on enzymatic hydrolysis by comparing specific surface area

Generally, enzyme hydrolysis yield increases with increasing specific surface area of substrate particles [2, 13,

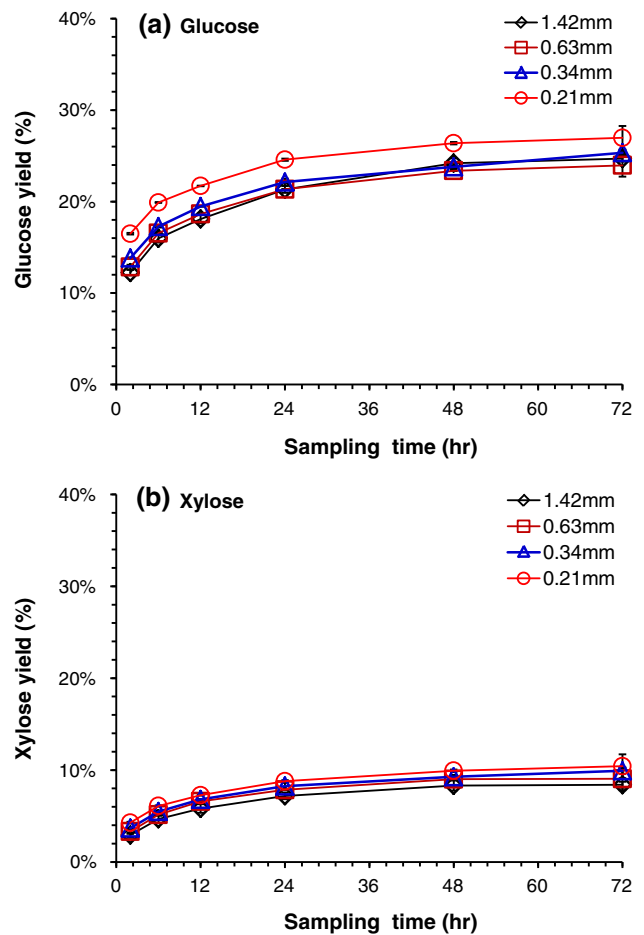


Fig. 2 Time courses of enzymatic hydrolysis of corn stover particles with different time points. Conditions: solids content was 5 % (w/w), cellulase enzyme dosage was 15 FPU/g DM, 50 °C, 72 h at 150 rotation rate

14]. The surface area of a particle is composed of two fractions, one is the outside surface area, and the other is the inner pore surface area. For a porous particle, the inner surface area may be much greater than the outside surface

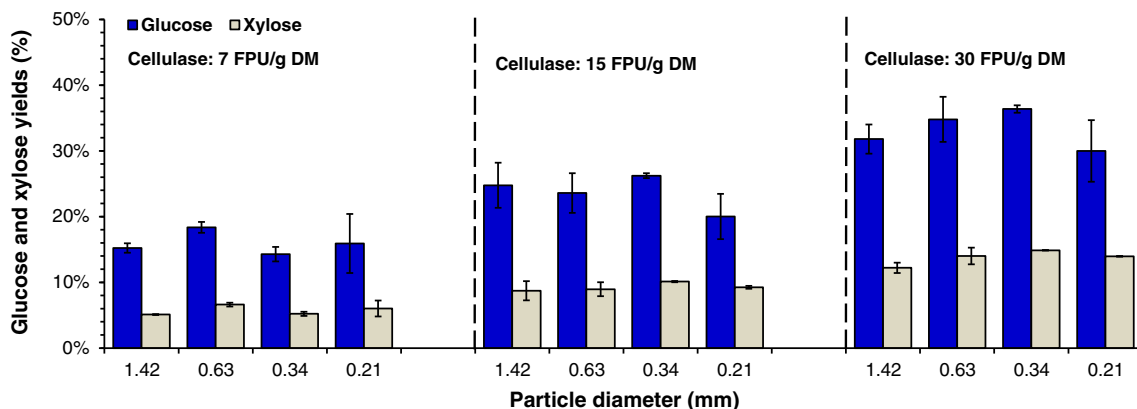


Fig. 3 Enzymatic hydrolysis of corn stover particles with different enzyme dosages. Conditions: cellulase enzyme dosages were 7, 15, and 30 FPU/g DM, respectively; solids content was 5 % (w/w), 50 °C, 72 h at 150 rotation rate

Table 1 Outside and total specific surface area of corn stove particles with different size

Particles diameter (mm)	1.42	0.63	0.34	0.21
Outside surface area (m ² /g)	0.0282	0.0635	0.1176	0.1905
Total surface area (m ² /g)	2.8156	3.0729	– ^a	– ^a

^a The total surface area was determined using physisorption analyzer. When the particle size of corn stover was reduced to 0.34 and 0.21 mm, the nitrogen adsorption curve and desorption curve were too large that were not reliable, thus the total specific surface area measurement was carried out only for the particles of 1.42 and 0.69 mm

area. Assuming the corn stover particles are uniform rigid spheres, the specific outside surface S_{outside} could be calculated by:

$$S_{\text{outside}} = \frac{A_{\text{outside}}}{m} = \frac{NA_{\text{single}}}{m} = \frac{V_{\text{stacking}}A_{\text{single}}}{V_{\text{single}}m} = \frac{\frac{m}{\rho}4\pi r^2}{\frac{4}{3}\pi r^3 m} = \frac{3}{\rho r} \quad (1)$$

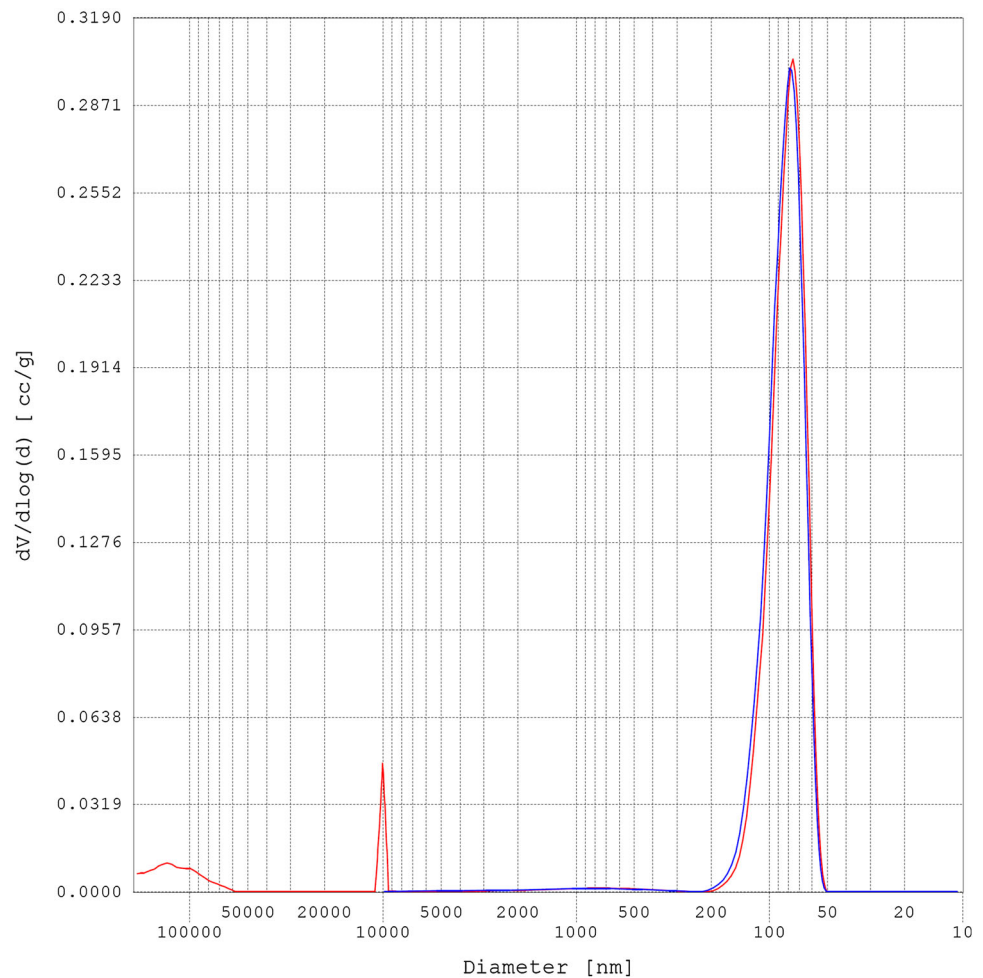
where A_{outside} is the total outside surface area, m is the corn stover sample mass, N is the total particle number, A_{single} is the outside surface area of one single particle, V_{stacking} is

the stacking volume of corn stover m , V_{single} is the volume of one single particle, ρ is the stacking density of corn stover (150 kg/m³), r is the mean diameter of corn stover particle. The specific outside surface area results of the four corn stover samples are shown in Table 1. The outside specific surface area increases from 0.0200, 0.0476, 0.0952, and 0.1905 m²/g with the decreasing particle size from 1.42, 0.63, 0.34, and 0.21 mm.

Table 1 also shows the overall specific surface area of the two corn stover samples measured by the nitrogen adsorption–desorption method. The overall specific surface area of the corn stover samples were found surprisingly larger than the outside specific surface area: 2.8156 and 3.0729 m²/g for the two corn stover samples (1.42 and 0.63 mm). The outside specific surface area only takes 1.00 and 1.55 % of the overall specific surface area for the two corn stover samples, respectively.

Figure 4 shows the size distribution of the inner pores of the corn stover sample of the size 1.42 mm using the mercury intrusion method. The result reveals that almost all the inner pores were in the range of 50–200 nm in diameter, with few larger pores scattered. Considering the

Fig. 4 Size distribution of the inner pores of the corn stover sample at the size of 1.42 mm. *Horizontal axis* diameter of the inner pores, *vertical axis* change of space volume with diameter



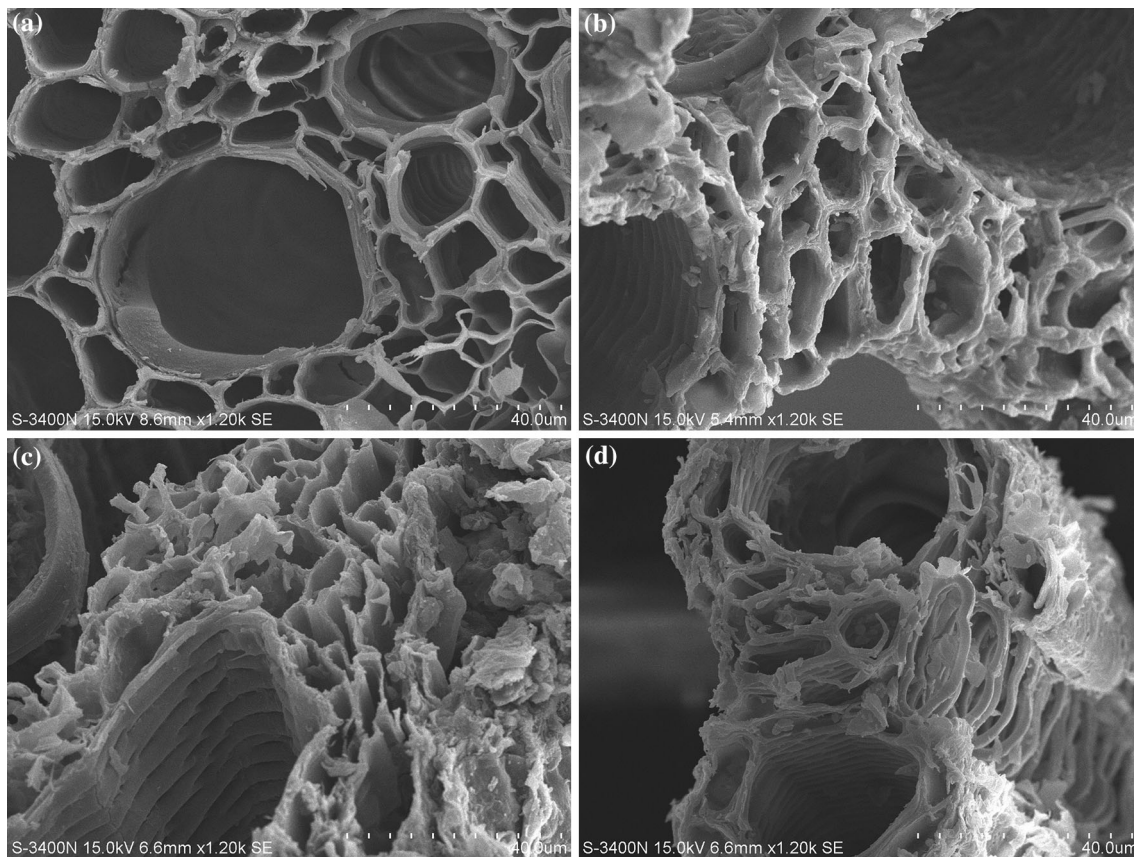


Fig. 5 Scanning electron microscope (SEM) photographs of corn stover with different particle size. **a** 1.42 mm, **b** 0.63 mm, **c** 0.34 mm, **d** 0.21 mm

dimension of cellulase enzyme proteins is only at 4.5 nm [15], the inner pores are large enough to let cellulase enzymes access freely and contact with cellulose substrate.

A direct observation of the inner pores of corn stover particles was recorded using scanning electron microscope (SEM) as shown in Fig. 5. The photographs of the intersection surfaces at changing particle size show that inner pores of the stem intersection of corn stover apparently kept constant from the particle size of 1.42–0.21 mm in diameter. The direct observation also gives an evidence of the greater inner pore surface area than the outside surface, regardless the particle size difference.

Figure 6 schematically indicates the contribution of size reduction of the corn stove particles on their overall specific surface area. Although both the inner and outside surface area provides the effective contact area of cellulase enzyme with cellulose substrate, the contribution of the outside surface area to the overall surface area is limited because most lignocellulose biomass is porous materials with much greater inner surface area. Therefore, the increase of the outside specific surface area by size reduction on the overall specific surface area is almost negligible, unless the particle size is reduced to extremely

fine scale to nanoscale which is practically not acceptable, according to the outside surface area calculation $S_{\text{outside}} = \frac{3}{\rho r}$. In the practical range of particle size of lignocellulose biomass, such as the corn stover particles in millimeter by conventional mechanic milling in this study, the effect of size reduction on the overall specific surface area is also negligible. This result could be an important factor for the constant glucose and xylose yields when particle size of corn stove is reduced.

This study only provides the size reduction case of virgin corn stover in enzymatic hydrolysis. Generally, a chemical or physiochemical pretreatment step is carried out before the enzymatic hydrolysis in the practical biorefinery processing. Thus, the result of this study is not directly applied to the hydrolysis of pretreated lignocellulose and a further investigation of size reduction on pretreated lignocellulose is required. However, the conclusion that the inner surface area of lignocellulose determines the hydrolysis yield should be valid even for pretreated lignocellulose materials. Considering the high power consumption of size reduction of lignocellulose materials, the intensive milling of lignocellulose into very fine particles is not necessary in biorefinery processes.

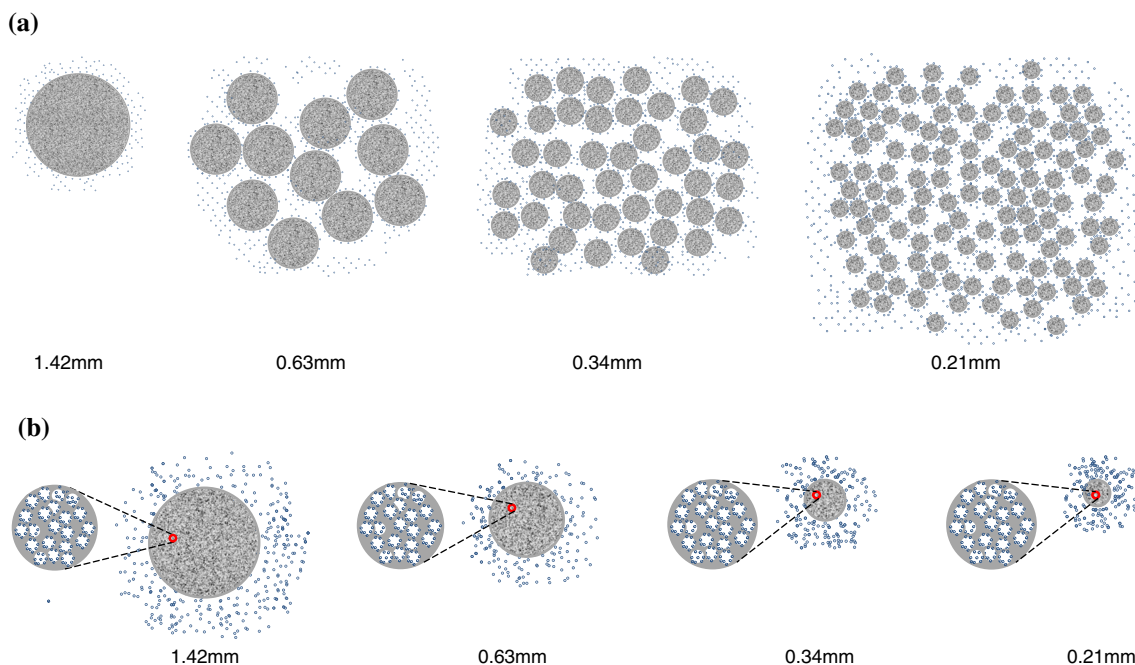


Fig. 6 Schematic illustration of corn stover particles at different size and inner surface area. **a** Milling of corn stover into smaller particles. **b** Inner surface area of the particles with different size

Conclusion

Corn stover was milled and sieved into four size fractions of 1.42, 0.63, 0.34, and 0.21 mm in mean diameter. The enzymatic hydrolysis results show that the hydrolysis yield is approximately the same for the four samples. The surface area measurement shows that the overall specific surface area is dominantly determined by the inner pore surface area and almost independent of size reduction of the particles. This result may give evidence for proper size determination in the biorefining operation.

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