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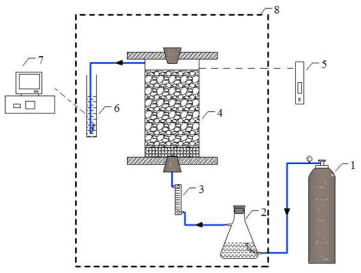

Solid simultaneous saccharification and fermentation of rice straw for bioethanol production using nitrogen gas stripping

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Abstract

A solid simultaneous saccharification and fermentation (SSSF) of rice straw for ethanol production using N_2 stripping was developed. The effects of N_2 flow rate, yeast inoculation volume, and substrate moisture content on SSSF were investigated. The highest total output of ethanol was 4.78 g, and the highest ethanol yield obtained was 56.3% using an N_2 flow rate of 30 mL/min, a yeast inoculation volume of 20 mL, and a moisture content 4.6 mL (water)/g (substrate). The low residuals of ethanol and glucose in the substrate demonstrate that the N_2 carrier gas effectively stripped the produced ethanol out of the packed bed, alleviating the inhibitions caused by evolved glucose and ethanol remaining in the bioreactor. With increases in N_2 flow rate or substrate moisture content, the total output of ethanol and ethanol yield initially increased and then decreased. However, with the increase in yeast inoculation volume, both indexes first rose and then remained relatively constant.

Keywords*:* solid-state fermentation, bioethanol, gas stripping, rice straw, *Saccharomyces cerevisiae*

1. Introduction

Renewable energy sources are a popular topic in the world because of increasing demands for energy sources and the decreasing reserves of fossil fuels. Of all the renewable energy sources, bioethanol is an ideal substitute for liquid fuel because it has a high energy content and originates from biomass.[1, 2] Currently, bioethanol is mainly produced from grain and creates competition with food stock grains for people and livestock.[3, 4] However, ethanol can also be produced from abundant renewable biomass sources such as agricultural and forestry residues.[5]

The saccharification and fermentation processes of cellulosic materials have been mainly separated for bioethanol production. Previous reports of this two-step method indicate that glucose accumulation may suppress the enzymatic hydrolysis of cellulose.[6, 7] To solve this problem, a method of simultaneous saccharification and fermentation for ethanol production has been developed.[8, 9] In this process, the reducing sugar derived from the saccharification of cellulose was immediately utilized by yeast cells for ethanol fermentation. Thus, little glucose was accumulated in the reactor and reduced the issue of glucose inhibiting cellulose saccharification. This operation is also more convenient because the two steps (saccharification and anaerobic fermentation) were integrated, and the efficiency of lignocellulose saccharification was improved. Nevertheless, the accumulated ethanol still inhibited the growth and activity of yeast cells in a noncompetitive manner, and the cellulase activity was also decreased, reducing ethanol yield.[10, 11] Thus, a novel ethanol stripping method using a carrier gas was developed to carry the generated ethanol out of the bioreactor to be recovered in an absorber.[12] Zhang et al [13] studied effects of different stripping gas on cell physiology and ethanol production during ethanol fermentation, and indicated that common-purity N_2 was the best choice for ethanol

fermentation and cell growth. Later research results indicated that both of the efficiencies of saccharification and fermentation were improved using N_2 as carrier gas.[9, 11] The ethanol stripping method has many advantages such as simple operation, safe for the yeast culture, requiring low energy input, and inexpensive capital investment for facilities.[14] Importantly, the two issues of inhibiting saccharification and fermentation can be alleviated simultaneously, enhancing the efficiencies of saccharification and ethanol fermentation overall.[15, 16]

Traditional simultaneous saccharification and fermentation processes have generally submerged cellulosic materials in liquid. Thus, an increasing cost is incurred as a result of a decreasing substrate density and a large quantity of waste water requiring treatment.[11] Additionally, more energy is required in this process, which becomes another disadvantage. Solid simultaneous saccharification and fermentation (SSSF) can overcome the above problems because of the many benefits such as high substrate concentration and product yield, simple and controllable operation, less effluent wastewater, and less energy consumption.[17] Thus, SSSF has vast potential in bioethanol production.[18] Some studies on SSSF for bioethanol production have been shown that the prehydrolysis of cellulosic materials prior to simultaneous saccharification and fermentation had a positive effect on the overall ethanol yield.[19,20] Mohanty et al.[21] investigated ethanol production using mahula flowers, and the fermentation time achieving peak ethanol concentration in solid-state fermentation was shorter than that in submerged fermentation. These investigations show that ethanol production through the SSSF of cellulosic materials can effectively reduce the production cost, improving the commercial application of bioethanol. However, most of these studies have not considered the inhibiting factors in ethanol fermentation during SSSF. There are few reports on the SSSF of cellulosic materials coupled with

carrier gas stripping of ethanol.

In the present study, a packed bed for the SSSF of rice straw coupled with N_2 sparging was designed to improve the saccharification of lignocellulose and bioethanol production. This study aimed to evaluate the performance of SSSF using ethanol gas stripping, and the effects of gas flow rate, inoculation amount of yeast, and substrate moisture content were analyzed.

2. Materials and methods

2.1 Materials and yeast strain

Rice straw collected in the suburb of Chongqing, China was cleaned with tap water and dried at room temperature. The straw was cut into segments 2–4 cm long. These segments were pretreated in 1% NaOH solution for 24 h, rinsed with water five times and dried. The treated rice straw was ground up and sieved using a 60-mesh sieve. The rice straw powder was sterilized at 121 ℃ for 15 min. Yeast strain, *Saccharomyces cerevisiae*, which has a high activity and high temperature resistance, was purchased from Yichang Angel Yeast Co. Ltd, Hubei Province, China. Prior to inoculation, 2 g of dried yeast powder was mixed with a glucose solution (2 g/L) at the ratio of 1 g to 25 mL and activated at 38 ℃ for 15 min, then at 33 ℃ for 1.5 h in a water bath, the OD_{630nm} value of the activated yeast solution was 4.05. Cellulase (135 u/mg dw) and cellobiase (≥250 u/g, Novozym188) were purchased from Worthington Biochemical Corporation (New Jersey, US). The culture medium for yeast growth was the following composition: 2 g/L $(NH_4)_2SO_4$, 2 g/L KH_2PO_4, 0.2 g/L $MgSO_4$, 0.2 g/L $CaCl_2$, and 3 g/L of yeast extract. The solution was adjusted to pH 4.8 using acetic acid–sodium acetate buffer solution and monitored using a pH meter.

2.2 Packed-bed test system

The test system for SSSF of rice straw with N_2 sparge included a packed-bed reactor, an N_2 canister (gas source), a humidifier, a gas flow meter, and an absorber (Fig.1). The cylindrical bioreactor (⌀48 mm × H190 mm) used a working volume of 280 mL and was made of transparent polymethylmethacrylate (5 mm wall thickness). Approximately 54 mL of sterile glass beads (30 mm stacking height) were placed into the bottom of the packed-bed reactor to distribute the carrier gas uniformly. The whole packed-bed bioreactor was fixed in an incubator with a constant temperature of 35±2 ℃. Before assembling the test system, the components were autoclaved at 121 ℃ for 15 min. The bioreactor body was sterilized using formalin solution. The assembled test system was cleaned three times with sterile distilled water. During SSSF, the N_2 gas was passed through a humidifier before sparging into the bioreactor. The N_2 flow rate was controlled using a gas valve. The effluent gas from the bioreactor was channeled into an absorber with distilled water to recover the stripped ethanol. Two test systems were employed and run in parallel.

2.3 Operational processes

A 25 g sterile rice straw powder was mixed with 20 mL acetate buffer solution (pH 4.8) containing 5 mL cellobiase and 0.025 g cellulase. The mixture was poured into a conical flask for enzymatic hydrolysis for 24 h at 50 ℃ in an incubator, and then the activated yeast solution was mixed with the substrate. The inoculated substrate was transferred into the packed-bed bioreactor for SSSF at 35 ℃. Nitrogen gas was sparged into the air-tight reactor from the bottom to strip the generated ethanol out of the bioreactor. During SSSF, ethanol concentration in the absorber was detected every 12 hours. At the end of SSSF, distilled water was added into the residual substrate to extract glucose and ethanol, and the supernatant was collected by centrifugation at 9500 ×g to detect the ethanol and glucose

concentrations. To avoid ethanol loss in the absorber, whenever the ethanol concentration in the absorber was detected the recovered solution of ethanol was replaced with fresh distilled water. The ethanol concentration in discharged gas from the absorber was also measured to evaluate ethanol loss.

2.4 Analytical methods

Ethanol concentration in the absorber was detected at an interval of 12 hours using a gas chromatograph (SC-3000, Chongqing, China) equipped with a flame ionization detector and a 1.5 m stainless-steel column packed with porous styrene particles. For operation, N_2 with a 30 mL/min flow rate was used as a carrier gas, H_2 with a 25 mL/min flow rate as a flammable gas, and air with a 150 mL/min flow rate as an oxidizing gas. The injector and oven temperatures were set to 160 ℃, and the detector temperature was set to 170 ℃.

The consumption amount of cellulose was calculated by subtracting the weight of the residual rice straw from its initial weight. The substrate was weighed using an electronic analytical balance (Sartorius BP114, Göttingen, Germany). The cellulose content of substrate was determined using Van Soest's method.[22] At the end of SSSF, the glucose content in the residual substrate was measured using the 3, 5-dinitrosalicylic acid method using a UV-Vis spectrophotometer (TU1950, Beijing, China). The pH value of culture medium was detected using a pH meter (Orion 3 Star, Waltham, MA, USA).

The recovered ethanol amount refers to the accumulative amount of ethanol stripped out of the bioreactor collected in the absorber and reveals the capacity of N_2 for stripping ethanol out of the packed bed.

The total output of ethanol is equal to the sum of the recovered ethanol amount from the absorber and the residual ethanol amount measured from the bioreactor.

The theoretical ethanol amount was obtained using the formula:

$$\text{Theoretical ethanol amount (g)} = 0.51f \times \text{initial biomass weight} \times 1.111,$$

where f is the cellulose fraction of dry biomass (g/g), 0.51 is the conversion factor for glucose to ethanol calculated from the stoichiometry and biochemistry of yeast, and 1.111 is the conversion factor for cellulose to equivalent glucose.

The ethanol yield (%) was calculated using the following expression taken from:[23]

$$\text{Ethanol yield} = \frac{\text{total output of ethanol (g)}}{\text{theoretical ethanol amount (g)}} \times 100\%.$$

3. Experimental results

3.1 Effect of gas flow rate

The flow rate of N_2 as the carrier gas was set at 10, 20, 30, and 40 mL/min, respectively, and 25 mL of activated yeast solution was inoculated into the substrate. The temperature for the SSSF was controlled at 35 ℃, and the results are shown in Fig. 2. The recovered ethanol amount in the absorber gradually increased over time at a fixed gas flow rate (Fig. 2 a), demonstrating that N_2 sparging stripped the evolved ethanol out of the bioreactor. After 96 hours of inoculation, the recovered ethanol amount increased slower than that after 12 h. The decrease in the performance of SSSF can be ascribed to the reducing activities of enzymes and yeast cells as a result of the production of intermediates.[24]

Fig. 2 b shows that with an increase in N_2 flow rate from 10 to 30 mL/min, the total output of ethanol increased from 1.57 to 2.09 g. However, when the gas flow rate was increased to 40 mL/min, the total output of ethanol was 1.93 g. Thus, the highest total output of ethanol, 2.09 g, was obtained using an N_2 flow rate of 30 mL/min. The increase in N_2 flow rate dispersed more ethanol into the gas phase from the fermented substrate, then the ethanol was stripped out of the bioreactor. Consequently, the total

ethanol output increased with increasing N_2 flow rate. However, an N_2 flow rate of 40 mL/min caused a decrease in substrate humidity because of water evaporating in the substrate, resulting in a decrease of total ethanol output from that of 30 mL/min.

The residual ethanol amount and residual glucose amount are shown in Fig. 2 c. When the gas flow rate was increased from 10 to 30 mL/min, the ethanol residue in substrate decreased from 0.40 to 0.10 g, and plateaued using a flow rate of 40 mL/min to a value of 0.08 g. This result further demonstrates that using N_2 as a carrier gas stripped the evolved ethanol from the SSSF of rice straw out of the bioreactor. Furthermore, the residual glucose amount in the fermented substrate was maintained within the range of 0.83–0.85 g for the different N_2 flow rates studied. Low concentrations of ethanol and glucose remained in the fermented substrate, suggesting that the SSSF of rice straw with N_2 sparge alleviated the issues of ethanol inhibiting glycolysis and glucose inhibiting enzymatic hydrolysis.

As depicted in Fig.2 d, the substrate consumption was initially increased slightly and then slightly decreased with increasing N_2 flow rates. The substrate consumption amount initially increased from 10.5 to 11.8 g with the increase in N_2 flow rate from 10 to 20 mL/min and then decreased to 10.9 g with an N_2 flow rate of 40 mL/min. The ethanol yield initially increased from 10 to 30 mL/min and then decreased slightly for 40 mL/min. The highest ethanol yield obtained was 25.7% at 30 mL/min, which was equivalent to producing 0.19 g of ethanol per gram of initial substrate. Therefore, it was considered that the optimal N_2 flow rate for SSSF was 30 mL/min in this experiment.

3.2 Effect of yeast inoculation amount

The yeast inoculation amount has a large influence on the fermentation process. Here, the yeast inoculation amount on SSSF were set at 10, 15, 20, and 25 mL. To maintain the same moisture content

of the substrate in each run, the yeast solution was made up to a total volume of 25 mL using sterile distilled water. During SSSF, the flow rate of N_2 was controlled at 30 mL/min, and the other operating parameters were the same as those given above.

The recovered ethanol amount in all four runs gradually increased with time (Fig. 3 a). The yeast inoculation amount affected the recovered ethanol amount by first showing step increases from 1.46 to 2.17 g from 10 to 20 mL of inoculation amount and maintained relatively similar values for 25 mL. For example, 108 h after inoculation, the recovered ethanol increased for the yeast inoculation amount rising from 10 to 20 mL, and then slightly increased to 2.19 g for 25 mL. This result also showed that by increasing the yeast inoculation amount from 10 to 20 mL, the total output of ethanol gradually increased from 1.64 to 2.34 g, and then with an inoculation amount of 25 mL, the total output of ethanol rose slightly to 2.36 g (Fig 2 b).

The residual ethanol amount in the fermented substrate using different yeast inoculation amounts were generally maintained between 0.17 and 0.18 g (Fig. 3 c). This result demonstrates that the inoculation amount only slightly affected the residual ethanol amount in the substrate during SSSF coupled with N_2 sparge. Furthermore, residual glucose in the fermented substrate was maintained between 0.79 and 0.81 g, confirming that the accumulation of glucose, which inhibits enzymatic hydrolysis, was almost eliminated during SSSF. The substrate consumption amount and ethanol yield for 20 mL yeast inoculation amount was 10.95 g and 27.47%, respectively. For 25 mL, the consumption and yield was slightly higher at 11.02 g and 27.79%, respectively (Fig. 3 d). The highest ethanol yield recorded of 27.79% indicates that 0.21 g ethanol can be produced for one gram of substrate in this experiment.

The above results indicate that with the increase in yeast inoculation amount to 20 mL, more yeast cells utilize the monosaccharides derived from the saccharification of rice straw to produce ethanol by anaerobic fermentation. Correspondingly, the hydrolysis of rice straw was partly enhanced because the problem of evolved monosaccharides inhibiting saccharification was alleviated in the process of SSSF. Consequently, the recovered ethanol amount, total output of ethanol and ethanol yield increased. However, a further increase of the yeast inoculation amount to 25 mL produced an ethanol yield rise of only 0.32% higher than that of 20 mL. The saccharification of rice straw did not provide any more monosaccharides for ethanol fermentation by more yeast cells because of the limited enzyme activities. Therefore, in this experiment although both peaks of total output of ethanol and ethanol yield of 2.36 g and 27.79%, respectively, were obtained using 25 mL yeast inoculation amount, the optimal yeast inoculation amount was considered to be 20 mL.

3.3 Effect of substrate moisture content

In the process of solid-state fermentation, moisture content of the substrate affects the efficiencies of saccharification and ethanol production.[25, 26] Here, the substrate moisture content was fixed at 3.6, 4.6, 5.6, and 6.6 mL (water)/g (substrate), respectively. The gas flow rate in the experiment was controlled at 30 mL/min, and the yeast inoculation amount was fixed at 20 mL. The other experiment conditions were the same as those given above.

The recovered ethanol amounts in the four runs increased gradually over time, and the rate of increase became slower after 84 h (Fig. 4 a). Moreover, by the end of the experiment, the recovered ethanol amount increased from a moisture content of 3.6 to 4.6 mL/g and then decreased with further increases in moisture content. Correspondingly, the total output of ethanol increased from 3.42 to 4.78

g with the increase in moisture content from 3.6 to 4.6 mL/g and then decreased to 3.64 g with further increases in moisture content up to 6.6 mL/g (Fig. 4 b).

Fig. 4 c shows that with the increase in moisture content from 3.6 to 6.6 mL/g, the residual ethanol amount in the substrate decreased gradually from 0.69 to 0.48 g. Furthermore, the substrate consumption amount decreased from 11.80 to 10.18 g. The ethanol yield first increased to 56.3% with an initial increase in moisture content to 4.6 mL/g and then dropped to 42.8% with further increases in moisture content up to 6.6 mL/g (Fig. 4 d).

The highest total output of ethanol and ethanol yield, 4.78 g and 56.3%, respectively, were obtained using a moisture content of 4.6 mL/g with an N_2 flow rate 30 mL/min and a yeast inoculation amount 20 mL. This result corresponds to generating 0.46 g of ethanol for every one gram of rice straw.

4 Discussion

To alleviate the problems of glucose inhibiting saccharification and ethanol inhibiting anaerobic fermentation in the conversion process of cellulosic materials to ethanol, SSSF with carrier gas sparging was used. The effects of gas flow rate, inoculation amount, and substrate moisture content on the performance of SSSF were investigated using rice straw as the substrate. The results indicate that the residual ethanol and glucose in the bioreactor were low throughout. The highest residual ethanol amount found was only 0.69 g in the fermented substrate, corresponding to 0.05 g ethanol per gram of dried residual rice straw. The highest ethanol yield obtained was 56.3%, generating 0.46 g of ethanol per gram of initial substrate, which is higher than those results in other solid-state fermentation studies.[25, 27, 28] All these results demonstrate the improved performance of the SSSF system.

The mass transfer of ethanol in the SSSF test system was divided into two steps. First, the evolved

ethanol dispersed from the solid substrate to the gas phase in the bioreactor. Thus, the ethanol concentration in the gas phase in the bioreactor gradually increased. A high gas flow rate enhanced ethanol mass transfer from the solid substrate to the gas phase because of an increasing concentration difference of ethanol at the gas–solid interface. Therefore, the recovered ethanol amount, total ethanol output and ethanol yield were increased for higher flow rates. However, further increases in gas flow rate above 30 mL/min reduced the recovered ethanol amount. In this case, the high gas flow rate led to a reducing substrate humidity as more water in substrate was stripped out of the bioreactor by the carrier gas, even though the carrier gas was humidified before entering the bioreactor.

The second step of ethanol transfer was recovery in the absorber. When ethanol concentration in the absorber is high, the absorption efficiency of ethanol may decrease because the carrier gas may strip ethanol out of the absorber and reduce recovery. Therefore, to avoid losing ethanol, the solution used for ethanol recovery was replaced with fresh distilled water just after ethanol was detected in the absorber. We also evaluated the ethanol loss caused by discharged gas from the absorber and found that the ethanol concentration was low. The highest ethanol loss was about 0.21 g during the SSSF, which accounted for only 4.4% of the total output of ethanol.

During SSSF, yeast cells adsorbed on the substrate surface, diffused into the substrate for growth, and formed a biofilm on the surface of the fermented substrate.[29] The formation of a bacterial biofilm on the substrate surface comprises three stages: adsorption, growth, and maturity.[30] Therefore, the growth phase of yeast biofilm was reflected in the recovered ethanol amount. For instance, as shown in Fig. 2 a, 12 h after inoculation, the recovered ethanol amount was low and accumulated slowly over time at a fixed inoculation amount, which indicates that the yeast biofilm was still in the adsorption

stage. Between 84 h and 96 h, the recovered ethanol amount was rising rapidly, which indicates that the yeast biofilm was in the growth stage. Consequently, the biomass of yeast cells increases rapidly in the packing layer, and increased the amount of ethanol generated during the SSSF of rice straw. Then, the increment of the recovered ethanol amount was mitigated and shows that the yeast biofilm was in the maturity stage. However, the intermediates from the enzymatic hydrolysis and ethanol fermentation processes may negatively affect the enzyme activities, which may be another reason that caused the recovered ethanol amount to decrease over time.[31] Thus, in conclusion, SSSF relies on the synergy of hydrolytic enzymes and yeast cells. Increasing enzymes loading or yeast inoculation amount cannot enhance the ethanol yield during SSSF. Hence, the ethanol yield did not increase with further rises of yeast inoculation amount in this study (Fig. 3 d).

Cellulases catalyzing cellulosic hydrolysis adsorb on the surface of the substrate during solid-state fermentation.[32] A low substrate moisture content affects the adsorption of cellulosic enzymes on the substrate surface and suppresses enzymatic hydrolysis.[33] Moreover, the growth and metabolism of yeast cells were affected at a low moisture content of substrate because low water levels affect activity. Consequently, a low total output of ethanol and ethanol yield were observed. However, with the increase in moisture content, the adsorption and activities of hydrolytic enzymes were significantly improved, and the growth and activity of yeast cells were enhanced. Therefore, obtaining the highest total ethanol output and ethanol yield (Fig. 4). However, with further increases in moisture content, the adsorption of enzymes on the substrate surface was reduced because of a diluted enzyme concentration. Thus, the resistance to mass transfer was increased because of an increasing thickness of liquid film on the surface of the fermented substrate.[34] These factors caused the total output of ethanol to decrease. In

the present study, a significant agglomeration of rice straw using the moisture contents of 5.6 and 6.6 mL/g was observed. The high moisture content led to a reduced porosity of the packing layer and an increase in the pressure drop of the bioreactor.[33] Thus, microbial growth was affected.[35] These effects are another contributing reason as to why the total output of ethanol and ethanol yield decreased with further increases in substrate moisture content during SSSF.

To summarize, the SSSF of lignocellulose coupled with the gas stripping of ethanol is a complicated process that can be influenced by many factors such as the activities and loadings of cellulase and yeast cells, the type and pretreatment methods of cellulosic material, the porosity of the packed bed, and the carrier gas flow rate. Although a high ethanol yield of 56.3% in this study was achieved, which was lower than those found in some literatures (Table 1). As shown above the synergy of hydrolytic enzymes, yeast cells, and carrier gas is important to the operation of an SSSF system. Therefore, more research into the enzymolysis mechanism of cellulose and the interaction of multiple factors will further improve the performance of SSSF.

4 Conclusions

The effects of gas flow rate, yeast inoculation amount, and substrate moisture content on SSSF employing the gas stripping of ethanol were investigated. The low residuals of ethanol and glucose in the substrate demonstrate that the carrier gas effectively strips the evolved ethanol out of the bioreactor during SSSF, alleviating the issues of the produced glucose and ethanol inhibiting enzymatic hydrolysis and fermentation. With increases in gas flow rate and moisture content, the total outputs of ethanol and ethanol yields initially increase and then decrease, whereas with increases in yeast inoculation amount, the total output of ethanol and ethanol yield initially increase and then remain

relatively constant. The results reveal that SSSF was mainly conducted by the synergy of hydrolytic enzymes and yeast cells.

Acknowledgements

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Figure Captions

Fig.1 Experimental system: 1) nitrogen canister, 2) humidifier, 3) flow meter, 4) packed-bed bioreactor, 5) pH meter and thermometer, 6) ethanol absorber, 7) computer, and 8) incubator.

Fig. 2 Effect of gas flow rate on SSSF

Fig.3 Effect of yeast inoculation amount on SSSF

Fig. 4 Effect of substrate moisture content on SSSF

Table caption

Table 1 Comparison of conversion efficiency of cellulose to ethanol

Fig. 1

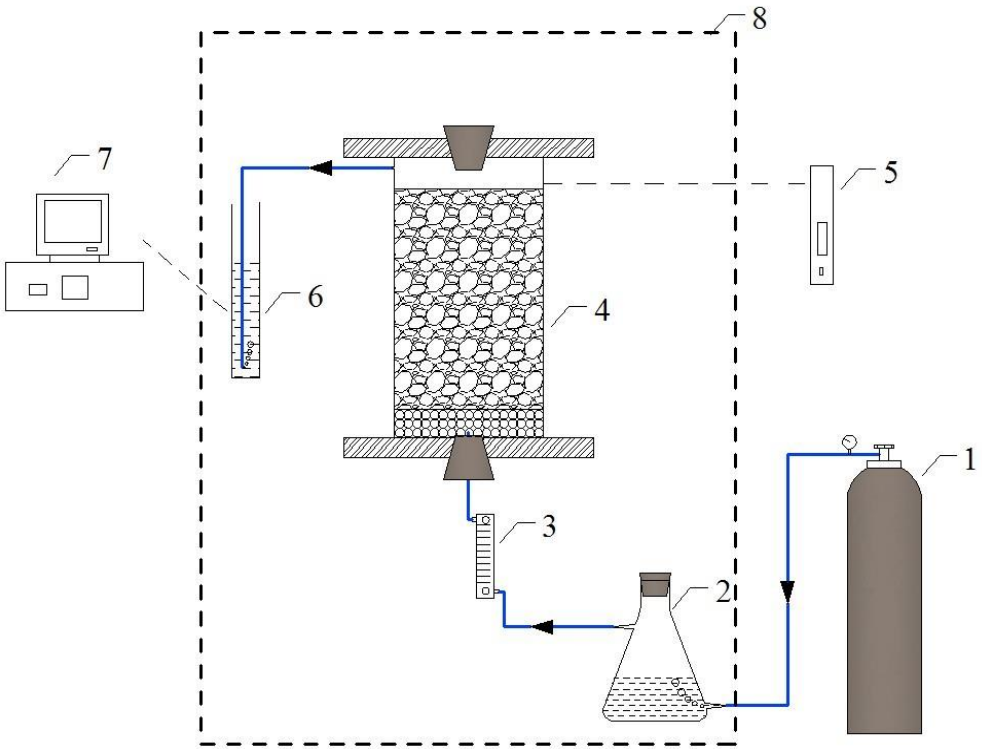

Fig. 2

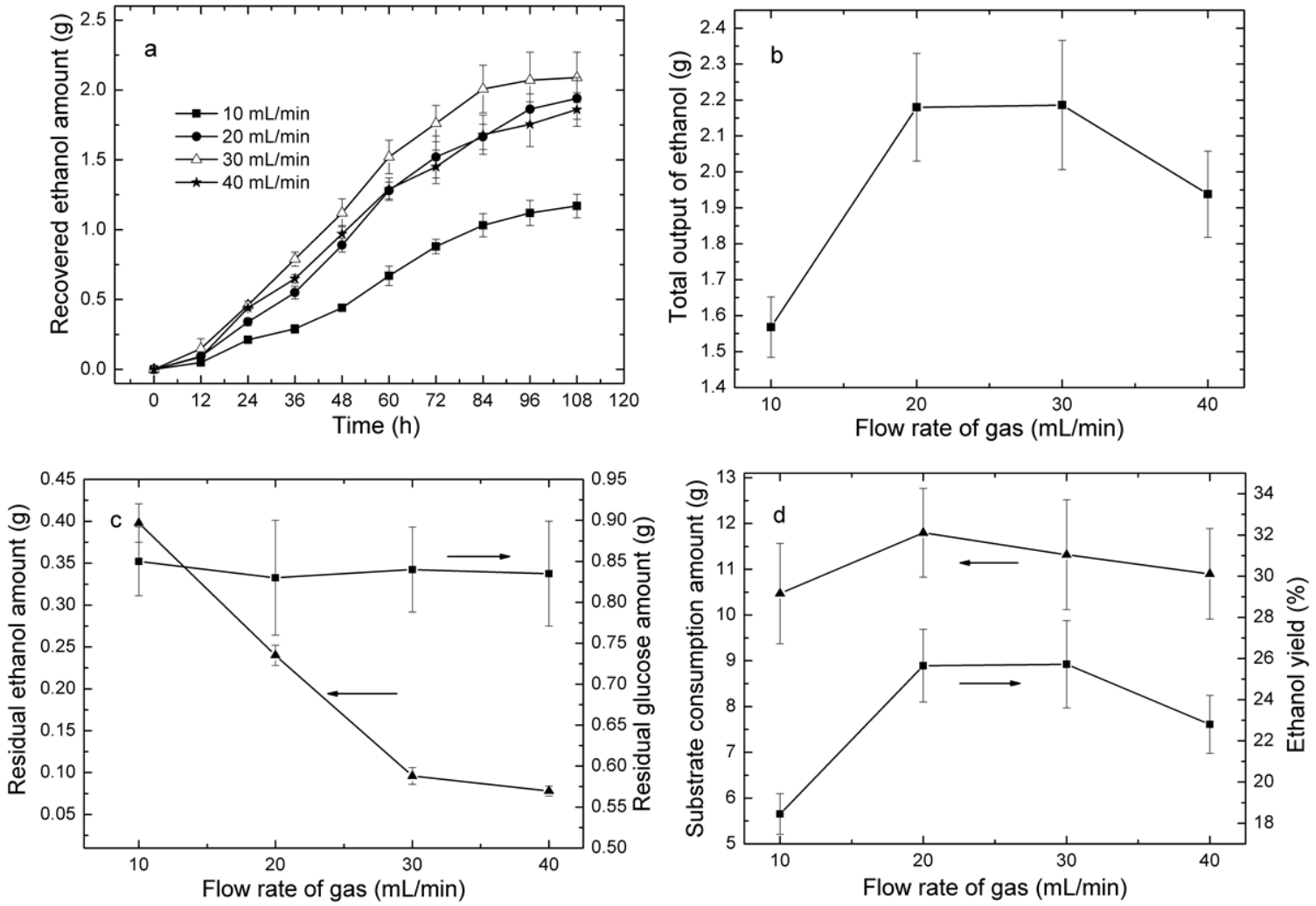

Fig. 3

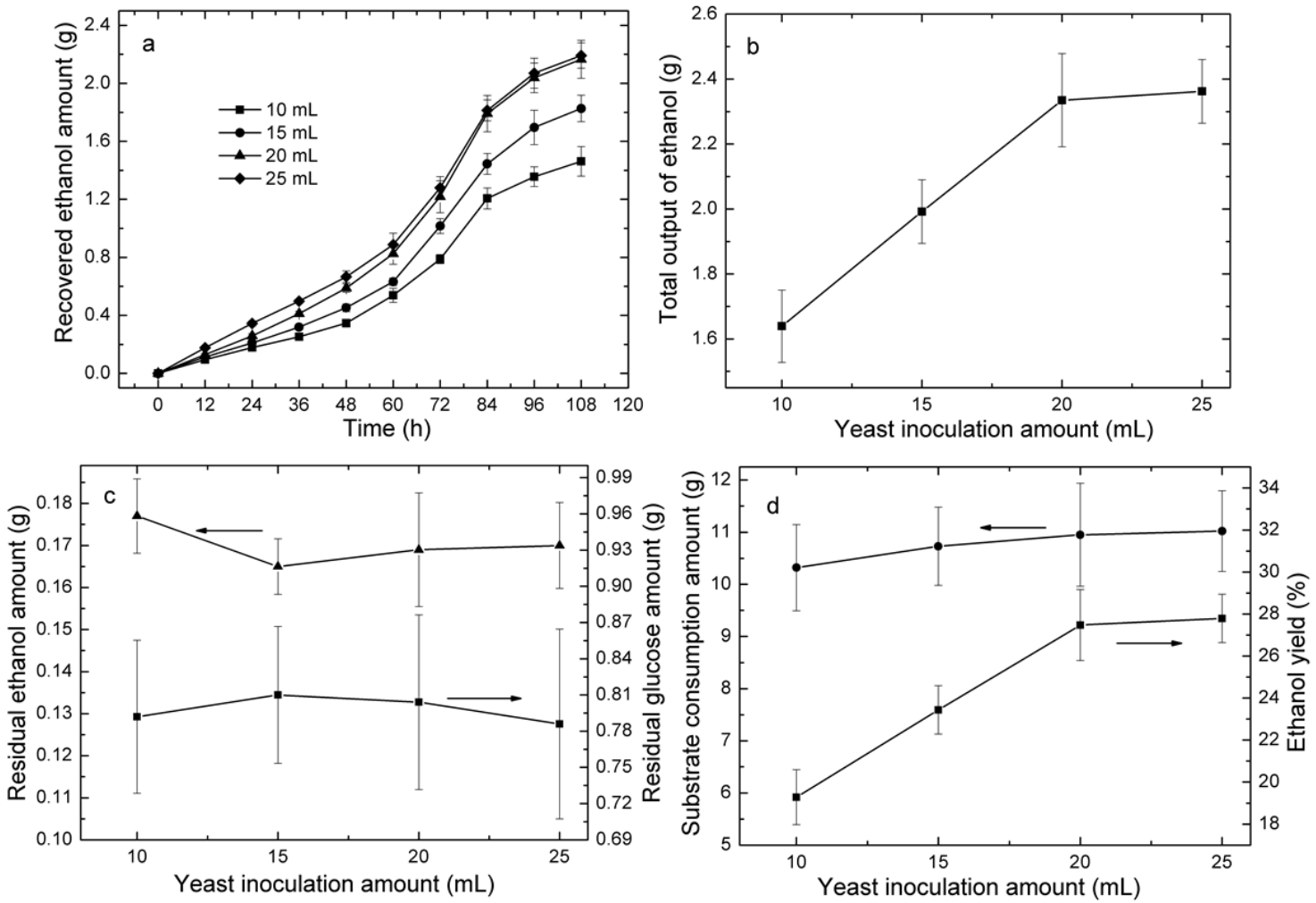

Fig. 4

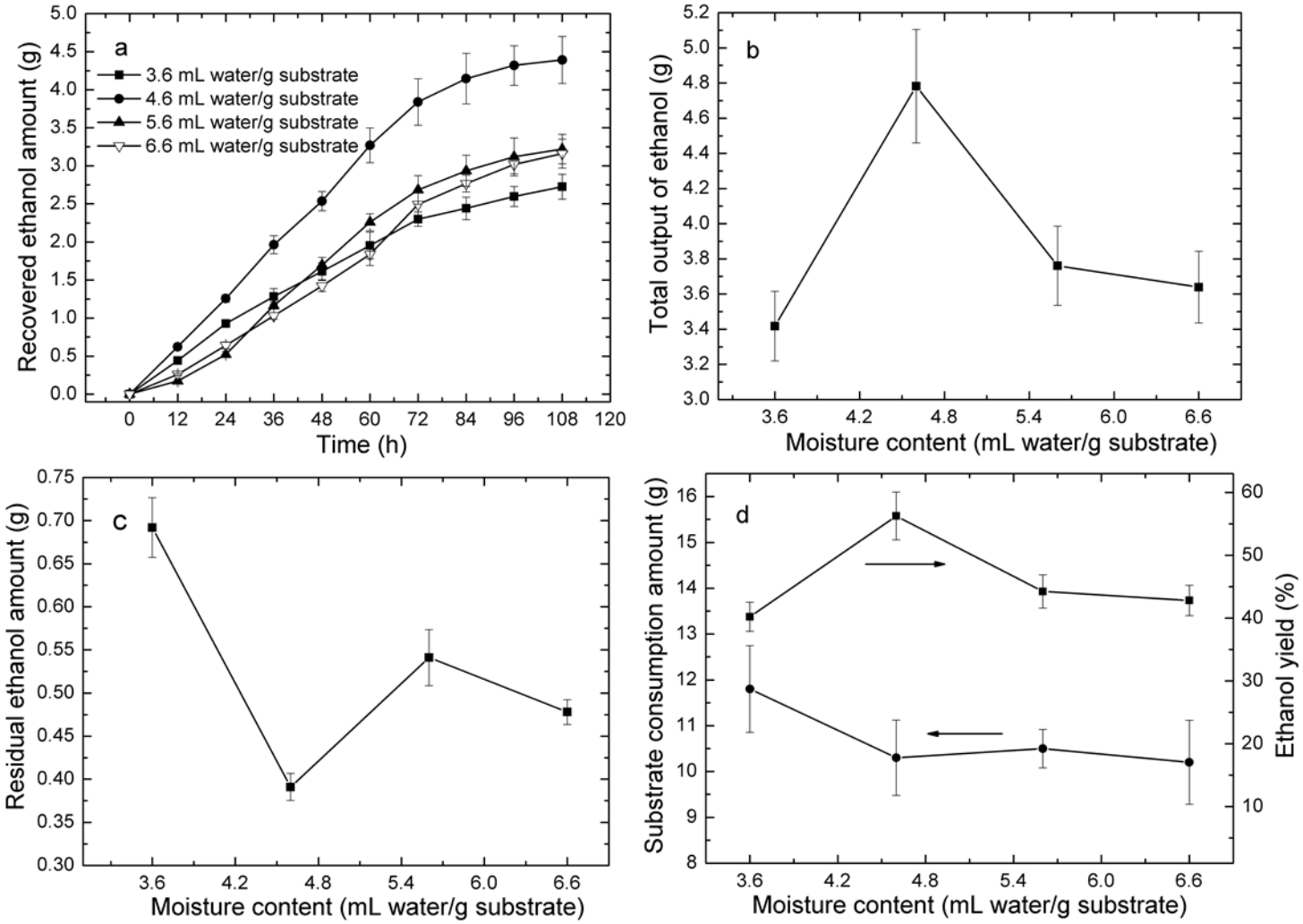

Table 1 Comparison of conversion efficiency of cellulose to ethanol

Substrate	Pretreatment conditions	Fermentation conditions	The highest theoretical ethanol yield (%)	The corresponding Ethanol content (g/L)	Ref
Miscanthus	1.5 M NaOH with stirring at 120 rpm and heated to 150 ℃ for 30 min	Liquid-state saccharification and fermentation at 42 ℃ with shaking at 150 rpm	86.3%	29.5	36
Paper bark tree	Subcritical water at 180 ℃ for 30min	anaerobic condition in an orbital shaker (150 rpm,37 ℃) for 120 h	91.25%	43.7	37
Corn stover	Steam explosion at 200 ℃ for 4min	Semi-continuous liquid-state simultaneous saccharification and fermentation(37 ℃ for 60 h)	52.1%	40.6	23
Olive tree pruning	liquid hot water pretreated at 210 ℃ with magnetic agitation	Liquid-state simultaneous saccharification and fermentation at 35 ℃ for 72 h and 150 rpm	38%	24.9	38
mature coconut fibre	sequential alkaline hydrogen peroxide (Alk-H2O2)–sodium hydroxide (NaOH)	Semi-simultaneous saccharification and fermentation at 30 ℃ for 40 h	89.15	9.32	39
Hinoki cypress	steam treatment (150 ℃ for 2 h,) with wet disk milling	yeast-based simultaneous saccharification and fermentation at 58 ℃ with shaking at 125 rpm	63.4% (calculated value)	--	40
Rice straw	dilute acid pretreatment, then delignification with 0.5% NaOH at 121 ℃ for 30 min	Simultaneous saccharification and fermentation with agitation at 120 rpm for 72 h at 42 ℃	84.6%	24.63	41
Rapeseed straw	liquid hot water pretreatment at 217 ℃ for 42 min)	Liquid-state simultaneous saccharification and fermentation in an orbital shaker at 150 rpm.	66.6%	17.2	42
Rice straw	1% NaOH solution for 24 h	Solid-state Simultaneous saccharification and fermentation at 35 ℃ for 108 h	56.3%	Equivalent to 21.05	This work