Combining *sestc* engineered *A. niger* with *sestc* engineered *S. cerevisiae* to produce rice straw ethanol via step-by-step and in situ saccharification and fermentation

Peizhou Yang¹ · Haifeng Zhang¹ · Lili Cao¹ · Zhi Zheng¹ · Dongdong Mu¹ · Shaotong Jiang¹ · Jieshun Cheng¹

Received: 23 July 2017 / Accepted: 4 December 2017 / Published online: 11 December 2017
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Abstract

The development of agricultural residue ethanol has a profound effect on the environment protection and energy supply. To increase the production efficiency of straw ethanol and reduce operation progress, the single-enzyme-system-three-cellulase gene (*sestc*) engineered *Aspergillus niger* and *sestc* engineered *Saccharomyces cerevisiae* were combined to produce ethanol using the pretreated rice straw as the substrate. The present results showed that both the step-by-step and in situ saccharification and fermentation can effectively produce ethanol using rice straw as the carbon substrate. The conversion rates of ethanol were 12.76 and 14.56 g per 1 kg of treated rice straw, respectively, via step-by-step and in situ processes. In situ process has higher ethanol conversion efficiency of rice straw and fewer operation processes as compared with step-by-step process. Therefore, in situ saccharification and fermentation is a more economical and effective pathway to convert rice straw into ethanol. This study provides a reference to the conversion of lignocellulosic residues into ethanol with a combination of two kinds of *sestc* engineered strains.

Keywords Cellulase gene · *A. niger* · *S. cerevisiae* · Rice straw · Ethanol · In situ · Saccharification · Fermentation

Introduction

The increasing demand of energy and gradually exhausting storage of fossil fuels has raised a great concern for the sustainable development of humans. Bioethanol provides an important supplement to face the inevitable decrease of petroleum reserve amounts. Previous studies indicated that starch rich material is not suitable for large-scale development of bioethanol for food safety, because excessive use of corn ethanol could result in food shortages (Solange et al. 2010). The high lignocellulosic containing materials, which are used to produce ethanol, are regarded to be an

economical and feasible alternative to achieve the application of marketization (Hahn et al. 2006). In addition, the utilization of agricultural biomass helps environmental protection, food safety, and harmonious relationship between the human and nature. In the major developing countries, the treatment of agricultural residues after harvest is a vexing issue to solve. The bio-ethanol conversion of agricultural residues is a feasible effort to protect the environment and enhance the utilization efficiency of the residues. However, the high cost of producing ethanol has severely limited its practical application. The hydrolysis of lignocellulose and utilization of hydrolyzed sugars are the main cost sources. Therefore, recent efforts of bioethanol development have mainly focused on decreasing the cost of ethanol production through modifying fermentation processes and engineering host strains to increase bioethanol yield.

The difficulty of converting lignocellulose into ethanol is associated with material characteristics and product properties. These whole processes are generally achieved using the following two models: step-by-step saccharification and fermentation (Badal et al. 2005) and simultaneous saccharification and fermentation (Ballesteros et al. 2004). The first model separates the whole process into independent sections

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s13205-017-1021-1>) contains supplementary material, which is available to authorized users.

✉ Peizhou Yang
yangpeizhou@163.com; yangpeizhou@hfut.edu.cn

¹ College of Food Science and Engineering, Anhui Key Laboratory of Intensive Processing of Agricultural Products, Hefei University of Technology, No. 193 Tunxi Road, Hefei 230009, China

of saccharification and fermentation; the lignocellulose of pretreated material is converted into sugars by cellulases (Juijen et al. 2013), acid, and an ionic liquid, and then, these sugars are fermented to produce ethanol under the other reactor system. The second model simultaneously hydrolyzes lignocellulose and ferment available reducing sugars to produce ethanol under the same reactor system. Both these two models need the lignocellulosic hydrolysis and ethanol fermentation process. Lignocellulosic hydrolysis using the enzymatic method is regarded as a feasible and effective method (Peizhou et al. 2011). However, low cellulase activity, slow reaction effect, and incomplete cellulase enzymic systems severely limit its wide application (Rakesh et al. 2014).

To increase the total cellulase activities, we have engineered *sestc* *Aspergillus niger* (*A. niger*) (Peizhou et al. 2016a) and *sestc* *Saccharomyces cerevisiae* (*S. cerevisiae*) (Peizhou et al. 2016b). The *sestc*-encoding protein has a function of endo-glucanase (endo-1,4- β -glucanases, EGs), exo-glucanase (exo-1,4- β -glucanases, CBHs), and D-xylanase. The total cellulase activities were significantly increased in this two engineered strains. In addition, wild types *A. niger* and *S. cerevisiae* are excellent and safe strains to express exogenous proteins, and both are widely used in food production (Sergi et al. 1998) and the medical industry (Schuster et al. 2002; Riquelme et al. 2003). Therefore, to further increase ethanol yield and decrease the cost, two models were investigated to produce ethanol with rice straw as the substrate; in addition, the specific parameters and technologies were redesigned based on the characteristics of the material and strains. This study provides an important reference to the conversion of lignocellulosic residues into ethanol using *sestc* engineered strains.

Materials and methods

Medium preparation

Spore culture medium was prepared with the components of bran 10% (w/v), ammonium sulfate 1% (w/v), dipotassium hydrogen phosphate 0.02% (w/v), magnesium sulfate heptahydrate 0.01% (w/v), agar powder 1.5% (w/v), and 300 $\mu\text{g ml}^{-1}$ of hygromycin (for the *sestc* engineered strains). Mandel's minimal solution was prepared with components of 0.3 g urea, 1.4 g $(\text{NH}_4)_2\text{SO}_4$, 2 g KH_2PO_4 , 0.4 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.3 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 5.0 mg $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 1.6 mg $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$, 1.4 mg $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, and 20 mg $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, added to 1000 ml with distilled water, adjusted to pH 7.0. Fermentation solution was prepared with the components of Mandel's minimal solution, 20 g l^{-1} of peptone, 10 g l^{-1} of yeast extract, 300 $\mu\text{g ml}^{-1}$ of hygromycin, and 0.11% of Tween-80 (w/v).

The *sestc* engineered strains

A single-enzyme-system-three-cellulase gene (*sestc*) isolated from *Ampullaria gigas* Spix encodes a protein possessing the activities of endo-glucanase, exo-glucanase, and β -xylanase. This gene is harbored in an expression vector promoted by the glyceraldehyde-3-phosphate dehydrogenase gene (*Gpd*) promoter of *Shiitake* mushroom; in addition, an anti-hygromycin gene cassette is also integrated into the expression vector (Supplementary material). This vector has been transferred into *A. niger* (Peizhou et al. 2016a) and *S. cerevisiae* (Peizhou et al. 2016b). The engineered strains have been screened and stored on the solid induction medium.

Rice straw and H_2O_2 -NaOH pretreatment

Rice is an important food crop in China, India, Indonesia, etc. The world rice production in 2017/2018 will reach 481.04 million metric tons, which was reported on the web page of worldriceproduction.com. As a by-product, rice straw is composed of lignocellulosic materials, which can release glucose and xylose under the treatment of cellulases. Therefore, the development of straw ethanol provides an effective supplement to the fossil energy and protects the environment. In this study, fresh rice straw was harvested from a local farm; the powder of straw was stirred in a mixture solution of 4% NaOH (w/v) and 13.4% 30% H_2O_2 (v/v) after being cleaned, grinded, and dried in a convection oven at 60 °C for 48 h; then, the structure of straw mixture was destroyed after incubation at 40 °C for 24 h in a water bath. Cellulose, hemicellulose, and lignin of the untreated rice straw, respectively, account for 49, 30, and 6% (w/w); correspondingly, cellulose, hemicellulose, and lignin of treated straw mentioned above account for 61, 24, and 3.7% (w/w).

The schematic graph of the conception

In this study, the pathways of straw ethanol were presented via an independent step-by-step and in situ method in Fig. 1. The step-by-step pathway performs the saccharification and fermentation in the independent reactors; in situ saccharification and fermentation is designed to produce ethanol in the same reactor system. In addition, this in situ pathway supplements an additional saccharification process to enhance the efficiency of lignocellulosic hydrolysis.

Saccharification and fermentation

Spore culture medium was used to increase the cell amount of the *A. niger* and the *S. cerevisiae* before fermentation.

Fig. 1 Pathways of step-by-step and in situ saccharification and fermentation to produce ethanol with rice straw as substrate

The cellulase induction medium prepared with 10 g of dry treated rice straw, 20 ml of Mandel's minimal solution, and 0.3 g tween-80 was loaded into a 500-ml Erlenmeyer flask and sterilized in an autoclave at 121 °C for 20 min. Three grams of wet *A. niger* mycelia were uniformly mixed in the sterile cellulase induction mixture, and then, the Erlenmeyer flask was statically incubated at 30 °C with a humidity of 70%. The activities of cellulases were measured using DNS methods (Peizhou et al. 2011). Cellulase activity is determined as a unit; one unit is defined as the amount of the enzyme that catalyzes the formation of 1 μ mole reducing sugars per minute. The catalysis substrates used to measure the activities of total cellulase, endo-glucanase, exo-glucanase, and D-xylanase, respectively, are Whatman No. 1 filter paper (Peizhou et al. 2011), carboxymethyl cellulose (Ghose 1987), avicel (Masa et al. 2015), and xylan (Cheng et al. 2009). The glucose content was measured by HPLC method (Haibo et al. 2016).

Step-by-step saccharification and fermentation

A volume of 50 ml of 0.05 mol l⁻¹ citrate buffer adjusted to pH 5 was added to the 500-ml Erlenmeyer flask, and the temperature was quickly increased to 50 °C for the lignocellulosic saccharification. The liquid containing sugars was

filtered with a 300-mesh filter cloth from the saccharification mixture of rice straw. The sugar contents increased by concentrating the hydrolysate of rice straw. When the glucose content reached around 80 g l⁻¹, 3 g of wet *sestc* engineered *S. cerevisiae* was added to the sugar liquid. In addition, the concentrations of peptone and yeast extract were, respectively, adjusted to 20 and 10 g l⁻¹. A total volume of 40 ml was used for fermentation at 30 °C under anaerobic condition. Ethanol concentration was determined using the method of gas chromatography as described previously (Reeta et al. 2015).

In situ saccharification and fermentation

The initial step of in situ saccharification and fermentation is the same as that of step-by-step saccharification and fermentation as mentioned above. For in situ saccharification and fermentation, the *sestc* engineered *S. cerevisiae* was added into the hydrolyzates without the removal of straw residues after saccharification. After the inoculation at 50 °C for 2 days, a mixture of 50 ml of the fermentation solution and 3 g of wet *sestc* engineered *S. cerevisiae* was poured into the 500-ml Erlenmeyer flask containing the hydrolyzates and straw residues. A water volume of at least 70 ml was available in the fermentation broth. With sufficient mixing, the

flasks were statically inoculated at 37 °C under an anaerobic condition to produce ethanol. The supernatant was obtained by a centrifugation at 8000g for 10 min to determine ethanol concentration.

Results

Step-by-step saccharification and fermentation

Cellulase expression of the combination of rice straw and *A. niger*

Figure 2 indicates that four different cellulase activities were influenced by the combination of rice straw and *A. niger* under the solid fermentation condition. The result showed that the combination of pretreated rice straw and *sestc* engineered *A. niger* increased the activities of total cellulase, endo-1,4- β -glucanases, exo-1,4- β -glucanases, and xylanase activities; in addition, the increased rates and times of highest enzymatic activities are different between the combination of pretreated rice straw and *sestc* engineered *A. niger* and other three combinations. The combination of pretreated rice straw and *sestc* engineered *A. niger* produced activities of 18.4 U g⁻¹ of filter paper activity (FPA) (Fig. 2a),

75.3 U g⁻¹ of endo-1,4- β -glucanase (Fig. 2b), 75.4 U g⁻¹ exo-1,4- β -glucanase (Fig. 2c), and 1644 U g⁻¹ xylanase activities (Fig. 2d), which, respectively, were 1.78, 1.23, 1.39, and 1.46-fold as compared to the combination of the native rice straw and the wild type *A. niger*. Therefore, the combination of pretreated rice straw and *sestc* engineered *A. niger* could effectively induce the expression of exogenous *sestc* cellulase gene and increase the total cellulase activities.

Residual reducing sugar profiles during cellulase production by *sestc* engineered *A. niger*

Figure 3 indicates that the contents of available reducing sugars were measured during the cellulase induction of *sestc* engineered *A. niger*. The combination of *sestc* engineered *A. niger* and the pretreated rice straw resulted in the highest content of released reducing sugar compared to three other combinations. Furthermore, the low content of released reducing sugar was detected in the initial fermentation stage of *sestc* engineered *A. niger*; released reducing sugar sharply increased to 156 mg g⁻¹ after a 2-day fermentation; then, the contents of released reducing sugar again decreased to a low content of 45 mg g⁻¹ after a 4-day fermentation. The following content of released reducing sugar maintained a low level with minor fluctuations. The

Fig. 2 Cellulase activities were affected by the different combinations. **a–d**, respectively, represented the effect of different treatment combinations on FPA, endo-1,4- β -glucanase, exo-1,4- β -glucanase, and D-xylanase

Fig. 3 Residual reducing sugar content profiles of different fermentation times

accumulation of high amount of reducing sugar is a basic guarantee to enhance the yield of bioethanol. This study indicated that an inconsistent fermentation time occurred between high activities of cellulase and high contents of released reducing sugar.

Comparing the sugar content released for rice straw via in situ saccharification

Figure 4 indicates that the content of the released reducing sugars by in situ saccharification at 50 °C for 48 h after the pretreated rice straw with an addition of the *sestc* engineered *A. niger* was incubated at 30 °C. After a 2-day fermentation at 30 °C, the released contents of reducing sugar reached the highest amount. The highest contents of reducing sugar, glucose, and xylose, respectively, reached 329, 232, and 88 mg g⁻¹ treated rice straw. Therefore, a 2-day fermentation at 30 °C was used to produce cellulase

Fig. 4 Content of released sugars via in situ saccharification following different fermentation times

in the following step-by-step and in situ saccharification and fermentation.

Ethanol production of the rice straw hydrolysates by *sestc* engineered *S. cerevisiae*

Figure 5 indicates that the yields of ethanol were from the hydrolysate of rice straw using the process of step-by-step saccharification and fermentation. The used carbon source for fermentation was the concentrated sugar from the cellulase hydrolysates. The initial glucose content used for fermentation was 71 g l⁻¹. Before the 36-h fermentation, the ethanol yields gradually increased; then, the ethanol content was kept stable after 36 h. *Sestic* engineered *S. cerevisiae* fermented the hydrolysate of rice straw to produce an ethanol content of 31.9 g l⁻¹ after an incubation for 36 h at 30 °C under an anaerobic condition. The conversion rate of ethanol was 12.76 g per 1 kg treated rice straw. The glucose content rapidly decreased before a 36-h fermentation, then kept a low content after a 36-h fermentation.

Ethanol production via in situ saccharification and fermentation

Effect of temperature on activities of two engineered strains

In this study, lethality rates were calculated based on the death rate of the strain at the set temperatures for 1 h under the submerged condition (yeast extract peptone dextrose medium prepared with 1% yeast extract, 2% peptone, and 2% glucose; 250 rpm) (Fig. 6). The result showed that the of *sestc* engineered *A. niger* had a stronger tolerance than *sestc* engineered *S. cerevisiae*. The lethality rate increased significantly when the temperature exceeds 42 °C. Temperature affected strain growth and activity in the liquid state. A

Fig. 5 Ethanol production from the hydrolysate of rice straw by *sestc* engineered *S. cerevisiae*

Fig. 6 Temperature affected lethality rate of two different engineered strains

too high temperature was inappropriate to perform in situ saccharification and fermentation. Therefore, a temperature of 42 °C was used to saccharify the straw and ferment to produce ethanol in the next experiment for ethanol production.

Half-life of total cellulase activity under different temperatures

The half-life of cellulase determined the efficiency of cellulase for sugar release. Enough half-life of cellulase was essential to hydrolyze the straw, especially at a relatively low temperature. The half-life of cellulase activity produced by *sestc* engineered *A. niger* under a different temperature is shown in Fig. 7. The results showed that the half-life rapidly decreased when the temperature exceeded 55 °C; meanwhile, the half-life of cellulase at 37 °C exceeds 168 h,

Fig. 7 Half-life of cellulase activity produced by *sestc* engineered *A. niger* under different temperatures

which meant that this temperature could provide enough time to hydrolyze the lignocellulose of straw. Therefore, a temperature of 37 °C was used to saccharify the substrate and produce ethanol.

Ethanol production via in situ technique

Figure 8 indicates the effect of fermentation time on ethanol and glucose contents at 37 °C after a 2-day saccharification via in situ technique, the result showed that ethanol content gradually increased to 20.8 g l⁻¹ before a 36-h fermentation, and then, the ethanol content was kept stable; in addition, the glucose content gradually decreased from 61 to 7 g l⁻¹ before a 24-h fermentation. Therefore, the consumed glucose was converted into ethanol by the *sestc* engineered *S. cerevisiae*. The conversion rate of ethanol was 14.56 g per 1 kg treated rice straw.

Discussion

Strategies of ethanol production of agricultural straw

The development of lignocellulosic ethanol is facing the bottleneck problem of application; the available technology makes it difficult to provide a competitive and cost-effective production of bio-ethanol. Therefore, the decrease of the cost provided an integration of cheaper substrates, improved recovery processes, excellent recombinant strains, effective pretreatment techniques, energy, and waste minimization, and effective exploitation of side products (Saumita et al. 2010). Feasible strategies determine the efficiency, value,

Fig. 8 Effect of fermentation time on the contents of ethanol and glucose after a 2-day saccharification

and sustainability of lignocellulosic ethanol (Mitsunori et al. 2013). Many strategies of lignocellulosic ethanol production have been reported; for instance, diluted sulfuric acid was used to soak rice straw in a boiling water bath for 1 h with the addition of filtration and pH 5.5 adjusted (Abbi et al. 1996); saccharification of rice straw using cytolase (Novozymes) at 50 °C for 120 h followed by steam exploding within the parameters of 3.5 MPa, 275 °C, and 2 min (Moniruzzaman 1995). These strong corrosive chemical reagents and highly energy-consuming techniques undoubtedly are effective ways to achieve the quick release of sugar. However, these less environmentally friendly means would bring an economic burden to remove the negative effect on the production of bio-ethanol. Therefore, a relatively low strength treatment method and less energy input should be feasible to convert low-value agricultural residues into bioethanol. In this study, two strains integrated by *sestc* cellulase gene possess high cellulase activity; in addition, in situ simultaneous fermentation and saccharification reduce the cost of transferring the lignocellulosic sugar. The main disadvantage is a need for a longer fermentation time to achieve the high conversion of lignocellulose into ethanol; however, the strategies provided by this study would produce agricultural ethanol via a low-energy-input, easy-to-control, and environmentally friendly pathway.

Technical pathways of producing bioethanol from agricultural residuals

To effectively convert lignocellulose into sugars and ferment released reducing sugars to ethanol, the development of an efficient process is urgently needed to decrease the total cost of lignocellulosic ethanol and increase its competitiveness in the market (Nopparat et al. 2013). Simultaneous saccharification and fermentation could reduce the cost of product via shortening product cycles and decreasing production processes. The strain combination is one of the most key factors to enhance the final effect of simultaneous saccharification and fermentation (Saravana et al. 2016). The present study showed that the conversion rate of a step-by-step technique is lower than that of in situ saccharification and fermentation; in addition, the step-by-step technique needs an additional step to separate the hydrolyzates from the catalysis reactors; in addition, the cellulase hydrolysate needs to be filtered from the straw residues and concentrated. In in situ process, the reason of relatively high glucose content is as follows: (1) before saccharification and fermentation, a 2-day saccharification has been performed, which can release reducing sugar from straw; (2) rice straw after H_2O_2 –NaOH pretreatment contains less lignin, and the lignocellulose is easy to release its reducing sugar under cellulase treatment; (3) in addition, in this study, the pretreated rice straw amount

was input (10 g), which could provide enough material for degradation.

Previous reports showed that the combination of different species of yeast (Nopparat et al. 2013), yeast, and cellulase producing fungi (Thomas et al. 2015; Gulten and Ali 2017), and multi-engineered strains (Jiaqing et al. 2014) could produce ethanol with agricultural residues as carbon sources via saccharification and fermentation pathways (Table 1). In addition, most agricultural wastes including rice straw (Nopparat et al. 2013), wheat straw (Hongzhang et al. 2008), *Brassica carinata* residue (Ballesteros et al. 2004), corn stover (Micky et al. 2015), sweet sorghum (Lijun et al. 2013), and potato waste (Gulten and Ali 2017) possess the characteristics of cheap price, low-value utilization, and easy to contaminate the environment. Their cellulose components are embedded into calcitrant lignocellulosic structure surrounded by the outer hard epidermis. Proper treatments are necessary to expose the cellulose accessible to cellulase. The lignocellulosic structure is easily loosened under an aqueous ammonia (Jiaqing et al. 2014), alkaline (Joginder et al. 2015), acid (Joginder et al. 2015), oxidation (Anne et al. 2013), steam explosion (Joginder et al. 2015), or ionic liquid condition. The mixed use of these two chemicals helped the effective removal of lignin (Anne et al. 2013; Hailong et al. 2015). Alkaline and H_2O_2 were combined to destroy the structure of rice straw in this study.

In the present study, the ethanol concentration and conversion rate were lower than those of starch sources (Joscelin et al. 2014; Gulten and Ali 2017). The main reasons were that starch sources were easy to saccharify with fewer inhibitors presented. The ethanol yield was higher than that of sodium hydroxide treated corn stover (Micky et al. 2015). In addition, the ethanol conversion rates were similar to that of pretreated olive stones (13/100 kg of raw material), which performed strong pretreated techniques including liquid hot water, diluted sulphuric acid, and de-lignification at 230 °C. Therefore, the present study provided economical and environmentally friendly techniques to produce ethanol from agricultural straw.

Conclusions

The combination of pretreated rice straw and *sestc* engineered *A. niger* produced higher FPA, endo-1,4- β -glucanases, exo-1,4- β -glucanases, and xylanase activities compared with a combination of the native rice straw and the wild type *A. niger*. Via step-by-step saccharification and fermentation, the ethanol content was 31.9 g l⁻¹ after an incubation under an anaerobic condition, with a conversion rate of 12.76 g l⁻¹ kg of treated rice straw. In addition, the ethanol conversion rate was as high as 14.56 g per 1 kg of treated rice straw via in situ saccharification

Table 1 Ethanol production by different strains using different agricultural sources

Combinations	Carbon sources	Application effect	Material type
<i>S. cerevisiae</i> / <i>S. stipitis</i>	Rice straw, simultaneous saccharification and fermentation	28.6 g l ⁻¹ (Nopparat et al. 2013)	Lignocellulose
<i>T. viride cellulase</i> / <i>S. cerevisiae</i>	Wheat straw, steam explosion, simultaneous saccharification and fermentation	51.5 g l ⁻¹ , yield of 81.1% (Hongzhang et al. 2008)	Lignocellulose
Commercial cellulase/ <i>K. marxianus</i>	<i>Sorghum</i> sp. wheat straw, <i>Brassica carinata</i> residue	16–19 g l ⁻¹ (Ballesteros et al. 2004)	Lignocellulose
<i>P. chrysosporium</i> / <i>G. trabeum</i> / <i>S. cerevisiae</i> / <i>E. coli</i>	Sodium hydroxide treated corn stover	2.88–3.09 g/100 g (Micky et al. 2015)	Lignocellulose
<i>A. niger</i> / <i>S. cerevisiae</i>	Potato waste, simultaneous saccharification and fermentation	37.93 g l ⁻¹ , 0.41 g ethanol/g starch yield (Gulten and Ali 2017)	Starch
<i>K. marxianus</i> / <i>S. cerevisiae</i>	Flour industrial sweet potato (ISP) feed-stocks 35 °C	0.31–0.34 g/g dry ISP (Joscelin et al. 2014)	Starch
<i>S. cerevisiae</i> IR2-9a	Pretreated olive stones, simultaneous saccharification and fermentation	13/100 kg of raw material (Manuel et al. 2015)	Lignocellulose
Enzyme loading at 25 FPU/g-glucan/yeast	Sweet sorghum (<i>Sorghum bicolor</i>) bagasse 37 °C	7% (Lijun et al. 2013)	Lignocellulose
<i>F. xyloporum</i> / <i>S. cerevisiae</i>	Liquefied wheat straw	58 g l ⁻¹ (Thomas et al. 2015)	Lignocellulose
Engineered <i>S. cerevisiae</i>	Aqueous ammonia pretreated corn stover	70.1 g l ⁻¹ (Jiaqing et al. 2014)	Lignocellulose
<i>Sestc</i> engineered <i>A. niger</i> / <i>Sestc</i> engineered <i>S. cerevisiae</i>	Alkaline-H ₂ O ₂ pretreated rice straw, via step-by-step saccharification and fermentation	31.9 g l ⁻¹ ; 12.76 g per 1000 g of treated rice straw, this study	Lignocellulose
<i>Sestc</i> engineered <i>A. niger</i> / <i>Sestc</i> engineered <i>S. cerevisiae</i>	Alkaline-H ₂ O ₂ pretreated rice straw via in situ saccharification and fermentation	20.8 g l ⁻¹ ; 14.56 g per 1000 g of treated rice, this study	Lignocellulose

and fermentation. Therefore, in situ saccharification and fermentation is a more effective technique. The present study would help the effective utilization of agricultural straw to produce bioethanol.

Acknowledgements This research was financially supported by the Anhui Provincial Natural Science Foundation (1408085MC67) and Key Science and Technology Program of Anhui Province (No. 1604a0702001). Many thanks for the grammar revision by Dominique C. from University of Illinois at Urbana-Champaign, Urbana (USA).

Author contribution Yang Peizhou contributed to the conception of the study and funding support; Zhang Haifeng performed the experiments; Zheng Zhi, Cao Lili, Jiang Shaotong, Mu Dongdong, and Cheng Jieshun analyzed the data and wrote the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest in the publication.

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