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Bioethanol production from the dry powder of Jerusalem artichoke tubers by recombinant *Saccharomyces cerevisiae* in simultaneous saccharification and fermentation

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Abstract It has been found that recombinant *Saccharomyces cerevisiae* 6525 can produce high concentration of ethanol in one-step fermentation from the extract of Jerusalem artichoke tubers or inulin. However, the utilization rate of raw materials was low and the fermentation process was costly and complicated. Therefore, in this study, after the optimum processing conditions for ethanol production in fed-batch fermentation were determined in flask, the recombinant *S. cerevisiae* 6525 was first used to produce ethanol from the dry powder of Jerusalem artichoke tubers in 5-L agitating fermentor. After 72 h of fermentation, around 84.3 g/L ethanol was produced in the fermentation liquids, and the conversion efficiency of inulin-type sugars to ethanol was 0.453, or 88.6 % of the theoretical value of 0.511. This study showed high feasibility of bioethanol industrial production from the Jerusalem artichoke tubers and provided a basis for it in the future.

Keywords Bioethanol · *Saccharomyces cerevisiae* · SSF · Jerusalem artichoke · Fed-batch fermentation

Introduction

With the less and less energy on the earth and the pollution of the environment, especially the global warming and energy crisis problems caused by the combustion of fossil fuels [16], people are eager to look for new energy to add or replace the normal energy. In recent years, bioethanol has attracted an increasing number of attentions as one of the most important renewable and environmentally friendly petroleum-based liquid fuels [5]. At present, derivatives from food crops such as corn grain and sugar cane are generally utilized in bioethanol production. However, it may lead to huge competition between food provision and their use for the production of bioethanol due to the limited supply of these crops, and some environmental problems such as serious destruction of vital soil resources and food shortages may also be caused [9, 20]. In this case, an increasing number of people around the world show great interest to look for new and cheap carbohydrate sources for bioethanol production [17].

Jerusalem artichoke, as a cheap and inexpensive carbohydrate alternative feedstock, has been studied for bioethanol production since the 1950s. The tuber of Jerusalem artichoke is rich in inulin which is present even more than 50 % in the dried tuber [19]. As a reserve carbohydrate, inulin is a linear polymer of D-fructose containing a terminal D-glucose which can be hydrolyzed to fructose, sucrose, glucose and polymers of fructo-oligosaccharides by inulinase [4]. Moreover, not only Jerusalem artichoke can resist many plant pests and diseases, but also grow well in barren land with its growing traits of drought tolerance and saline tolerance [2]. With these advantages, Jerusalem artichoke can be used as a cheap and inexpensive carbohydrate alternative feedstock for bioethanol production in addition to its usual uses as single-cell oil or oligofructose syrup [10, 23, 28]. Remize et al. [22] used the extract from Jerusalem artichoke tuber as the substrate to produce ethanol by *Saccharomyces cerevisiae* strain. Ethanol was also produced from Jerusalem artichoke tubers using recycled immobilized cells of *Kluyveromyces fragilis* [14]. Therefore,

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Jerusalem artichoke is a potential biomass source for ethanol fermentation.

Currently, the most commonly used strain in bioethanol fermentation is *S. cerevisiae* which can produce high concentration of ethanol from reducing sugar like glucose or fructose and has high ethanol tolerance [1, 11]. However, ordinary *S. cerevisiae* cannot use the inulin directly to produce bioethanol. It needs pretreatment of hydrolyzing the inulin included in Jerusalem artichoke tubers to fructose and glucose by inulinase or acids before the process of ethanol fermentation [7], called SHF (separate hydrolysis and fermentation), which is long-running and complicated. To produce bioethanol effectively and economically with *S. cerevisiae* from Jerusalem artichoke, a recombinant *S. cerevisiae* strain which can produce exo-inulinase was constructed successfully in our previous studies [21, 26]. Using the recombinant *S. cerevisiae*, Jerusalem artichoke tuber can be utilized to produce bioethanol in SSF (simultaneous saccharification and fermentation) with exo-inulinase which can hydrolyze inulin to fructose effectively [3, 24]. Ethanol fermentation using the recombinant *S. cerevisiae* from the raw extract of Jerusalem artichoke tubers containing 26 % total sugars has also been carried out in our previous studies [8]. The final ethanol concentration reached 102.1 g/L under the optimum conditions. However, using pure inulin or raw extract of Jerusalem artichoke tubers as substrate in previous studies is too costly and complicated, and has low feasibility of bioethanol industrial production.

In our present study, to develop cost-effective and sustainable technologies for industrial bioethanol production and increase the utilization rate of Jerusalem artichoke tubers, the dry powder of Jerusalem artichoke tubers was used directly to produce bioethanol by the recombinant *S. cerevisiae*. In addition, the fermentation medium containing the dry powder of Jerusalem artichoke tubers usually has a high viscosity, which is detrimental to high-gravity ethanol fermentation. Therefore, the optimum cooking pretreatment of medium before fermentation and the optimum fermentation conditions in flask based on fed-batch fermentation were investigated in this study. Then, scale-up test in 5-L agitating fermentor was carried out based on the optimum conditions of flask fermentation, which could provide a basis for our next pilot fermentation test in 50- and 500-L agitating fermentor for industrial production.

Materials and methods

Materials

Jerusalem artichoke was harvested in maturity from Dafeng in China at the end of November. The tubers were washed in running water and cut into pieces. Then, they were heated at 70 °C until reaching a constant weight in an oven and were milled to powder. The powder was stored at ambient temperature. The total sugar comprised about 62 % of dry powder according to the phenol–sulfuric acid method [6].

Yeast strains

The recombinant *S. cerevisiae* 6525 with the exo-inulinase gene from *Penicillium janthinellum* was already constructed in our laboratory [25, 26]. It is chose as the producer of ethanol from the dry powder of Jerusalem artichoke tubers in this study. The recombinant *S. cerevisiae* 6525 was found to be able to produce 1.1 U mL^{-1} inulinase activity within 72 h and 14.0 % (v/v) ethanol in fermentation medium containing inulin and 1 % (w/v) $(NH_4)_2SO_4$ in a 5-L fermentor [25].

Media

The strains were kept at 4 °C in medium YPD containing (in g/L) yeast extract, 10; peptone, dextrose, 20; and peptone, 20. The YPI medium which was used for exo-inulinase production by the recombinant *S. cerevisiae* 6525 contained (in g/L): yeast extract, 10; peptone, 20; and inulin, 20. The medium used for ethanol fermentation by the recombinant *S. cerevisiae* 6525 contained 240 g/L dry powder of Jerusalem artichoke tubers, the fermentation medium composition was varied based on the fermentation experiments. The pH value of the fermentation medium was between 5.3 and 5.6. For flask study, pH was adjusted to 5.5.

Preparation of the exo-inulinase produced by the recombinant *S. cerevisiae* 6525 carrying the inulinase gene

200 μl of recombinant *S. cerevisiae* 6525 inoculum was added to 100 mL YPI medium, cultured at 30 °C and 180 rpm for 72 h to produce enough exo-inulinase. The cell density was 1×10^8 cells/mL after counting the cells using a hemocytometer. The cell culture was used as both the exo-inulinase and ethanol producer.

Cooking pretreatment of fermentation medium

Fermentation medium was cooked at the optimum temperature before fermentation to decrease the medium viscosity and accelerate the release of inulin in the dry powder of Jerusalem artichoke tubers. 100 mL of fermentation medium containing 240 g/L dry powder of Jerusalem artichoke tubers in the 250-mL flask was heated at 30, 50, 70, 80, 90 °C in water rocker (150 r/min) for 30 min and heated at 115 °C for 20 s in high-pressure steam sterilization,

Table 1 Four kinds of fed-batch methods and the concentration of the dry powder of Jerusalem artichoke tubers in initial time, 24, 48 and 72 h

Fed-batch method	Initial time (g/L)	24 h (g/L)	48 h (g/L)	72 h (g/L)
① Fed-batched at 24 h	240	300	300	300
② Fed-batched at 24 and 48 h	240	280	300	300
③ Fed-batched at 24 and 48 h	240	270	300	300
④ Fed-batched at 24, 48 and 72 h	240	270	300	330

respectively. After cooling the medium to room temperature, 10 % (v/v) seed culture was inoculated into the 250-mL flask with the final volume of 100 mL fermentation medium. The flask was incubated at 30 °C and 180 rpm, for 96 h at anaerobic conditions.

Fed-batch fermentation for ethanol production

10 % (v/v) seed culture was inoculated into a 250-mL flask with 100 mL fermentation medium containing 240 g/L dry powder of Jerusalem artichoke tubers. The flask was incubated at 30 °C and 180 rpm, for 96 h at anaerobic conditions. As the fermentation proceeded, additional Jerusalem artichoke dry powder was supplemented. Four kinds of fed-batch methods are shown in Table 1: fed-batched at 24 h, the final concentration reached 300 g/L; fed-batched at 24 h, the concentration reached 280 g/L, then fed-batched at 48 h again, the final concentration reached 300 g/L; fed-batched in two equal amounts at 24 and 48 h, the final concentration reached 300 g/L; and fed-batched in three equal amounts at 24, 48 and 72 h, the final concentration reached 330 g/L. The final ethanol concentration and residual total sugar in the fermented media were determined as described below.

Ethanol production from the dry powder of Jerusalem artichoke tubers in 250-mL flask

10 g/L yeast extract, 10 g/L peptone, 10 g/L corn steep liquor, 10 g/L NH_4NO_3, and 10 g/L $NH_4H_2PO_4$ were added to the fermentation medium, respectively, and no nitrogen source was added to the control group in the experiment. 10 % (v/v) seed culture was inoculated into a 250-mL flask containing 100 mL fermentation medium. The flask was cultured at 30 °C and 180 rpm, for 96 h at anaerobic conditions. As fermentation proceeded, we fed-batched in two equal amounts at 24 and 48 h to make the final concentration of dry powder reach 300 g/L.

5, 10, 15 and 20 % (v/v) of seed culture were inoculated into the fermentation medium in a 250-mL flask with 100 mL of fermentation medium containing 240 g/L dry powder of Jerusalem artichoke tubers and 10 g/L yeast extract, respectively. The flask was cultured at 30 °C and 180 rpm, for 96 h at anaerobic conditions. As fermentation proceeded, we fed-batched in two equal amounts at 24 and 48 h to make the final concentration of dry powder reach 300 g/L.

The initial medium pH was adjusted at a unit of 0.5–4.0, 4.5, 5.0, 5.5 and 6.0, respectively. 10 % (v/v) seed culture was inoculated into a 250-mL flask with 100 mL fermentation medium containing 240 g/L dry powder of Jerusalem artichoke tubers and 10 g/L yeast extract. The flask was cultured at 30 °C and 180 rpm, for 96 h at anaerobic conditions. As fermentation proceeded, we fed-batched in two equal amounts at 24 and 48 h to make the final concentration of dry powder reach 300 g/L.

10 % (v/v) seed culture was inoculated into a 250-mL flask of fermentation medium (pH 4.5) with the bottle load of 75, 100, 125 and 150 mL (the final fermentation medium volume was 30, 40, 50 and 60 %), respectively. The flask was incubated at 30 °C and 180 rpm, for 96 h at anaerobic conditions. As fermentation proceeded, we fed-batched in two equal amounts at 24 and 48 h to make the final concentration of dry powder reach 300 g/L.

10 % (v/v) seed culture was inoculated into a 250-mL flask with 125 mL fermentation medium (pH 4.5) containing 240 g/L dry powder of Jerusalem artichoke tubers and 10 g/L yeast extract. The flask was incubated at 28, 30, 32, 34 and 36 °C, respectively, 180 rpm, for 96 h at anaerobic conditions. As fermentation proceeded, we fed-batched in two equal amounts at 24 and 48 h to make the final concentration of dry powder reach 300 g/L.

Scale-up test in 5-L agitating fermentor

After fermentation condition was optimized in flask, fermentation curves were investigated using 5-L agitating fermentor (i.d. 15.1 cm, height 28.0 cm, 3.0 L working volume, BIOTECH-5JG, Zhenjiang, China) under the optimum conditions. The initial medium (2.5 L) containing 240 g/L dry powder of Jerusalem artichoke tubers and 10 g/L yeast extract was used with the initial pH adjusted to 4.5. The fermentation medium was cooked at 115 °C for 20 s before being used. 10 % (v/v) seed culture was inoculated into the fermentation medium, and the culture was carried out at 34 °C and 180 rpm, for 96 h at anaerobic conditions. As fermentation proceeded, we fed-batched in two equal amounts at 24 and 48 h so as to make the final concentration of dry powder reach 300 g/L. The final ethanol concentration and residual total sugar in the fermented liquids were determined as described below.

Table 2 Analysis of the dry powder of Jerusalem artichoke tubers

	Dried weight (%)	Total sugar (%)	Reducing sugar (%)
The dry powder of Jerusalem artichoke tuber	100	61.9 ± 2.4	5.9 ± 1.2

Table 3 Effect of different cooking pretreatments on ethanol fermentation from the dry powder of Jerusalem artichoke tubers

Cooking method	Viscosity of medium (mPa s)	Total sugar after cooking (g/L)	Ethanol (g/L)
30 °C 30 min	1.78×10^5	125.17 ± 2.28	53.34 ± 4.04
50 °C 30 min	1.24×10^5	131.12 ± 2.63	56.00 ± 3.61
70 °C 30 min	1.55×10^4	134.05 ± 2.95	61.34 ± 3.51
80 °C 30 min	1.33×10^4	138.16 ± 2.09	63.00 ± 2.65
90 °C 30 min	1.29×10^4	139.02 ± 2.11	62.34 ± 1.53
115 °C 20 s	1.32×10^4	144.08 ± 2.05	66.34 ± 3.06

Analytical methods

Ethanol concentration was determined using a biosensor (SBA-40C) from Biology Institute of Shandong Academy of Science (Jinan, China). Viscosity was determined using a rotational viscosimeter (NDJ-1) from Shanghai Jinke Company (Shanghai, China). The total sugar was measured according to the phenol–sulfuric acid method [6]. Reducing sugar, mainly fructose in the fermentation liquids detected in our previous researches [25, 26], was quantified by the 3,5-dinitrosalicylic acid method [15].

Statistical analysis of ethanol concentration, total sugar and reducing sugar was processed by SPSS 20.0. The comparisons were tested after analysis of variance (one-way ANOVA) using Duncan's test. Data are presented as the mean ± S.E. for each treatment. Different letters indicate statistical difference at $p < 0.05$.

The ethanol yield in this study was evaluated by the conversion efficiency of inulin-type sugars to ethanol:

$$Y_p = P/(C \times 62\,\%)$$

where P was the total ethanol produced (g/L), C was the final concentration of the dry powder of Jerusalem artichoke tubers in medium (g/L), 62 % was the total sugar in the dry powder of Jerusalem artichoke tubers determined experimentally by the method described in the "Materials and methods" section. And the ethanol yield in the figure was expressed as the percentage of the theoretical value of 0.511.

Results and discussion

The most suitable cooking pretreatment of fermentation medium for ethanol production

Jerusalem artichoke is a non-food crop which grows extensively in many parts of China and the yield of its tuber is very high (5.4 ton/ha) [13]. The tuber of Jerusalem artichoke is rich in inulin which can be used as substrate for ethanol production. The previous studies have indicated that the extract of Jerusalem artichoke tubers or inulin can be fermented into high concentration of ethanol by the recombinant *S. cerevisiae* 6525 [8, 25]. However, the utilization rate of raw materials was low and the fermentation process was costly and complicated. To increase the utilization rate of Jerusalem artichoke tubers, this study used the dry powder of Jerusalem artichoke tuber directly to produce ethanol. After analysis of the dry powder of Jerusalem artichoke tubers used in this study, it can be observed from the results in Table 2 that the dry powder was containing 61.9 ± 2.4 % (w/w) of total sugar and 5.9 ± 1.2 % (w/w) of reducing sugar.

Since the fermentation liquor containing the dry powder of Jerusalem artichoke tubers has a high viscosity, cooking pretreatment of fermentation medium was carried out in this study before the process of fermentation. The results in Table 3 showed that the viscosity of medium in treatments heated at 70, 80, 90 °C for 30 min and 115 °C for 20 s was much lower than the other treatments. Although the viscosity of medium did not continue to decrease at the treatment cooked at 115 °C for 20 s, the sugar concentration dissolved in the medium was higher than the other treatments. As can be seen in Fig. 1, after 48 h of fermentation, the fermentation medium heated at 70, 80 and 90 °C, which could provide germicidal effect similar to pasteurization, had relatively higher ethanol yield than the treatments heated at 30 and 50 °C, but no significant difference of the ethanol yield was observed between them. And the highest ethanol yield of 87.2 % was achieved at the treatment cooked at 115 °C for 20 s. It was possible that the medium cooked at 115 °C for 20 s could improve the lixiviating effect and kill more other harmful microorganisms. In this way, the fermentation efficiency was improved under the sterile conditions. Thus, in this study, the fermentation medium was cooked at 115 °C for 20 s before fermentation.

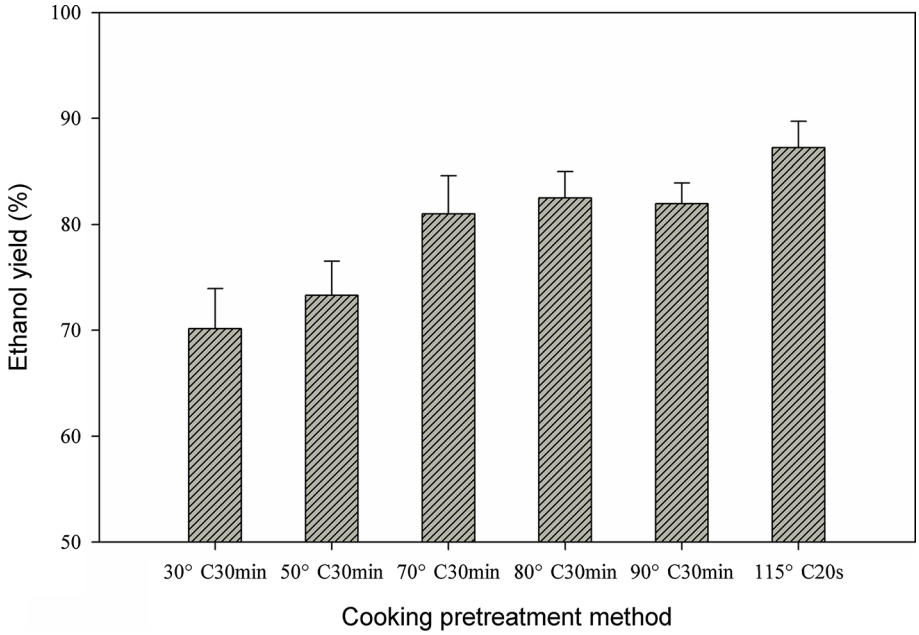

Fig. 1 Effect of different cooking pretreatments on ethanol fermentation from the dry powder of Jerusalem artichoke tubers. Ethanol yield was expressed as the percentage of the theoretical value of 0.511

The most suitable fed-batch method for ethanol fermentation

Zhang et al. [27] used the *Saccharomyces* sp. W0 to produce ethanol from the hydrolysate of inulin, and fed-batched inulin at 48 and 96 h. Under this condition, 14.6 mL ethanol in 100 mL fermented medium was produced within 120 h of fermentation. In our study, as fermentation proceeded, we fed-batched the dry powder of Jerusalem artichoke tubers to keep a suitable concentration of total sugar in the medium. The results in Fig. 2 showed that the method 3 with fed-batched in two equal amounts at 24 and 48 h was the most suitable condition for ethanol production by the recombinant *S. cerevisiae* 6525. Under the condition, ethanol concentration increased continuously within 72 h of the fermentation and then decreased. The highest ethanol concentration of 79.0 g/L was achieved at 72 h and 90.8 % of total sugar was used. As can be seen in Table 4, the ethanol yield of method 3 was 83.12 %, which was the highest one than the other three methods. In accordance with our observations, supplement could dissolve in fermentation liquor quickly and the treatments with fed-batched in two equal amounts at 24 and 48 h could keep a relatively low viscosity. This demonstrates that the dry powder of Jerusalem artichoke tubers can also be fermented into high concentration of ethanol by the recombinant *S. cerevisiae* 6525 in fed-batch fermentation. The treatment with fed-batched in three equal amounts at 24, 48 and 72 h achieved the highest final dry powder concentration of 330 g/L and ethanol concentration of 83.67 g/L at 84 h, but the ethanol yield was only 79.87 %, which was lower than the treatment with method 3. It may be possible that in the fermentation anaphase,

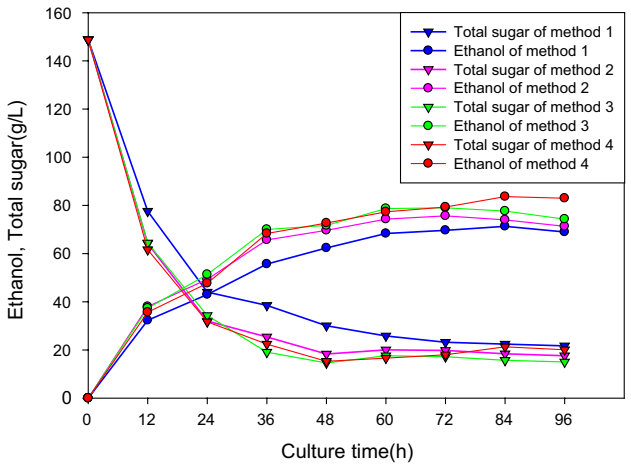

Fig. 2 Time course of fed-batch fermentation for ethanol production from the dry powder of Jerusalem artichoke tubers at different fed-batch methods

some metabolites had a protective effect on ethanol production. The method with more than two times fed-batched would be left behind for further research.

Effect of different nitrogen sources on ethanol production

After different nitrogen sources were added to the fermentation medium, the recombinant *S. cerevisiae* 6525 was inoculated into the fermentation medium in the 250-mL flask. It can be observed from the results in Fig. 3a that when 10 g/L yeast extract was added to the fermentation medium, the highest ethanol yield of 83.12 % was achieved, which was higher than the other treatments. In

Table 4 Effect of four kinds of fed-batch methods on ethanol fermentation from the dry powder of Jerusalem artichoke tubers

Fed-batch method	Theoretical content of total sugar (g/L)	Ethanol (g/L)	Ethanol yield (%)
Method 1	186	71.34	75.06
Method 2	186	75.67	79.61
Method 3	186	79.00	83.12
Method 4	205	83.67	79.87

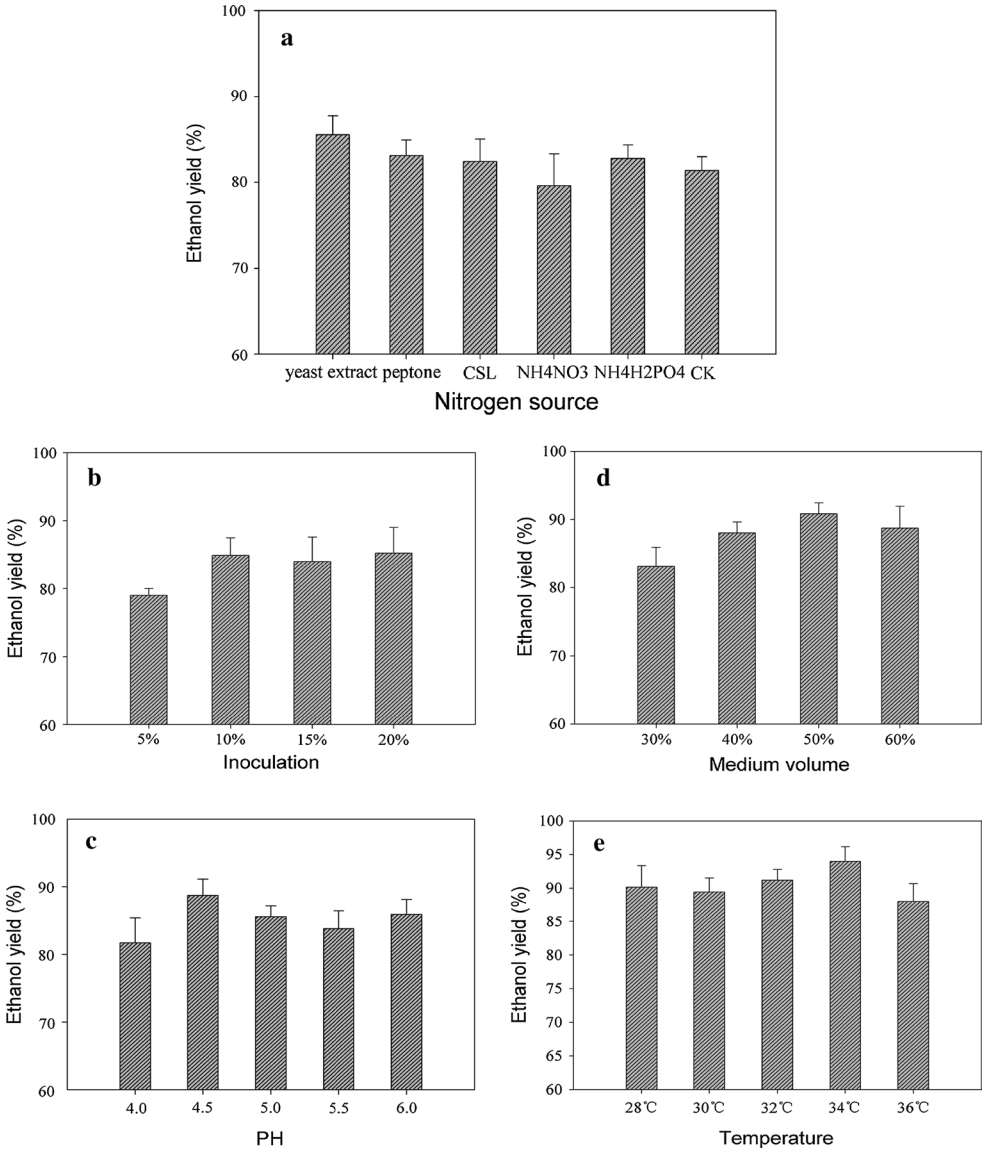

Fig. 3 Effect of nitrogen source, inoculation, initial medium pH, medium volume and temperature on ethanol production in fed-batch fermentation from the dry powder of Jerusalem artichoke. Ethanol yield was expressed as the percentage of the theoretical value of 0.511. **a** Nitrogen source, **b** inoculation, **c** initial medium pH, **d** medium volume, **e** temperature

addition, $NH_4H_2PO_4$, as inorganic nitrogen, was much cheaper than other organic nitrogen for ethanol industrial production, which had a good prospect of utilization and will be studied in further research. Finally, for flask study, we chose yeast extract as nitrogen source in the experiment.

Effect of inoculation on ethanol production

It can be seen from the data in Fig. 3b that there was no significant difference for the ethanol yield at the inoculation from 10 to 20 % after 72 h of fermentation (data not shown), and the ethanol yield of the treatment with 5 % inoculum was lower than the other treatments. Therefore, the inoculation number we chosen in this study was 10 %.

Effect of initial medium pH on ethanol production

The results in Fig. 3c indicated that the ethanol concentration in the medium was the highest while the initial medium pH value was 4.5. Under the conditions, 84.33 g/L

ethanol was achieved after 72 h of fermentation, and the ethanol yield was over 88.73 %. Much lower ethanol yield was obtained at the pH values of 4.0, and no significant difference for the ethanol yield was found at the pH values of 5.0, 5.5 and 6.0. This means that the initial medium pH value of 4.5 was the most suitable pH for ethanol production by the recombinant *S. cerevisiae* 6525 and was also the optimal pH of the recombinant exo-inulinase, which is consistent with the results in Wang et al. [26]. However, the reason for this phenomenon still needs to be further studied from the impact of the medium pH on the growth of recombinant *S. cerevisiae* 6525, different processes of respiration and exo-inulinase production, especially the formation of some by-products in the process of fermentation.

Effect of medium volume on ethanol production

As can be seen in Fig. 3d, treatments with medium volume of 30, 40, 50, 60 % produced 79.0 ± 2.4, 83.7 ± 1.5, 86.3 ± 1.5 and 84.3 ± 3.1 g/L ethanol after 72 h of fermentation, respectively. The highest ethanol yield of 90.83 % was achieved at the treatment with medium volume of 50 %. It indicated that excess oxygen supply was detrimental to ethanol production by the recombinant *S. cerevisiae* 6525. Thus, the most suitable medium volume we temporarily chose would be 50 % in this study.

Effect of temperature on ethanol production

The flask was incubated at 28, 30, 32, 34 and 36 °C, respectively. It was found that when the cultured temperature was 34 °C, the ethanol concentration in the fermentation medium reached the highest. It can also be seen from the result in Fig. 3e that the most suitable cultured temperature for ethanol production by the recombinant *S. cerevisiae* 6525 was 34 °C, at which the highest ethanol yield of 93.98 % was obtained. The reason for this phenomenon may be related to the inulinase activity, the growth of recombinant *S. cerevisiae* 6525 and the solubility of the dry powder of Jerusalem artichoke tubers, which needs further studies.

Scale-up test in 5-L agitating fermentor

In the previous studies [18], the flour of Jerusalem artichoke tubers was used to produce ethanol by *A. niger* 817 and *S. cerevisiae* 1200 in simultaneous saccharification and fermentation, and 20.1 % of ethanol was obtained within 120 h of fermentation after complementing flour at 15 and 24 h. However, the process of fermentation was complicated and time consuming, and the utilization rate of sugar was only 80 %. In our study, after fermentation condition was optimized in flask, dry powder of Jerusalem artichoke tubers was used for ethanol production by the recombinant *S. cerevisiae* 6525 in 5-L agitating fermentor as described in "Materials and methods". The results in Fig. 4 showed that the process of ethanol production proceeded very rapidly in the first 36 h and the ethanol concentration still increased slowly from 36 to 60 h with fed-batched in two equal amounts at 24 and 48 h. The highest ethanol concentration of 84.3 g/L was obtained at 72 h, and the conversion efficiency of inulin-type sugars to ethanol was 0.453, or 88.6 % of the theoretical value of 0.511 [12]. The total sugar in the fermentation liquids was decreased continuously after the start of the fermentation, while the reducing sugar in the fermentation liquids was increased slightly within 12 h of fermentation and then still kept a relatively lower concentration until 60 h of fermentation, which helped prevent substrate inhibition. After that, the reducing sugar was decreased until the end of fermentation. At the end of the fermentation, only 14.6 g/L total sugar and 5.5 g/L reducing sugar were remained in the fermentation liquids.

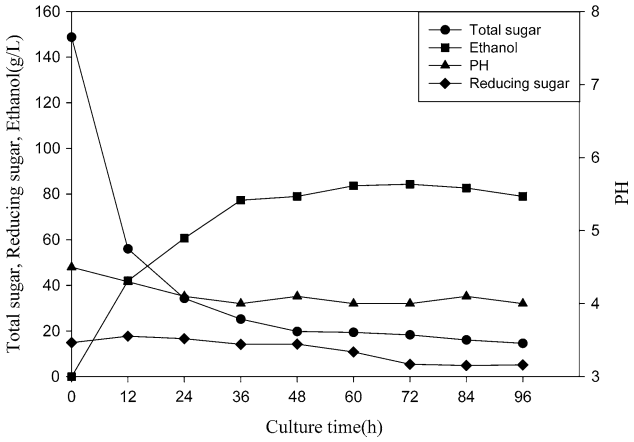

Fig. 4 Time course of fed-batch fermentation for ethanol production from the dry powder of Jerusalem artichoke tubers with recombinant *S. cerevisiae* 6525 in 5-L agitating fermentor

At present, as the dry powder of Jerusalem artichoke tubers is rich in inulin and has a much lower processing cost for industrial production, more and more researchers are interested in using the dry powder of Jerusalem artichoke tubers as substrate for ethanol production. Zhang et al. [27] used the tuber meal of Jerusalem artichoke [the concentration of the tuber meal in the fermentation medium was 50 % (w/v)] to produce ethanol by *Saccharomyces* sp. W0 in 5-L agitating fermentor, 12.05 mL ethanol in 100 mL fermented medium was obtained within 144 h, and the ethanol productivity was 0.319 ± 0.9 g of ethanol/g of sugar. However, because the fermentation medium containing the dry powder of Jerusalem artichoke tubers usually has a high viscosity, the utilization rate of

the sugar included in dry power is too low and the process of fermentation is time consuming, which makes it difficult to use this medium for high-gravity ethanol fermentation. In our study, the recombinant *S. cerevisiae* 6525 was first used to produce ethanol from the dry powder of Jerusalem artichoke tubers. The result indicated that proper cooking pretreatment and fed-batch fermentation could help solve the problem of high viscosity of the fermentation medium containing Jerusalem artichoke dry powder and had a better performance for high-gravity ethanol fermentation. In addition, the results in Fig. 4 also showed that the Jerusalem artichoke dry powder could be used efficiently and fermented to high concentration of ethanol within a relatively short time by the recombinant *S. cerevisiae* 6525 in fed-batch SSF. Therefore, this shows that using the dry powder of Jerusalem artichoke tubers as substrate for ethanol production by the recombinant *S. cerevisiae* 6525 has high feasibility in industrial production.

Conclusions

After the optimum processing conditions for ethanol production in fed-batch fermentation were determined in flask. The dry powder of Jerusalem artichoke tubers was fermented into ethanol by recombinant *S. cerevisiae* 6525 in 5-L agitating fermentor, about 84.3 g/L ethanol was obtained within 72 h of fermentation, and the conversion efficiency of inulin-type sugars to ethanol was 0.453, or 88.6 % of the theoretical value. Therefore, this study provided a basis for our next fermentation test in 50- and 500-L fermentor and showed high feasibility of bioethanol industrial production from the dry powder of Jerusalem artichoke tubers.

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

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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