Bioethanol production from sweet sorghum stalk juice with immobilized yeast

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Abstract

The experiments of ethanol fermentation from sweet sorghum stalk juice with immobilized yeast at different conditions, including temperature, pH, particles stuffing rate and initial substrate concentration, were carried out in 250ml shaking flasks to obtain suitable parameters by analyzing the values of ethanol formation rate (rp/s) and ethanol-sugar conversion ratio (Yp/s), respectively. The results indicated that fermentation temperature of 33°C, pH of 4.5, particles stuffing rate of 25% and the initial sugar concentration of 218.1 mg·ml⁻¹ could be selected. The results of verification experiments in 250ml shaking flasks with the corresponding conditions showed that the value of rp/s and Yp/s were 0.0486 g·g⁻¹·h⁻¹ and 0.4005 g·g⁻¹, respectively. In addition, the results of rp/s and Yp/s in the further verification experiments of 1L shaking flasks were 0.0465 g·g⁻¹·h⁻¹ and 0.4248 g·g⁻¹, respectively, which could be deduced that the selected conditions were suitable and reliable for ethanol production from sweet sorghum using immobilized yeast.

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Keywords: Sweet sorghum; Immobilized yeast; Ethanol fermentation; Suitable conditions;

1. Introduction

Due to the diminishing fossil fuel reserves, alternative energy sources needs to be renewable, sustainable, efficient, cost-effective, convenient and safe [1,2]. In this context, biomass energy has emerged as one of the most attractive and promising energy carried to fossil fuels [3]. Ethanol, both renewable and environmentally friendly, is believed to be one of the best alternatives [4], especially the ethanol refined from biomass materials. As far as the biomass materials are concerned, sweet sorghum (Sorghum bicolor {L.} Moench) is a C₄ plant characterized by a high photosynthetic efficiency, high biomass- and sugar-yield, genetic diversity and climatic adaptation, and, hence, it has been considered as an important source for the ethanol production [5-8]. Overall, out of many “new crops” that are investigated as potential raw materials for energy and industry, sweet sorghum seems to be the most promising one [9,10].

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As for the conversion technologies, fermentative processes stand out, where microbial metabolism is used for the transformation of simple sugars (sucrose, glucose, and fructose, etc.) in raw materials into ethanol [11]. The traditional conversion method was ethanol fermentation with free yeast \((S.\ cerevisiae)\), which has higher yeast cost, and lower production efficiency, and it will be gradually replaced by immobilized yeast. Immobilized microbial cell system offers advantages over free cell system in terms of ethanol productivity and stability of cell activity [12], because cell washout in continuous operation is prevented, and, hence, cell separation and/or recycle are not required for maintaining high cell density in the bioreactor [13].

Many factors have influence upon the ethanol fermentation process, such as fermentation temperature, pH, initial sugar concentration (ISC) and particles stuffing rate (PSR) that is defined as a ratio of immobilized yeast particles weight to fermentation solution weight. The immobilization process changes the environmental, physiological and morphological characteristics of cells, along with the catalytic activity [14]. Hence, the fermentation conditions are different between free yeast and immobilized yeast fermentation.

The aims of the current work were to investigate the effect of main variables (fermentation temperature, pH, PSR and ISC) on \(r_p/s\) and \(Y_p/s\) that could stand for fermentation rate and ethanol-sugar conversion ratio, respectively, and to determine suitable conditions for ethanol fermentation by immobilized yeast from stalk juice of sweet sorghum.

2. Materials and methods

2.1 Sweet sorghum and organisms

Ethanol-Sweet No.2 sweet sorghum cultivar was cultivated in the farm of Shanghai Jiao Tong University. Laboratory strain of \(S.\ cerevisiae\) CICC 1308 (obtained from Centre of Industrial Culture Collection of China) was used.

2.2 Culture media and microorganism culture

The composition of culture media is shown in Table 1. The \(S.\ cerevisiae\) was inoculated according to the protocols in reference [8].

| Chemicals (%) | Solid medium | Liquid medium | Fermentation medium |
|---------------|--------------|---------------|---------------------|
| Glucose       | 5.0          | 5.0           | 10.0                |
| Yeast extract | 0.5          | 0.5           | 0.5                 |
| Peptone       | 0.5          | 0.5           | 0.5                 |
| \(\text{K}_2\text{HPO}_4\) | 0.1 | 0.1 | 0.1 |
| \(\text{MgSO}_4\cdot7\text{H}_2\text{O}\) | 0.1 | 0.1 | 0.1 |
| Agar          | 2.0          | –             | –                   |

2.3 Yeast cells immobilization and proliferation

Na-alginate powder was used for immobilization. The detailed protocol of immobilization and proliferation was based on the reference [15].
2.4 Fermentation

The single-factor experimental design was listed in the Table 2. (NH₄)₂SO₄ of 0.2%, K₂HPO₄ of 0.125% and MgSO₄ of 0.05% were added into the sterilized stalk juice[15]. The fermentation were carried out in the 250ml and 1L shaking flasks (for verification).

Table 2 The single-factor experimental design

| Factors         | Levels          |
|-----------------|-----------------|
| Temp. (℃)       | 24 27 30 33 36 -|
| pH              | 3.0 3.5 4.0 4.5 5.0 5.5 |
| PSR(%)          | 15 20 25 30 35 - |
| ISC(mg·ml⁻¹)    | 111.8 174.9 218.1 268.4 320.9 - |

2.5 Analysis

The total soluble sugar concentration of stalk juice was determined by 3,5-dinitrosalicylic acid (DNS) method [16]. Ethanol concentration in fermentation mash was measured by an alcoholimeter and the measured results were adjusted to 20℃ ethanol concentration [17]. The pH of juice were determined with a pH meter.

2.6 Definition

In the study, r_p/s and Y_p/s were applied for the judgment of suitable parameters at different conditions, and the equation definition of them were listed at follows:

\[
r_{p/s} = \frac{1}{C_{s,initial}} \frac{C_{eth,final} - C_{eth,initial}}{t_{total}}
\]

\[
Y_{p/s} = -\frac{C_{eth,final} - C_{eth,initial}}{C_{s,final} - C_{s,initial}}
\]

Where \(r_{p/s}\) is the fermentation rate (g·g⁻¹·h⁻¹); \(Y_{p/s}\) is the ethanol-sugar conversion ratio (g·g⁻¹); \(C_{eth,final}\) is the final ethanol concentration (g·L⁻¹); \(C_{eth,initial}\) is the initial ethanol concentration (g·L⁻¹); \(C_{s,initial}\) is the ISC of sweet sorghum juice (g·L⁻¹); \(t_{total}\) is the total fermentation time (h);

3. Results and Discussion

3.1 Ethanol fermentation at different temperatures

Batch fermentation experiments in the 250ml shaking flasks for ethanol production were carried out in duplicate with ISC of 109.1 mg·ml⁻¹. Fermentation temperature was maintained constant at 24℃, 27℃, 30℃, 33℃, 36℃ with water bath. The agitation rate, pH and PSR were controlled at 150 r·min⁻¹, 5.0 and 20%, respectively. As shown in Fig.1, the value of \(Y_{p/s}\) was increased markedly with the increase of fermentation temperature from 24℃ to 33℃, however it decreased from 33℃ to 36℃. As far as the values of \(r_{p/s}\) were concerned, the same trend was appeared with the fermentation temperature changed...
from 24°C to 36°C. The maximum values of Y_{ps} and r_{ps} were achieved at 33°C, which were 0.3970 g·g^{-1} and 0.0496 g·g^{-1}·h^{-1}, respectively. According to the results of Y_{ps} and r_{ps} at different temperatures, it could be deduced that the suitable temperature for ethanol fermentation by immobilized yeast could be determined as 33°C. Generally speaking, the ethanol formation in the fermentation processes is dependent on fermentation temperature, and an increase in fermentation temperature results in an increased concentration of total ethanol \[18,19\]. But too higher temperature will restrain the growth and metabolism of the yeast cells since the activity of enzyme in yeast cell was decreased at higher temperature \[20\], which will decrease the ethanol yield. Moreover, the fermentation rate of yeast is enhanced at higher temperature, but the lifetime of yeast is decreased evidently, and too high fermentation temperatures cease fermentation \[21,22\]. As a result, a suitable temperature of 33°C should be selected for immobilized \emph{S. cerevisiae} fermentation.

![Figure 1](image1.png)

**Figure 1** Y_{ps} and r_{ps} at different temperatures

### 3.2 Ethanol fermentation at different PHS

The pH of the juice was adjusted to 3.0, 3.5, 4.0, 4.5, 5.0 and 5.5 by 1 M HCl and 1 M NaOH, and temperature, agitation rate and PSR were kept constant at 30°C, 150 r·min^{-1} and 25%, respectively. The ISC of the juice was 109.1 mg·ml^{-1}. As shown in Fig. 2, both the values of Y_{ps} and r_{ps} were evidently increased when pH was increased from 3.0 to 4.5, and decreased notably when pH was increased from 4.5

![Figure 2](image2.png)

**Figure 2** Y_{ps} and r_{ps} at different pHs
to 5.5. The juice pH of 5.5 was seriously negative for ethanol fermentation processes, resulting in lower ethanol production and fermentation rate. The maximum values of $Y_{ps}$ and $r_{ps}$ were 0.3783 g·g$^{-1}$ and 0.0454 g·g$^{-1}$·h$^{-1}$, respectively, at pH of 4.5. The pH has also been described as a factor that strongly interferes in the fermentative processes [11]. Overall, the lower pH in fermentation medium will inhibit the yeast cells growth and nutrition materials exchange between the cells and medium. And the higher pH will enhance microbial contamination [12,20]. Both of them will decrease the ethanol yield, consequently. Therefore, based on the results, there is a suitable pH for ethanol fermentation using immobilized $S. cerevisiae$, which should be 4.5. At this condition, a higher ethanol-sugar conversion ratio and fermentation rate would be obtained.

3.3 Ethanol fermentation at different PSRs

The PSR of immobilized $S. cerevisiae$ was chosen at 15%, 20%, 25%, 30% and 35%. Specified temperature, pH and agitation rate were 30$^\circ$C, 5.0 and 150 r·min$^{-1}$, respectively. The ISC of the juice used for fermentation was 118.1 mg·ml$^{-1}$. According to Fig.3, the $Y_{ps}$ and $r_{ps}$ were both increased with the increase of PSR before 25%, and sharply decreased from 25% within the designed PSR range of 15%-35%. The maximum values of $Y_{ps}$ and $r_{ps}$ were up to 0.3805 g·g$^{-1}$ and 0.0476 g·g$^{-1}$·h$^{-1}$, respectively, at PSR of 25%. The probably reason was that the growth and metabolism of yeast cells would be restricted when too high immobilized yeast PSR is offered in the ethanol fermentation because the nutrition material is not infinite. On the contrary, when the PSR is in a lower value, the quantity of immobilized yeast used for fermentation would be in fall. Hence, fermentation efficiency would be decreased at a lower particles stuffing rate. Thus, the fermentation rate would be boosted with the increased PSR from 15% to 25%, but the too high PSR in a certain concentration fermentation solution would result in a large part of sugar in the juice being consumed for the yeast growth, which led to the decrease of ethanol-sugar conversion ratio. As a result, a suitable PSR could keep the yeast cells in the particles with robust metabolism, which would enhance the fermentation rate and ethanol production. Therefore, PSR of 25% would be a suitable one for ethanol fermentation by immobilized $S. cerevisiae$.

![Figure 3 $Y_{ps}$ and $r_{ps}$ at different particles stuffing rates](image)

3.4 Ethanol fermentation at different ISCs

The juice of sweet sorghum was condensed with a rotary evaporator into different concentrations before the experiments were performed. ISC of the condensed juice were 111.8 mg·ml$^{-1}$, 174.9 mg·ml$^{-1}$, 218.1 mg·ml$^{-1}$, 268.4 mg·ml$^{-1}$, and 320.9 mg·ml$^{-1}$, respectively. The other fermentation conditions, such as
temperature, pH, PSR and agitation rate were fixed at 30°C, 5.0, 20%, and 150r·min⁻¹, respectively. As shown in Fig.4, the values of $Y_{ps}$ were increased, when the ISC increased from 111.8 mg·ml⁻¹ to 218.1 mg·ml⁻¹, and decreased from 218.1 mg·ml⁻¹ to 320.9 mg·ml⁻¹. As for the value of $r_{ps}$ at different ISCs, it was increased from 111.8 mg·ml⁻¹ to 218.1 mg·ml⁻¹, and decreased from 218.1 mg·ml⁻¹ to 320.9 mg·ml⁻¹. The maximum values of $Y_{ps}$ and $r_{ps}$ reached at 0.3501 g·g⁻¹ and 0.0491 g·g⁻¹·h⁻¹, respectively, at ISC of 218.1 mg·ml⁻¹. When the ISC was lower, the sugar and the produced ethanol in fermentation mash has no or little inhibition on yeast fermentation processes. Meanwhile, based on the Michaelis-Menten equation, the fermentation rate will be increased, when the ISC was increased within a certain range [23, 24]. The decrease in ethanol production at high sugar concentration occurred due to an increase in the osmotic pressure that is one of the main factors for the cells dewater resulting in plasmolysis, and the death of yeast, consequently [11]. In addition, the produced ethanol could not be removed on time, which will have toxic effect on the yeast cells resulting in the decrease of fermentation rate [25]. Hence, based on the inhibitory effect of high initial substrate and produced ethanol concentration, the ISC should be selected at 218.1 mg·ml⁻¹ for immobilized yeast ethanol fermentation.

Finally, the suitable conditions for immobilized *S. cerevisiae* ethanol fermentation of stalk juice of sweet sorghum should be selected as temperature of 33°C, pH of 4.5, PSR of 25%, and ISC of 218.1 mg·ml⁻¹.

3.5 The verification experiments in 250 ml and 1L shaking flasks

The verification experiments in 250 ml shaking flasks with the corresponding conditions were carried out in triplicate. The results indicated that the total fermentation time was 9 h. And the average values of $Y_{ps}$ and $r_{ps}$ were achieved 0.4005 g·g⁻¹ and 0.0486 g·g⁻¹·h⁻¹, respectively. Both of which were higher than those of signal-factor experiments. Additional experiments with the selected conditions were also performed in 1L shaking flasks in triplicate. The results of ethanol fermentation in 1L shaking flasks for verification is shown in Fig.5. It showed that the ISC of the juice was 215.9 mg·ml⁻¹. The final ethanol concentration, and the residual sugar concentration were 90.34 mg·ml⁻¹, and 3.30 mg·ml⁻¹ at the end of fermentation of 9 h, respectively. The obtained average values of $Y_{ps}$ and $r_{ps}$ were 0.4248 g·g⁻¹ and 0.0465 g·g⁻¹·h⁻¹, respectively, which were also higher than those of single-factor experiments. According to the results of verification experiments in 250ml and 1L shaking flasks, the selected conditions were reasonable, reliable, and suitable for ethanol fermentation of stalk juice of sweet sorghum with immobilized *S. cerevisiae*. To sum up, the suitable conditions for immobilized *S. cerevisiae* ethanol
fermentation of stalk juice of sweet sorghum could be finally determined as: the temperature 33°C, pH 4.5, PSR 25% and ISC 218.1 mg·ml⁻¹.

Figure 5 The verification results in 1L shaking flasks

4. Conclusions

In order to attain a higher yield of ethanol and fermentation rate, main conditions of ethanol fermentation by immobilized *S. cerevisiae* including temperature, pH, particles stuffing rate, and initial sugar concentration were investigated. According to the results of shaking flasks, the suitable conditions were determined as temperature of 33°C, pH of 4.5, particles stuffing rate of 25%, and initial sugar concentration of 218.1 mg·ml⁻¹. The suitable conditions for immobilized *S. cerevisiae* technology could be beneficial for application in ethanol production from stalk juice of sweet sorghum in order to enhance ethanol yield, shorten the ethanol fermentation interval, and decrease the production cost.

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