Repeated-batch Ethanol Fermentation of Kitchen Refuse by Acid-tolerant Flocculating Yeast Under the Non-sterilized Condition

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The ethanol production performance of the flocculating yeast S. cerevisiae ATCC26602 from non-sterilized kitchen refuse medium in repeated-batch culturing was investigated. Although no pH modification or asepsis technology was applied, no contamination occurred. Twenty cycles of repeated-batch fermentation over a period of 11 days were successfully carried out without any loss of productivity by the self-flocculating strain ATCC26602. In addition, from the third batch, the flocculent strain achieved the reduction of the fermentation time to half, which resulted in an increasing of ethanol productivity to 3.7 g/L/h. These results of ethanol fermentation from kitchen refuse under the non-sterilized condition by this acid-tolerant and self-flocculation yeast may pave the way to low-cost ethanol production in pilot applications.

Key words: kitchen refuse, repeated-batch ethanol fermentation, Saccharomyces cerevisiae, self-flocculation, non-sterilization

1. Introduction

Although the fermentative process for ethanol production has been well researched for several decades, the selection of cheap substrates which do not compete with food production is still the key issue for global application. Various agricultural products containing carbohydrates have been applied as substrates for ethanol fermentation, such as cellulosic materials (straw, bagasse, waste paper), starch-containing materials (corn, wheat, rice), sugar cane and sugar beet [1-4]. However, the emerging demand for ethanol as fuel has caused a price rise of food-oriented agricultural products. Moreover, additional operations, e.g. external nutrient addition, pH control and sterilization of the medium, centrifugation or immobilization of cells when using non-flocculating strains, and so on, are also factors which raise the cost of capital investment and processing energy in industrial-scale ethanol fermentation. To solve these problems, more and more researchers are focusing on producing ethanol from waste biomass, which may reduce the dependence on agricultural products; research is also being conducted into ways of reducing the cost of ethanol production by employing new and more efficient fermentation systems [5,6].

Kitchen refuse has drawn great attention in Japan as an effective and low-cost fermentation substrate in recent years [7,8]. Efficient utilization of kitchen refuse can bring special advantages from both the economic and the environmental viewpoints. In our previous study, we have examined batch ethanol fermentation from kitchen refuse by four ATCC strains of S. cerevisiae in flask culturing [9]. The major fermentation parameters, e.g. the glucose concentration in the medium, the initial pH value and nitrogen supplementation, were discussed. The following conclusions were reached: (1) the optimal glucose concentration in the fermentation medium was around 120 g/L; (2) the nutritional content of the kitchen refuse was sufficient for ethanol fermentation; (3) no contamination occurred during the fermentation process because of the naturally acidic pH of the kitchen refuse medium, which means that sterilization was unnecessary; (4) ATCC 26602 showed a higher and more stable ethanol production capability than the three other strains under the same fermentation conditions. The initial acidity of the kitchen refuse medium had little impact on the growth and ethanol fermentation
capability of ATCC 26602, due to this strain’s acid tolerance. In addition, it is a self-flocculation yeast strain, so S. cerevisiae ATCC26602 was proposed as the best strain for use in ethanol production from kitchen refuse medium.

In this study, the main objective was to evaluate the feasibility of repeated-batch ethanol fermentation from kitchen refuse medium. In addition, in order to realize industrial-scale ethanol production via the fermentation of kitchen refuse in the future, we tried to find out effective ways to reduce the cost and improve the productivity. Two aspects were focused on. The first was repeated-batch fermentation of kitchen refuse medium under conditions as natural as possible, i.e. no pH adjustment or sterilization. The second was the selection of the best microorganism for repeated-batch fermentation. S. cerevisiae ATCC26602 was chosen for its merits, mentioned above. In particular, since the adoption of self-flocculating yeast for ethanol fermentation has been well documented, it is expected to bring the advantage of its flocculent characteristics to repeated-batch fermentation from kitchen refuse medium as well [10,11].

2. Materials and Methods

2.1 Preparation and saccharification of the kitchen refuse

Food waste was provided by the local supermarket (Wakamatsu, Kitakyushu and Fukuoka, Japan). About 70 kg of kitchen refuse were collected in spring in the Kyushu area. We separated it into 3 groups by hand: the carbohydrate group (i.e. rice, bread, pasta, noodles, etc.), the protein group (i.e. fish, beef, pork, chicken, etc.) and the vegetable/fruit group. Thereafter, the kitchen refuse was again mixed in the following proportions: 43%(w/w) carbohydrates, 19%(w/w) protein and 38%(w/w) vegetables and fruits. The subsequent saccharification and pretreatment for medium preparation were done as previously described [9]. The saccharified solution was called “kitchen refuse medium,” and was adjusted to various concentrations for inoculum preparation and fermentation.

2.2 Strains

S. cerevisiae ATCC26602 was purchased from the American Type Culture Collection (Rockville, MD). The yeast cells were maintained at 4°C on YM agar slants containing yeast extract 3 g/L, malt extract 3 g/L, peptone 5 g/L, glucose 10 g/L and agar 20 g/L, with fortnightly sub-culturing until usage.

2.3 Inoculum preparation

One loopful of S. cerevisiae from the YM agar slants was transferred to a 300-ml Erlenmeyer flask containing 80 ml of sterilized kitchen refuse medium at a glucose concentration of 30 to 50 g/L at natural pH. In order to stimulate the growth of the yeast strain, 2 g/L of yeast extract and 1 g/L of peptone were also added. The Erlenmeyer flask was sealed with a cotton wool plug. The culture was incubated at 30°C on an orbital incubator with shaking at 100 rpm for 20 h.

2.4 Repeated-batch fermentation in a jar bioreactor

Repeated-batch fermentation was first carried out using S. cerevisiae ATCC26602. The incubations were conducted in a 1-L jar bioreactor; 10%(v/v) inoculum broth was added, with a final working volume of 800 ml. The reactor was maintained at 30°C, with agitation at 100 rpm for 24 h. An air-flow rate of 0.1 vvm was supplied for the first 8 h to stimulate yeast growth. The pH of the culture was recorded constantly but not regulated. The CO2 evolution rate was used as an indicator of the progress and completion of fermentation. When the CO2 evolution rate slowed down obviously, the agitation was stopped and the culture was allowed to stand for 20 min in order to sediment the suspended yeast. Seventy-five percent of the fermented broth was drained off via an internal tube and the fermentor was refilled with fresh medium, after which the next fermentation cycle was started. This procedure was continued for 20 cycles in this study.

The kitchen refuse medium used was not sterilized, its initial pH value was around 4.0, and its glucose concentration was around 120 g/L.

2.5 Analysis method

Samples for analysis were drawn at various times. The dry cell mass was determined by centrifuging the broth, followed by washing and drying of the cell pellet in a 105°C oven for 24 h. The viable cells were counted after incubation on YMA at 30°C for 48 h. Ethanol was assayed by the internal standard method (with isopropanol) using a gas chromatograph (Model GC-17A, Shimadzu) with BPX capillary columns and a flame ionization detector. The measurement of the glucose and lactic acid concentration was conducted as previously described [7].
3. Results and Discussion

The ethanol production performance of the self-flocculating yeast \textit{S. cerevisiae} ATCC26602 from non-sterilized kitchen refuse medium in repeated-batch culturing was investigated. Figure 1(a) shows the repeated-batch kinetic profile of the flocculating yeast in the non-sterilized kitchen refuse medium. A total of 20 cycles were completed, and the repeated-batch culturing was successful. The glucose consumption and ethanol production were stable during the fermentation period. The final ethanol concentration of each batch varied from 55 to 61 g/L over a total of 20 runs. The fermentation time was reduced from 24 h in the first batch to 12 h in the third batch and remained at that duration up to the last run, while the ethanol productivity increased from 2.33 g/L/h in the first batch to 3.64 g/L/h in the third batch, paralleling the decrease in fermentation time. The average fermentation yield was 0.49 g/g, corresponding to 96% of the theoretical maximum. The high flocculating activity of the cells was maintained well during the whole fermentation process, which was reflected by the biomass concentration and settling time. At the end of each batch cycle, the flocs settled rapidly and the settling time became almost constant from the third batch onward. Meanwhile, as shown in Fig. 1(b), the increase in biomass concentration from the forth run onward remained at 1.81 g/L per cycle on average. The cell recovery by sedimentation was about 95%.

Although no pH control and sterilization were implemented, contamination did not occur during the fermentation process, according to the data of the pH monitor and the concentration of lactic acid. The natural pH of the kitchen refuse medium was around 4.0. During the fermentation process, the pH was checked before and after each cycle. Figure 1(c) shows the pH variation during the fermentation. No obvious fluctuations, except slight changes of 0.1 and 0.2 units, were observed. On the other hand, the lactic acid concentration remained between 2000 and 3000 mg/L, which is close to the initial concentration in the medium, indicating that the growth of bacteria, especially \textit{Lactobacillus} species, was effectively repressed by the natural acidity of the kitchen refuse medium throughout the experimental period [5].

In practical industrial ethanol production, cost reduction is very important. The advantage of using the self-flocculating yeast strain ATCC26602 was proven. Because of the natural acidity of kitchen refuse medium, no contamination occurred during the long-term fermentation. Therefore, additional asepsis technologies or pH control can be avoided. Thus, we propose ethanol fermentation of kitchen refuse medium by the self-flocculating strain ATCC26602 as a practical and economical fermentation system which can be applied in future large-scale industrial ethanol production.

4. Conclusion

In this study, the feasibility of repeated-batch fermentation of non-sterilized kitchen refuse medium by the self-flocculating yeast strain ATCC26602 was proven. Because of the natural acidity of kitchen refuse medium, no contamination occurred during the long-term fermentation. Therefore, additional pH control or medium sterilization can be avoided. The self-flocculating yeast strain showed its superiority over the non-flocculating one in terms of productivity. Higher glucose uptake and ethanol productivity within a shorter time were achieved. The viability of the self-flocculating yeast cells was maintained during the entire fermentation process, which was reflected by the stable settling time and the merely minor changes in biomass concentration between successive batches. Taking into consideration the other merits of kitchen refuse medium, e.g. sufficient nutrient content and no seasonal limitation, repeated-batch fermentation of kitchen refuse medium by this acid-tolerant, self-flocculating yeast strain may be a promising option for industrial-scale ethanol production, in combination with food recycling.
Fig. 1 Repeated-batch kinetic profiles of the acid-tolerant flocculating yeast strain *Saccharomyces cerevisiae* ATCC26602 in non-sterilized kitchen refuse. Variations of (a) glucose and ethanol concentration, (b) cell concentration and (c) pH with each batch. (◆ Glucose concentration, ■ ethanol concentration, ● initial cell concentration, ○ final cell concentration, ▲ initial pH, ▲ final pH)
Table 1 Experimental results of the repeated-batch fermentation by the self-flocculating yeast strain.

| Fermentation parameter | ATCC26602 |
|------------------------|-----------|
| $P_f$ [g/L]            | 58.36±1.55|
| $X_f$ [g/L]            | 21.17±2.34|
| $t$ [h]                | 12        |
| $Q_p$ [g/L/h]          | 3.66±0.06 |
| $Y_{p/s}$              | 0.49±0.01 |

a : These results were obtained from the third run onward.

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酸耐性凝集性酵母を用いた食品ゴミからの籍返し回分エタノール発酵

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地球温暖化対策と枯渇性化石資源代替の観点から、バイオマス由来の自動車用燃料製造に、近年世界的関心が急増している。ガソリン代替としてのエタノールにせよ、軽油代替のバイオディーゼルにせよ、バイオマス由来の自動車用燃料製造において、持続可能性の見地が重要となる。すなわち、エタノール製造におけるトウモロコシやサトウキビ、またバイオディーゼルにおけるオイルマや原料作物と食糧との競合、あるいは需要増に対応する土地利用変化による生物多様性の減少などの問題が指摘される。また、発酵原料化が期待される木質バイオマスについては、前処理に要する投入エネルギー削減、五炭糖の利用に関する技術的課題の克服が必要な状況にある。

著者らは、炭素源やその他栄養源が豊富に含まれる食品廃棄物を発酵原料とする資源化プロセス、すなわちバイオマスプラスチックであるポリ乳酸やポリブチルコハク酸の原料となる有機酸の発酵法による製造を報告してきた。

さらに、再生可能エネルギーであるバイオ燃料を、食品廃棄物を原料として、ATCC株のエタノール発酵により製造するプロセスを、ビーカースケールで詳細に検討し、良好な発酵原料足り得ることを先に報告した。本研究では、スケールアップを念頭に、生産性向上の観点から、耐酸性を示す凝集性酵母 ATCC26602 株の籍返し回分培養の比較検討を行った。

食品ゴミを培地とするエタノール発酵の際に、耐酸性の凝集性酵母 ATCC26602 株を用いて、コンサシレーションや生産性の低下なく11日間同2サイクルに及ぶ籍返し回分培養に成功した。凝集性酵母 ATCC26602 株において 3.7 g/L/h の生産性を示した。

耐酸性の凝集性酵母を用いた籍返し回分培養により、食品ゴミを基質として、無滅菌条件下でも高い生産性で養成可能であることはコスト削減の観点から、食品廃棄物からエタノールの商業生産に大きな展望を拓くものである。今後は、商業生産に向けたプロセス検討とスケールアップ実証研究について報告する予定である。