ETHANOL PRODUCTION FROM YAM BEAN USING YEAST *Saccharomyces cerevisiae* TISTR 5339

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Abstract
The efficiency of ethanol production from yam bean by *Saccharomyces cerevisiae* TISTR 5339 using batch, fed-batch and repeated-batch fermentation were investigated. The result revealed that the highest ethanol concentration and ethanol yield was obtained from 5% inoculum which was subsequently used in all experiment. Repeated-batch fermentation using immobilized cell system was the most effective which showed ethanol concentration of 12.01 ± 0.33 g/L and ethanol yield of 0.59 ± 0.02 g ethanol/g sugar. Moreover, the reuse possibility of immobilized cells was also studied. The possibility of reuse of the immobilized cells was up to 10 cycles or 160 h. For fed-batch fermentation, sugar consumption rate and ethanol concentration were not significantly affected by substrate feeding strategy and cell system.

Keywords: Cell immobilization, Ethanol, Fed-batch, Repeated-batch, *Saccharomyces cerevisiae* TISTR 5339, Yam bean

Introduction

Yam bean (*Pachyrhizuserosus* L. Urban) is a tuber-root climbing plant. It is also called Jicama and Mexican yam bean. Nowadays, it has been widely cultivated in Thailand about 55 provinces which is more than 40,000,000 square meters yielding commonly 0.625 to 1.25 kilograms/square meter. However, extremely the price depression of yam bean affects farmers. Therefore, it is important to encourage their production and an opportunity for the effective utilization. Yam bean is considered to be a potential biological material according to chemical composition which consists of 90.07% water, 0.09% fat, 0.72% protein, 4.9% fiber, 8.82% carbohydrate and 1.8% sugar (USDA National Nutrient Database 2012). Biological processes for the conversion of biomass to fuels including ethanol fermentation by yeast or bacteria are more attractive. The continuous growth of global population has led to a rapid increase in the world's demand for energy. Various fossil energy sources, such as oil, coal, natural gas, are being used for electricity, motor vehicle, and industrial machine (Uihlein and Schbek 2009). However, these are non-renewable resources which are consumed faster than they can be replaced. Bioethanol has been considered as a promising alternative fuel because it can be produced from various sources of renewable raw materials. Sugar cane and sugar beet are the sucrose-containing feedstock which has been interested for biological transformation into ethanol (United Nations Conference on Trade and Development 2006) whereas yam bean has not been utilized for ethanol production yet. In Thailand, sugar cane molasses and cassava are the main raw material used as the substrates for ethanol production.

Thai government plans to enlarge ethanol production in 15 years from 3 to 9 million L/day (year 2008-2022) (Silalertruksa and Gheewala 2010). Therefore, the demand of raw materials for ethanol production will be increased. Yam bean may be an alternative raw material for ethanol production especially in the Northeastern part of Thailand because of its low price.

The objectives of this study were to compare the efficiency of ethanol production from yam bean using batch and fed-batch fermentation and to investigate the stability of immobilized cells of *S. cerevisiae* TISTR 5339 in repeated-batch ethanol fermentation. The effect of various substrate feeding strategies in fed-batch fermentation were also evaluated.

Methodology

Yeast strain and inoculum preparation

A commercial *S. cerecisiae* TISTR 5339 was purchased from Thailand Institute of Scientific and Technology (TISTR), Bangkok, Thailand. The inoculum was prepared by cultivation of yeast in 250-mL Erlenmeyer flask containing 200 mL of YM medium (yeast extract, 3 g/L; peptone, 5 g/L; malt extract, 3 g/L and glucose 10 g/L). The flask was incubated at 30°C for 24 h with shaking at 180 rpm.

Raw materials and ethanol production medium

Yam beans were obtained from Kamphaeng Saen local market, Nakhon Pathom, Thailand. After peeling, yam beans were cut into small pieces and water was added with ratio of 1:1 (w/w) then it was homogenized with blender. Juice was filtered through cheesecloth and 1% (w/v) of yeast extract was added into the filtrate before adjusting pH to 5. The medium was transferred into 250-ml Erlenmeyer flask with a final working volume of desired experiment (100, 75, 50, 25 ml) and autoclaved at 121°C for 15 min. The initial sugar concentration ranged from 21 to 28 g/L.

Cell immobilization

The inoculum was added into 2% (w/v) of sterilized sodium alginate solution and well mixed. The mixture was transferred to a syringe in order to extrude dropwise to 0.1 M stirred calcium chloride solution at 0°C to form estimated 2 mm-diameter beads. The beads were allowed to harden for 12 h at 4°C before used.

Fermentation of yam bean to ethanol

Batch fermentation

The fermentation was carried out in 250-ml Erlenmeyer flask with 100 ml the sterile yam bean juice which was inoculated with variety of inoculum sizes (5, 10, 15% inoculum). The batch fermentation was operated at 30°C under static condition. The samples were collected at time intervals until 22 h to determine the total reducing sugar and ethanol production.

Repeated-batch fermentation

The repeated-batch fermentation was carried out in batch mode as mentioned above but immobilized cells were used instead of free cell. After fermentation for 16 h, all fermented culture was withdrawn and then the gel beads were washed three times with sterilized water before transferred to the equal amount of fresh medium. The sample of each cycle was collected until the concentration of ethanol was approximately decreased to 50% of the first batch.

Fed batch

Fed batch fermentation was carried out by free cell and immobilized cell with three substrate feeding strategies (50:50, 50:25:25, 75:25). Briefly, the initial substrate volumes were prepared at 50, 50 and 75% of the total designed volume. After 6 h, the fresh medium was added 50, 25, 25% for 50:50, 50:25:25, 75:25, respectively. Another 25% fresh medium was added for 50:25:25 after 10 h. The samples were collected at appropriate time intervals of the fermentation for further analysis.

Analytical method

The sample collected at different time intervals were centrifuged at 10,000 rpm for 5 min. The supernatant was used to determine the total reducing sugar and ethanol production. Total reducing sugar was determined by using DNS method (Miller 1959) and the ethanol concentration was analyzed on high performance liquid chromatography (Shimadzu Class LC10, Japan) using SugarPax column (Bio-Rad) and refractive index detector. The column temperature was set at 85°C. Samples were eluted with deionized water at aflow rate of 0.5 mL/min. The volumetric ethanol productivity (Q_p) was calculated by using the following equation:

$$Q_p = \frac{P}{t}$$

Where; P is the concentration of produced ethanol (g/L) and t is the fermentation time (h) giving the highest ethanol concentration for batch, fed-batch fermentations and repeated batch fermentation.

$$\text{The ethanol yield } (Y_{p/s})\ (\text{g/g}) = \frac{\text{The concentration of produced ethanol } (P)}{\text{The concentration of utilized sugar}}$$

Percentage of conversion efficiency or yield efficiency (E_y) was also calculated as the following equation:

$$E_y = \frac{Y_{p/s} \times 100}{0.51}$$

Where; 0.51 is the maximum theoretical ethanol yield of glucose consumption.

Data analysis

The experiments were conducted in triplicate. Statistical comparisons between the groups were performed using one-way analysis of variance (ANOVA) which was performed by using SPSS software (v.17) (IL, U.S.A.). Statistical significance was set a priori at $p<0.05$.

Results and discussion

Batch fermentation by free cell

The effect of inoculum sizes on ethanol production by *S. cerevisiae* was investigated. Total reducing sugar and ethanol produced by free cell with different inoculum sizes (5, 10, 15%) were shown in Figure 1. The initial sugar concentration without adjustment was 28 g/L. The result showed that the inoculum size affected the substrate consumption rate and final ethanol concentration. For 5% inoculum, the sugar concentration was shown to remain for first 2 h whereas no lag phase was observed for 10 and 15% inoculum. The highest ethanol concentration obtained from 5% inoculum after 13 h was12.89 g/L. Moreover, it exhibited the highest ethanol yield and productivity of 0.49 g/g and 0.99 g/L h, respectively. At 10 and

15% inoculums, the low level of ethanol was observed which might be caused by the reduction of sugar tend to be used for energy and cell mass formation. Too high inoculum size can adversely affected ethanol production due to the decrease of the viability of yeast population and inadequate development of biomass and ethanol production (Powchinda et al. 1999). The summary of parameters of ethanol production from yam bean juice at various inoculums was shown in Table 1. Therefore, the 5% inoculum was further used in all subsequent experiments.

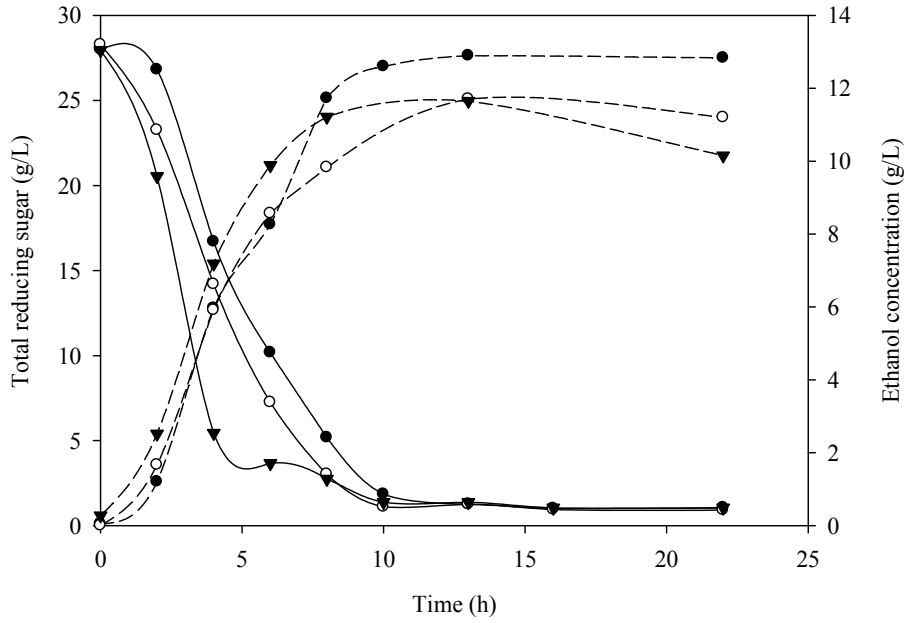

Figure 1 Effect of inoculums size on total reducing sugar and ethanol production from yam bean juice by *S. cerevisiae* TISTR 5339. 5% (●), 10% (○), 15% (▼), total reducing sugar (—) and ethanol (---)

Table 1 Parameters of ethanol production from yam bean juice at various inoculums size of *S. cerevisiae* TISTR 5339 in batch fermentation

Cell inoculum size	Parameter				
	P (g/L)	Q_p (g/l h)	$Y_{p/s}$ (g/g)	E_y (%)	Time (h)
5%	12.89	0.99	0.49	96.67	13
10%	11.7	0.90	0.43	84.44	13
15%	11.64	0.90	0.44	85.88	13

Repeated-batch fermentation by immobilized cell

Repeated-batch fermentation was carried out by using calcium-alginate as gel carrier for cell immobilization which is the most widely used in laboratory scales (Santos et al. 2008). The stability of calcium-alginate for cell immobilization was evaluated. The ethanol concentration remained almost constant in range from 11.01 to 12.01 g/L and also the ethanol yield ranged

from 0.54 to 0.59 g/g which were no significant difference between the 1st cycle to the 10th ($p<0.05$) (Table 2). The instability of immobilized cell was observed after cycle 10th. At the end of 10th cycle, partial gel degradation on the surfaces of calcium-alginate was observed. The strength of alginate beads can improve by increasing of alginate concentration; however, it led to lower mass transfer (Idris and Suzana 2006). The ethanol concentration slightly dropped in the 11th cycle and strongly decreased in the 12th to 14th cycle. Hence, the stability of calcium-alginate for cell immobilization in the present study is possible to use up to ten cycles or 160 h. Production of ethanol by using the immobilized cells is more effective than free cell system when compares the ethanol yield with batch fermentation result; moreover, the immobilized cells can be reusable.

Table 2 Parameters of ethanol production from yam bean juice by immobilized cell system of *S. cerevisiae* TISTR 5339 in repeated-batch fermentation

Cycle	Parameters (mean ± S.D.)			
	P (g/L)	Q_P (g/l h)	$Y_{p/s}$ (g/g)	E_y (%)
1	11.22 ± 0.45[a]	0.70 ± 0.03[a]	0.55 ± 0.02[a]	108.61 ± 4.39[a]
2	11.41 ± 0.17[a]	0.71 ± 0.01[a]	0.56 ± 0.01[a]	110.48 ± 1.69[a]
3	11.73 ± 0.22[a]	0.73 ± 0.01[a]	0.58 ± 0.01[a]	113.53 ± 2.09[a]
4	12.01 ± 0.33[a]	0.75 ± 0.02[a]	0.59 ± 0.02[a]	116.22 ± 3.22[a]
5	11.22 ± 0.18[a]	0.70 ± 0.01[a]	0.55 ± 0.01[a]	108.62 ± 1.75[a]
6	11.01 ± 0.46[a]	0.69 ± 0.03[a]	0.54 ± 0.02[a]	106.55 ± 4.49[a]
7	11.29 ± 0.58[a]	0.71 ± 0.04[a]	0.56 ±0.03[a]	109.25 ± 5.60[a]
8	11.04 ± 0.67[a]	0.69 ± 0.04[a]	0.55 ± 0.03[a]	106.86 ± 6.52[a]
9	11.05 ± 0.37[a]	0.69 ± 0.02[a]	0.55 ± 0.02[a]	106.92 ± 3.55[a]
10	11.05± 0.45[a]	0.69 ± 0.03[a]	0.55 ± 0.02[a]	106.97 ± 4.33[a]
11	8.23 ± 1.35[b]	0.51 ± 0.08[b]	0.41 ± 0.07[b]	79.68 ± 13.02[b]
12	6.88 ± 0.20[c]	0.43 ± 0.01[c]	0.34 ± 0.01[c]	66.57 ± 1.91[c]
13	6.13 ± 0.21[cd]	0.38 ± 0.01[cd]	0.30 ± 0.01[cd]	59.29 ± 2.03[cd]
14	5.91 ± 0.38[d]	0.37 ± 0.02[d]	0.29 ± 0.02[d]	57.18 ± 3.66[d]

* The different letters indicate the statistically significant difference at 0.05 probability level.

Fed-batch fermentation by free and immobilized cell

According to the batch experiment, the initial inoculum size of 5% was used in fed-batch fermentation by free and immobilized cell. The time course of ethanol production using different substrate feeding strategies with the estimated initial sugar concentration of 23 g/L was shown in Figure 2. Nutrient feeding strategies may significantly improve production in fermentations (Altintas et al. 2002). Comparisons of the parameters of ethanol production between free cell and immobilized cell system in fed-batch fermentation with different substrate feeding strategies were shown in Table 3. The results revealed that the sugar consumption rate and ethanol concentration were no significantly affected by substrate feeding strategy and cell system. The third feeding strategy showed lowest ethanol yield and productivity. All of the reported fed-batch fermentations by using immobilized cell gave greater ethanol yield than that of 13-h batch fermentation by using free cell. However, ethanol productivity in batch fermentation was significantly higher than that of fed-batch fermentation. To consider the operation cost, the substrate feeding strategies of 50:50 was the optimum condition for ethanol production because only once substrate feeding is reqiured.

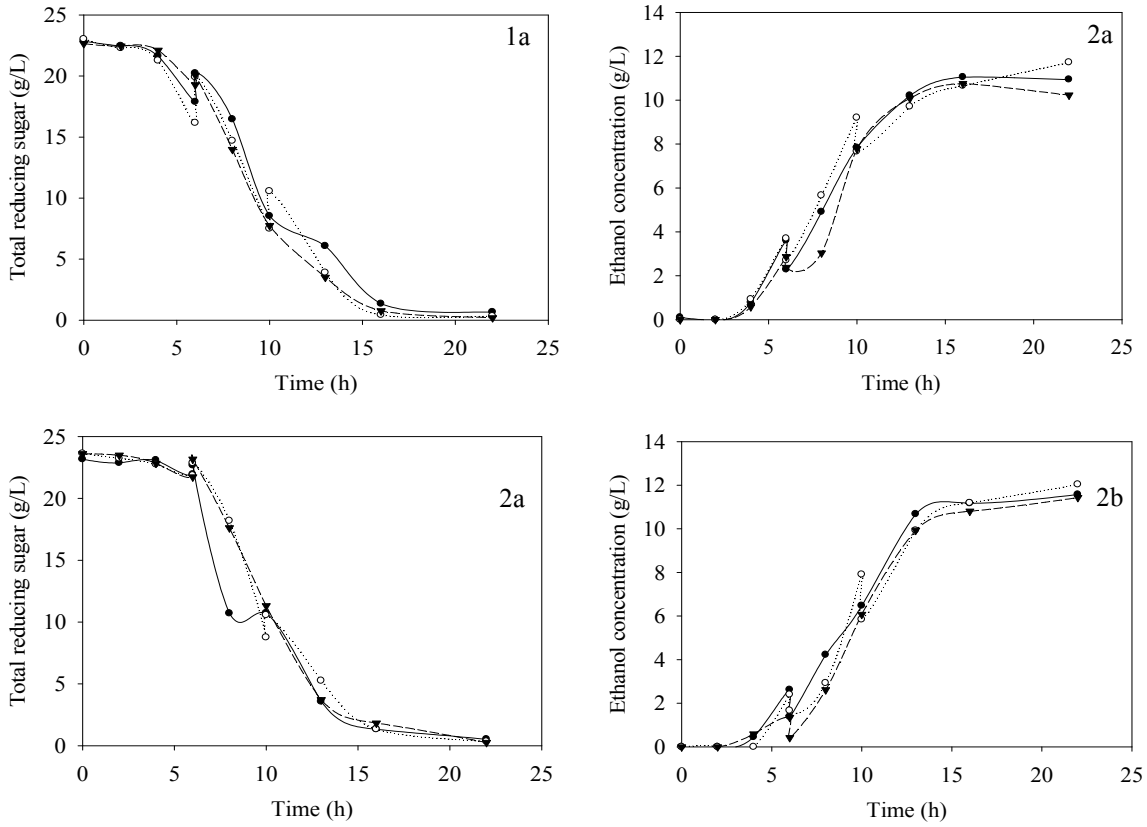

Figure 2 Effect of feeding strategy on sugar consumption by using free cell (1a), immobilized cell (2a) and ethanol concentration by using free cell (2a), immobilized cell (2b) from yam bean juice by *S. cerevisiae* TISTR 5339. 50:50:50 (●), 50:25:25 (○), 75:25 (▼).

Table 3 Parameters of ethanol production from yam bean juice by free cell (FC) and immobilized cell (IC) system of *S. cerevisiae* TISTR 5339 in repeated-batch fermentation

Feeding strategy	Parameters (mean ± S.D.)								Time (h)
	P (g/L)		Q_p (g/l h)		$Y_{p/s}$ (g/g)		E_y (%)		
	FC	IC	FC	IC	FC	IC	FC	IC	
50 : 50	10.92 ± 0.16^{b}	11.58 ± 0.27^{ab}	0.50 ± 0.01^{b}	0.53 ± 0.01^{ab}	0.50 ± 0.01^{a}	0.51 ± 0.01^{a}	98.26 ± 1.43^{a}	100.29 ± 2.31^{a}	22
50 : 25 : 25	11.71 ± 0.19^{ab}	12.01 ± 0.12^{a}	0.53 ± 0.01^{ab}	0.55 ± 0.01^{a}	0.49 ± 0.02^{a}	0.52 ± 0.01^{a}	96.65 ± 3.46^{a}	101.13 ± 1.03^{a}	22
75 : 25	10.00 ± 1.04^{c}	11.33 ± 0.28^{ab}	0.45 ± 0.05^{c}	0.51 ± 0.01^{ab}	0.45 ± 0.05^{b}	0.48 ± 0.01^{a}	87.38 ± 9.91^{b}	95.08 ± 2.37^{a}	22

* The different letters indicate the statistically significant difference at 0.05 probability level

Conclusion

This study demonstrated that yam bean could be an alternative raw material for ethanol production due to its low price and renewable property. The efficient ethanol production from yam bean is a simple preparation process with adding small amount of a nitrogen source into the juice. Moreover, yam bean contains high sugar content in case of pure juice. Cell immobilization system improves the efficiency of ethanol production including ethanol concentration and ethanol yield in repeated-batch fermentation but not in fed-batch fermentation. Repeated fed-batch is needed to further study to improve ethanol productivity. Furthermore, large scale fermentation will be required to confirm the results of the small scale.

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