

# Preliminary Study of Continuous Fermentation for Kaew Mango Wine Production in a Packed Bed Bioreactor with Immobilized Yeasts

## การศึกษาเบื้องต้นของการผลิตไวน์มะม่วงแก้วแบบต่อเนื่องในถังปฏิกรณ์ชีวภาพแบบเบดบรรจุโดยใช้เซลล์ยีสต์ตระหง่าน

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### ABSTRACT

The cells of *Saccharomyces cerevisiae* strain Sweden were immobilized in calcium alginate beads. The optimum immobilization conditions for Kaew mango wine production in batch process was at 2% sodium alginate and 0.1 M  $\text{CaCl}_2$ . The immobilized yeasts were packed in a glass column bioreactor with the working volume of 740 ml and the process was operated at 20 °C. The effect of bead volume packed in the column (20, 35 and 50% of the working volume) and dilution rate of Kaew mango juice fed in the column (25, 50 and 75% of maximum specific growth rate,  $\mu_{\max}$ ) were studied for Kaew mango wine production. The results showed that the bead volume at 35% and the dilution rate at 25%  $\mu_{\max}$  were suitable for continuous Kaew mango wine production in terms of ethanol production and stability of bead morphology. At the optimum operating condition, however, the ethanol concentration was only  $4.42 \pm 0.41\% (\text{v/v})$ .

**Key Words :** continuous fermentation, Kaew mango wine, packed bed bioreactor, immobilized yeast

**คำสำคัญ :** การหมักแบบต่อเนื่อง ไวน์มะม่วงแก้ว ถังปฏิกรณ์ชีวภาพแบบเบดบรรจุ ยีสต์ตระหง่าน

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## บทคัดย่อ

การตรึงยีสต์ *Saccharomyces cerevisiae* สายพันธุ์ Sweden ด้วยแคลเซียมอลจีเนต พบร่วมกับความเข้มข้นของโซเดียมอลจีเนตและแคลเซียมคลอไรด์ที่เหมาะสมในการตรึงยีสต์เพื่อผลิตไวน์มะม่วงแก้วโดยกระบวนการหมักแบบกึ่งคือ 2% และ 0.1

โนลาร์ ตามลำดับ จากนั้นเซลล์ยีสต์ตรึงรูปถูกนำไปบรรจุในถังปฏิกรณ์ชีวภาพแบบเบดบรรจุ ซึ่งเป็นคอลัมน์แก้วที่มีปริมาตรทำงาน 740 มิลลิลิตร หมักแบบต่อเนื่องที่อุณหภูมิ 20 องศาเซลเซียส โดยใช้ปริมาตรเม็ดเซลล์ตรึงเท่ากับ 20, 35 และ 50% ของปริมาตรทำงานทั้งหมด และแปรผันอัตราการเจือจางของการป้อนน้ำมะม่วงเข้าสู่ระบบเป็น 25, 50 และ 75% ของอัตราการเจริญสูงสุด ( $\mu_{max}$ ) ผลการทดลองพบว่าปริมาตรเม็ดเซลล์ตรึงและอัตราการเจือจางที่เหมาะสมในการหมักไวน์มะม่วงแก้วแบบต่อเนื่องในถังปฏิกรณ์ชีวภาพแบบเบดบรรจุในแต่ละความเข้มข้นเอทานอลที่ได้และความคงตัวของเม็ดเซลล์ตรึงคือ 35 และ 25%  $\mu_{max}$  ตามลำดับ อย่างไรก็ตามที่สภาวะนี้ความเข้มข้นเอทานอลมีค่าเพียง  $4.42 \pm 0.41\%$  (โดยปริมาตร)

## INTRODUCTION

Continuous wine production with immobilized cells offers several advantages compared to the traditional fermentation by free cells such as increase in productivity and reduction in production cost (Kourkoutas et al., 2002). The system can be operated beyond the nominal washing-out flow rate and smaller bioreactor volumes that may decrease capital costs (Nedovic et. al., 2000; Nigam, 2000). Among the different immobilization methods, gel entrapment by calcium alginate is the most common (Arasaratnam, 1994). The aim of this research was to determine optimum Na-alginate and CaCl<sub>2</sub> concentrations of cell immobilization for Kaew mango wine making in continuous process using packed bed bioreactor. The influence of the bead volume packed in the bioreactor and the dilution rate of mango juice fed to the column for continuous fermentation were also investigated.

## MATERIALS AND METHODS

### 1. Yeast strain

*Saccharomyces cerevisiae* strain Sweden was kept on yeast extract and malt extract (YM) agar slant at 4 °C.

### 2. Ethanol production medium

The ethanol production medium for batch and continuous culture consisted of (g l<sup>-1</sup>) yeast extract, 3; peptone, 5; glucose, 150; MgSO<sub>4</sub>.7H<sub>2</sub>O, 0.025; KH<sub>2</sub>PO<sub>4</sub>, 0.5; CaCl<sub>2</sub>.2H<sub>2</sub>O, 1; (NH<sub>4</sub>)<sub>2</sub>PO<sub>4</sub>, 1; MnSO<sub>4</sub>.6H<sub>2</sub>O, 0.5 and Zn(NO<sub>3</sub>)<sub>2</sub>, 0.2. The pH of the medium was adjusted to 4.5 by adding 0.1 M tartaric acid before sterilization at 110 °C for 20 min.

### 3. Mango juice for wine production

Total soluble solids and total acidity of the mango juice were adjusted to 22 oBrix and 6.5 g l<sup>-1</sup>, respectively. The mango juice was supplemented with (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> 1 g, MgSO<sub>4</sub> 20 mg and vitamin B 6 mg per 1 liter of the juice. Then it was sterilized by adding potassium metabisulfite (KMS) at 0.2 g l<sup>-1</sup> of juice for 12 hours before use.

#### 4. Cell immobilization and batch fermentation

The yeast cells were grown in YM medium at 200 rpm, 30 °C for 18 hours. The cells were harvested by centrifugation at 6,000 rpm for 10 min. The pellets were suspended in 0.85% NaCl. An appropriate volume of the cell suspension was mixed with sterile sodium alginate at desired concentrations (1.0, 1.5 and 2.0%) to obtain the initial cell concentration  $0.8 \times 10^8$  cells  $\text{ml}^{-1}$ . The mixer was added dropwise to gently stirred  $\text{CaCl}_2$  solution of desired concentrations (0.1 and 0.2 M). The beads were stored at 4 °C in the fresh  $\text{CaCl}_2$  solution for 24 hours before use.

The immobilized yeasts at each condition grown on the ethanol production medium for 24 hours were added into a 500 ml-airlock erlenmeyer flask containing 350 ml of the mango juice. The volume of the immobilized yeast in the flask was 20% of the working volume. The batch fermentation was carried out at 20 °C.

#### 5. Packed bed bioreactor and continuous operating conditions

The bioreactor is shown in Fig 1. The immobilized cells at optimum Na-alginate and  $\text{CaCl}_2$  concentrations were packed in the columns. The mango juice was fed into the bottom part of the columns. The bioreactor was operated as a batch system for 24 hours before starting a continuous process. The operating conditions were as follows:

#### 6. Analytical methods

The samples from 4) and 5) were taken everyday to determine total soluble solids with a hand refractometer. Ethanol concentration in the samples was detected with a gas chromatography (Shimadzu Model 14B) equipped with flame ionization detector (FID) and GC packed column

Working volume (per column)
: 740 ml
Dilution rate (D)
: 0.25, 0.50 and 0.75 $\mu_{\text{max}}$
Retention time
: 16, 8 and 5.3 min
Bead volume in the bioreactor
: 20, 35 and 50% of working volume
Fermentation temperature
: 20 °C

$\mu_{\text{max}}$  of the yeasts grown in the mango juice at 30 °C was 0.25  $\text{h}^{-1}$ .

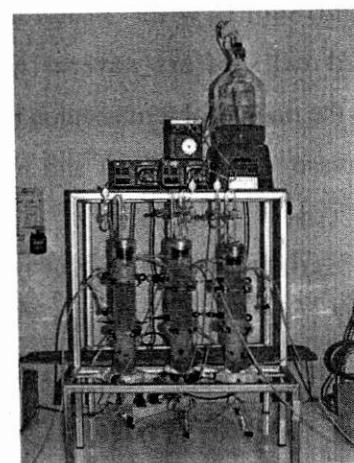


Fig 1 Continuous fermentation of Kaew mango wine production by packed bed bioreactor with immobilized yeasts.

(Shimadzu, Japan). The oven, injector and detector temperatures were 150, 180 and 250 °C respectively. Nitrogen gas was used as a carrier gas.

Bead morphology before and after fermentation was also observed by visual observation.

## RESULTS AND DISCUSSION

### 1. Effect of sodium alginate and calcium chloride concentrations

$\text{CaCl}_2$  at 0.1 M was more suitable for cell immobilization than 0.2 M due to shorter fermentation time (Fig 2). At the same  $\text{CaCl}_2$  concentration, changes in sugar in terms of total soluble solids and ethanol concentration during fermentation were not significantly different when the yeasts were immobilized at the various Na-alginate concentrations (Fig 2A and B). However, the bead shape at 1 and 1.5% Na-alginate was changed after fermentation (Fig 3A and B) while the beads at 2.0% Na-alginate were still spherical (Fig 3C). The change was possibly due to the fact that the beads did not have enough strength to withstand the rigor during the fermentation.

Therefore, the cells immobilized on 2% Na-alginate and 0.1 M  $\text{CaCl}_2$  were prepared for continuous fermentation.

### 2. Continuous fermentation in a packed bed bioreactor

The bead volume at 50% and the dilution rate at 25%  $\mu_{\text{max}}$  gave the best results in terms of sugar utilization and ethanol production (Table 1). However, the bead morphology at 50% bead volume was changed after the fermentation (Fig 4).

Therefore, the bead volume at 35% and the dilution rate at 25%  $\mu_{\text{max}}$  were more suitable for continuous Kaew mango wine production in terms of ethanol production and stability of bead morphology.

At the optimum operating conditions, the ethanol concentration was only  $4.42 \pm 0.41\%$  (v/v) which was too low for traditional wine production. Further studies should be carried out to

achieve higher ethanol level at least to the normal level in wine. This could be done by increasing the retention time of the juice in the bioreactor through the use of a larger bioreactor or using more columns.

## CONCLUSIONS

1) The optimum immobilization conditions for Kaew mango wine production in batch process was at 2% sodium alginate and 0.1 M  $\text{CaCl}_2$ .

2) The bead volume at 35% and the dilution rate at 25%  $\mu_{\text{max}}$  were suitable for continuous Kaew mango wine production in terms of ethanol production and stability of bead morphology.

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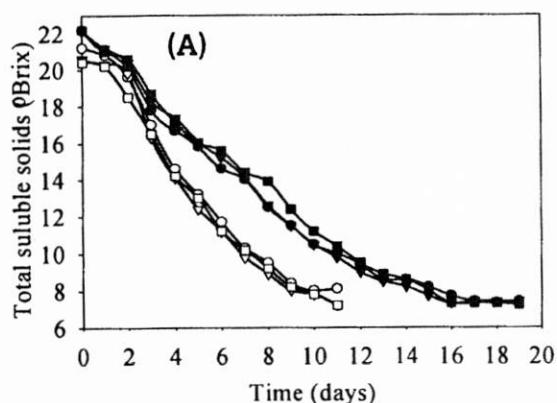
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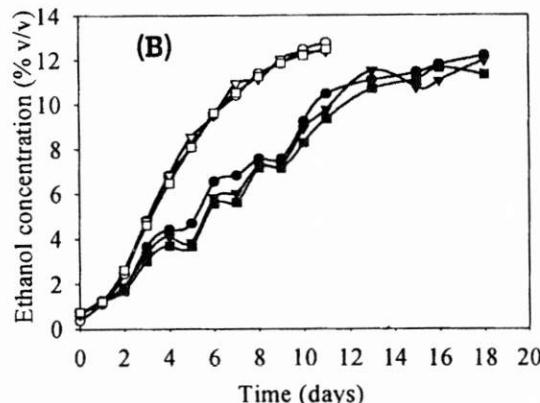
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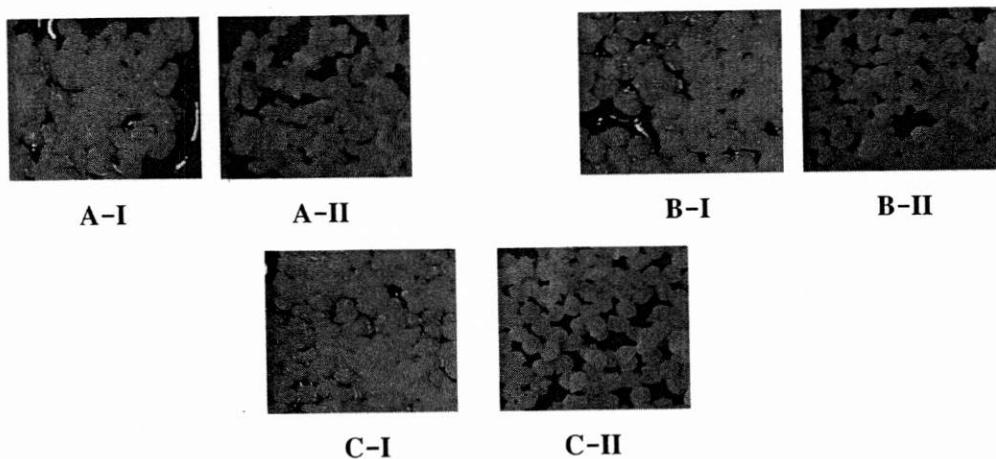


(A) Total soluble solids



(B) Ethanol concentration

**Fig 2** Kaew mango wine production in batch fermentation by *S. cerevisiae* immobilized on different Na-alginate (%) and  $\text{CaCl}_2$  (M) concentrations. (A) Total soluble solids (B) Ethanol concentration. (●) 1% and 0.2 M; (▼) 1.5% and 0.2 M; (■) 2% and 0.2 M; (○) 1% and 0.1 M; (▽) 1.5% and 0.1 M; (□) 2% and 0.1 M

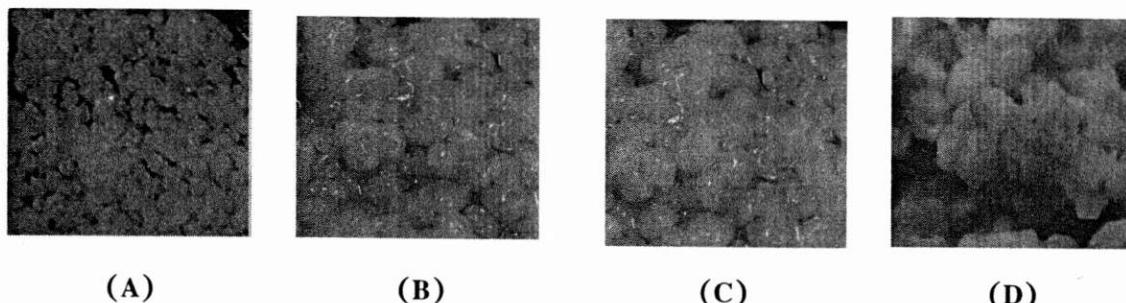


**Fig 3** Bead morphology at 1 (A), 1.5 (B) and 2.0% (C) Na-alginate and 0.1 M  $\text{CaCl}_2$  before (I) and after (II) the fermentations.

**Table 1** Total soluble solids and ethanol concentration during continuous fermentation for Kaew mango wine production by a packed bed bioreactor with immobilized yeasts at different operating conditions.

Composition	20% bead volume at D			35% bead volume at D			50% bead volume at D		
	0.25 $\mu_{\text{max}}$	0.50 $\mu_{\text{max}}$	0.75 $\mu_{\text{max}}$	0.25 $\mu_{\text{max}}$	0.50 $\mu_{\text{max}}$	0.75 $\mu_{\text{max}}$	0.25 $\mu_{\text{max}}$	0.50 $\mu_{\text{max}}$	0.75 $\mu_{\text{max}}$
Total soluble solid (oBrix)	19.75 $\pm 0.23$	20.23 $\pm 0.24$	19.92 $\pm 0.24$	18.48 $\pm 0.34$	19.68 $\pm 0.30$	19.37 $\pm 0.41$	17.04 $\pm 0.44$	18.60 $\pm 0.32$	18.04 $\pm 0.34$
Ethanol concentration (%v/v)	3.36 $\pm 0.32$	3.12 $\pm 0.17$	3.09 $\pm 0.28$	4.42 $\pm 0.41$	3.42 $\pm 0.22$	3.31 $\pm 0.37$	5.89 $\pm 0.34$	4.46 $\pm 0.27$	4.67 $\pm 0.31$

: The results were expressed as mean and standard deviation for at least 5 values during steady state fermentation.



**Fig 4** Bead morphology before (A) and after fermentation at bead volumes of 20 (B), 35 (C) and 50% (D).