ผลของความเข้มข้นกลูโคสต่อการผลิตเอทานอลโดย *Saccharomyces cerevisiae* Sc90

Effect of glucose concentrations on ethanol production by *Saccharomyces cerevisiae* Sc90

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บทคัดย่อ

เอทานอลที่ผลิตจากเซลลูโลสถูกยอมรับว่าเป็นทางเลือกที่มีประสิทธิภาพในการทดแทนเชื้อเพลิงจากผลิตภัณฑ์ปิโตรเลียม ซึ่งจะช่วยลดสภาวะเรือนกระจกที่ปกคลุมอยู่ชั้นบรรยากาศ ในทางปฏิบัติชีวมวลทางการเกษตรและไฮโดรลิซิสสารตั้งต้นดังกล่าวที่ได้จากการไฮโดรไลเซทมีความเข้มข้นกลูโคสที่แตกต่างกัน *Saccharomyces cerevisiae Sc90* ถูกเพาะเลี้ยงในอาหาร YPD ที่อุณหภูมิ 40^{O}C อัตราการกวนเท่ากับ 150 รอบต่อนาทีโดยการแปรผันความเข้มข้นกลูโคสเท่ากับ 74 120 170 220 และ 270 กรัมต่อลิตรสำหรับการผลิตเอทานอล ผลการศึกษาพบว่าความเข้มข้นกลูโคสเหมาะสมที่สุดเท่ากับ 220 กรัมต่อลิตร อัตราการเจริญจำเพาะเท่ากับ 0.327 ต่อชั่วโมง อัตราการผลิตเอทานอลเท่ากับ 1.779 กรัมต่อลิตรต่อชั่วโมง ความเข้มข้นเอทานอลเท่ากับ 85.87 กรัมต่อลิตรและประสิทธิภาพของการหมักเท่ากับ 84.90 เปอร์เซ็นต์ จากผลลัพธ์นี้ *S. cerevisiae* Sc90 เป็นสายพันธุ์ที่มีศักยภาพสำหรับการหมักที่ความเข้มข้นสูง

ABSTRACT

Cellulosic ethanol has been recognized as a potential alternative to petroleum fuel productions. This reduces the net contribution of greenhouse gases to the atmosphere. In practice, because of various types of agricultural biomass and their hydrolysis method, the glucose concentrations in their hydrolysates from different materials were different among them. *Saccharomyces cerevisiae* Sc90 were cultivated in YPD medium at 40^{O}C, 150 rpm agitation rate with various glucose concentrations at 74, 120, 170, 220, and 270 g/L for ethanol production. The results showed that the optimal glucose concentration was 220 g/L. The specific growth rate, ethanol production rate, ethanol concentration and yield efficiency were 0.327 1/h, 1.779 g/L h, 85.87 g/L, and 84.90 %, respectively. From these results, *S. cerevisiae* Sc90 has shown as a potential strain for high gravity fermentation.

Key Words: glucose concentrations, *Saccharomyces cerevisiae*, ethanol production

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INTRODUCTION

Ethanol has been recognized as a potential alternative to petroleum-derived transportation fuels. This reduces the net contribution of greenhouse gases to the atmosphere (Martin *et al.,* 2002). For sustainable development, lignocellulosic ethanol has been worldwide studied for more than decade. Wood and crop residues have been investigated as a source of substrate for ethanol production, and the predominant composition is cellulose, hemicellulose, and lignin.

Cellulose is a linear polymer of glucose units linked by beta-1, 4-glucosidic bonds. It constitutes 35–50% dry weight in agricultural biomass (Sun and Cheng 2002). The efficacy of microorganisms to ferment glucose in agricultural biomass to ethanol is of importance for an economically possible process (Lynd *et al.* 1999). The most efficacies ethanol-producing yeast is *Saccharomyces cerevisiae*. The optimal temperature for S. *cerevisiae* is 30-35^{o}C (Slaa *et al.* 2009). S. *cerevisiae* SC90 was able to produce the ethanol at 40^{o}C in simultaneous saccharification and fermentation process (Pan-utai *et al.* 2010). Moreover, it was found that 97.67 g/L ethanol concentration was also produced with using initial glucose concentration at 225 g/L, 30^{o}C (Laopaiboon *et al.* 2011).

In practice, because of various types of agricultural biomass and their hydrolysis method, the glucose concentrations in their hydrolysates from different materials were different among them. In addition, glucose and cellobios show product inhibitors of cellulolytic enzyme. Therefore, glucose concentrations have an important role to the hydrolysis and ethanol fermentation step. In this paper, the effects of glucose on cell growth, substrate utilization, ethanol concentration, ethanol yield, and ethanol production rate were studied using *Saccharomyces cerevisiae* Sc90

MATERIAL AND METHODS

1. Preparation of microorganism, media, and fermentation

Saccharomyces cerevisiae Sc90 was generously supplied by Liquor Distillery Organization in Thailand. Stock cultures were maintained on 40% glycerol at -20^{o}C. *S. cerevisiae* Sc90 were cultivated on YPD agar plates: 10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose, and 20 g/L agar at 30^{o}C for 2 days. The inoculum cells was prepared from the colony which grown in YPD broth at room temperature, 200 rpm for 18 h. YPD medium were used in ethanol production by fermentation, including 10 g/L yeast extract, 20 g/L peptone, and various glucose concentration at 74, 120, 170, 220, and 270 g/L. The cells were inoculated into 300 mL YPD broth at 40^{o}C with agitation rate at 150 rpm to produce ethanol.

2. Analytical methods

Fermentation was monitored for 4 days by taking 2 mL of samples at 0, 2, 4, 6, 8, 10, 12, 15, 18, 24, 36, 48, 60, 72, 84, and 96 h for analyses. The concentrations of glucose and ethanol were determined using an Agilent HPLC System with an analytical BIO-RAD Aminex HPX-87H column and a

BIO-RAD Cation H refill guard column. The spread plate technique was used to determine the number of viable cells. Serial dilutions of the samples were performed, and after the incubation time at 30°C, colonies grown in petri dishes were used to count the number of viable cells. The calculations of fermentation parameters follow method by Agbogbo *et al.* 2006

RESULT AND DISCUSSION

1. Fermentation on various glucose concentrations

The highest number of viable cell was 8.57 x 10^{11} CFU/L in 270 g/L glucose concentration, while 220 g/L glucose gave the lowest viable cell was 1.20 x 10^{11} CFU/L (Fig.1). In 270 g/L glucose concentration, the viable cells were abruptly decreased at 18 h and completely dead at 60 h. However, the viable cell (5.22 x 107 CFU/L) was able to survive for 84 h in the initial glucose concentrations at 220 g/L. *S. cerevisiae* Sc90 consumed completed glucose of 74, 120, 170, and 220 g/L glucose concentrations at 20, 36, 96, and 96 h, respectively. However, the glucose concentration at 270 g/L showed the substrate inhibition (Fig.2) because of decreased metabolic activity and the increased osmotic pressure (Panchal and Stewart 1980; Reddy and Reddy 2006). High glucose concentration obtained for ethanol fermentation reduced the viable cells activity and glucose consumption.

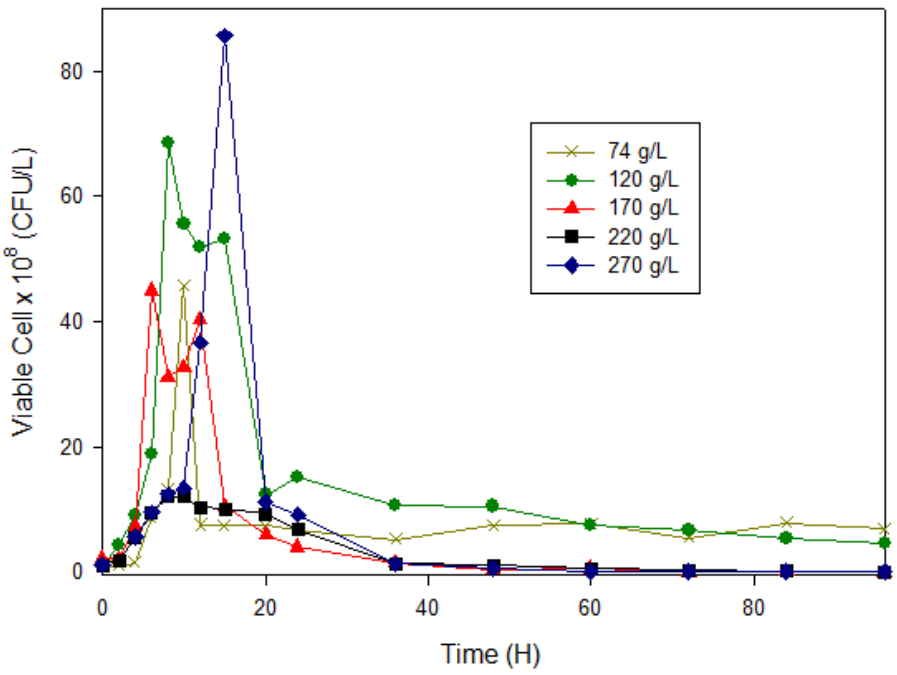

Figure 1 Effect of glucose concentration on cell viability in the fermentation to ethanol at 40°C. (x) 74 g/L glucose; (●) 120 g/L glucose; (▲) 170 g/L glucose; (■) 220 g/L glucose; and (♦) 270 g/L glucose.

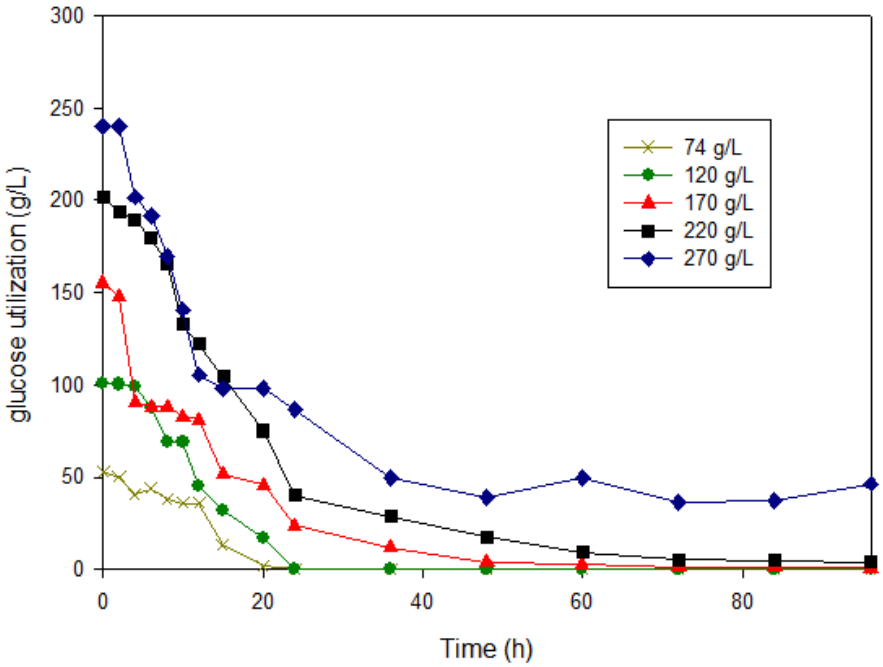

Figure 2 Effect of glucose utilization in the fermentation to ethanol at 40°C. (x) 74 g/L glucose; (●) 120 g/L glucose; (▲) 170 g/L glucose; (■) 220 g/L glucose; and (♦) 270 g/L glucose.

2. Fermentation parameters

The highest ethanol concentration (Table1) was 85.87 g/L in 220 g/L glucose concentration, while 74 g/L glucose gave the lowest ethanol concentration at 24.24 g/L (Fig.3). The maximum ethanol production time in 74, 170, 220, and 270 g/L glucose concentrations were 20, 36, 48, and 60 h, respectively. However, the highest ethanol concentration was obtained from the initial glucose concentration at 220 g/L. In addition, yields efficiency in 220 g/L glucose was 84.90 %, which was higher than those in other glucose concentration (Table 1). The maximum ethanol production rate was 1.779 g/L h in 220 g/L glucose concentration, while 74 g/L glucose concentration had the minimum ethanol production rate at 0.331g/L. However, the specific growth rate was decreased because of high concentration of glucose in ethanol fermentation and high temperature at 40°C. However, ethanol production rate was increased excepted at 270 g/L glucose concentration.

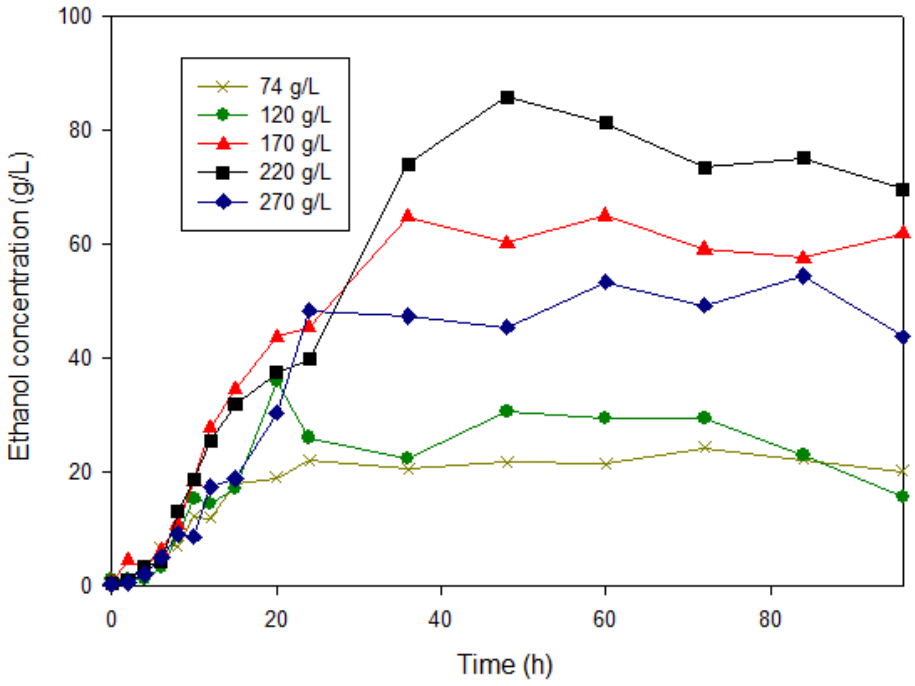

Figure 3 Effect of glucose concentrations in the fermentation to ethanol at 40°C. (x) 74 g/L glucose; (●) 120 g/L glucose; (▲) 170 g/L glucose; (■) 220 g/L glucose; and (♦) 270 g/L glucose.

Table 1 Fermentation parameters on various glucose concentrations using *S. cerevisiae* Sc90 at 40°C.

Glucose concentrations	74 g/L	120 g/L	170 g/L	220 g/L	270 g/L
Specific growth rate (1/h)	0.536	0.516	0.521	0.327	0.273
Maximum ethanol concentration (g/L)	24.24	30.61	65.01	85.87	54.49
Ethanol production rate (g/(L h))	0.331	0.613	1.073	1.779	0.647
Ethanol yield on substrates (g/g)	0.466	0.293	0.417	0.433	0.279
Glucose consumption rate (g/(L h))	2.559	4.183	1.608	2.057	2.016
Yield efficiency (%)	91.37	57.45	81.76	84.90	54.70

Note: As percentage of conversion efficiency 0.51 g ethanol/g glucose

CONCLUSION

In this study, viable cell, substrates consumption, and ethanol concentration, ethanol production rate, and yield efficiency (%) were affected by different proportions of glucose concentration in the media. These results showed that the highest ethanol production of high glucose concentration was 220 g/L glucose concentration at 40°C. The specific growth rate, ethanol production rate, ethanol concentration and Yield efficiency were 0.327 1/h, 1.779 g/L h, 85.87 g/L, and 84.90 %, respectively. Therefore, *S. cerevisiae* Sc90 has shown as a potential strain for high gravity fermentation.

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