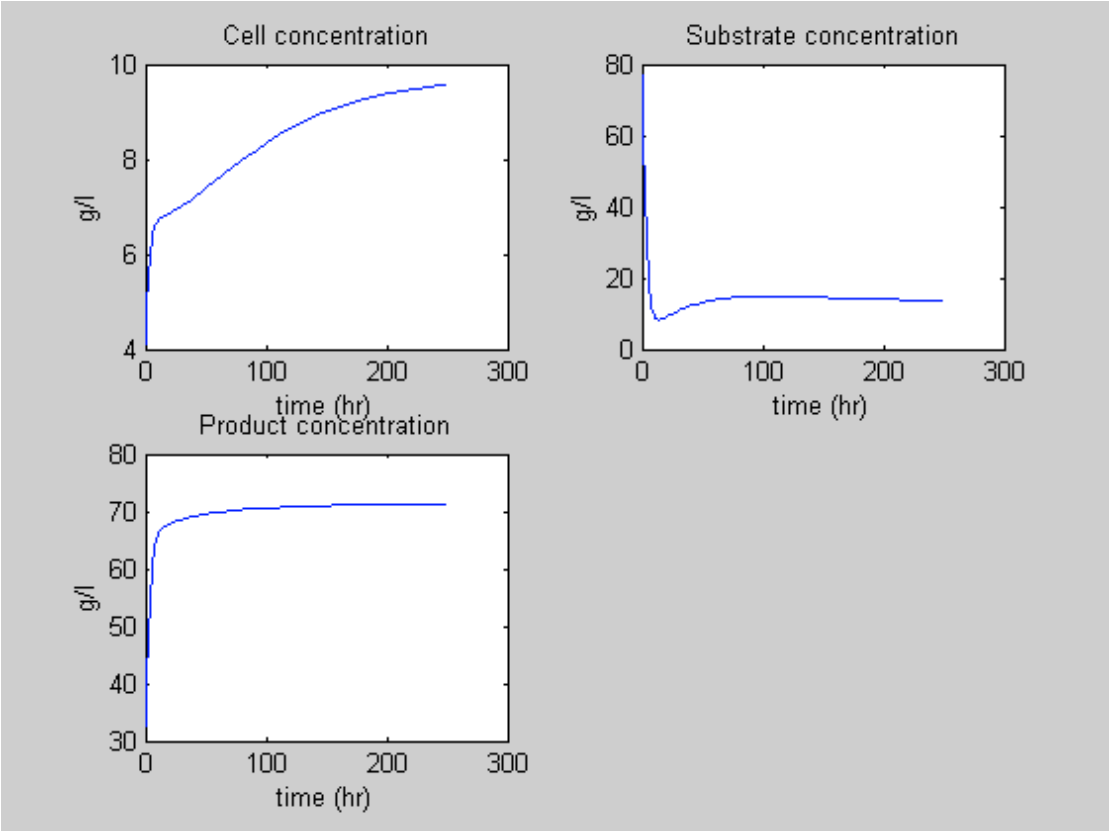


(b)

At time= 250 hr: cell conc^{n} = 9.56 g/L, glucose conc^{n}= 13.59 g/L, and ethanol conc^{n} = 71.24 g/L

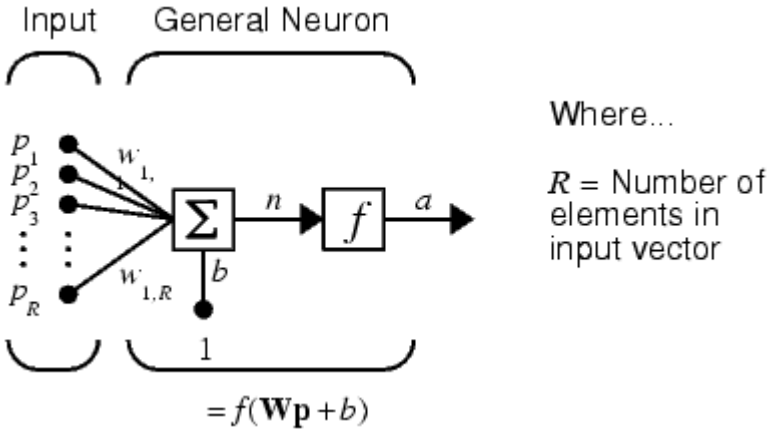
Figure 8.1.11 Simulation results from the continuous process with 70% cell recycle; reducing sugar in feed= 180 g/L with dilution rate of (a) 1/24 hr^{-1} and (b) 1/38 hr^{-1}.

8.2 การสร้างแบบจำลองแบบข่ายงานนิวรัล (Neural Networks) ของการผลิตเอทานอลจากกากน้ำตาลโดย *Saccharomyces cerevisiae*

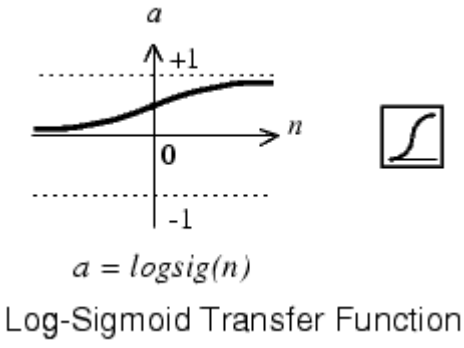
แม้ว่าแบบจำลองทางจลนศาสตร์ที่พัฒนาขึ้นจะใช้ทำนายและอธิบายผลของพารามิเตอร์ต่างๆที่มีต่อการหมักกากน้ำตาลเพื่อเปลี่ยนเป็นเอทานอลได้เป็นอย่างดี อย่างไรตามเมื่อต้องการใช้แบบจำลองในการทำนายกระบวนการหมักในระดับอุตสาหกรรม ที่มีการปรับเปลี่ยนขั้นตอนสภาวะต่างๆในระหว่างการหมัก มีความแตกต่างของวัตถุดิบตั้งต้นหรือสายพันธุ์ยีสต์ที่ใช้ และการแตกต่างทางรูปทรงและขนาดถังหมัก อัตราการกวน การไหลเวียนของสาร ตลอดจนอัตราการให้อากาศ (ออกซิเจน) การนำค่าจลนพลศาสตร์พารามิเตอร์และแบบจำลองที่สร้างขึ้นในการทดลองขวดเขย่าในห้องปฏิบัติการมาใช้อาจไม่ค่อยสะดวก เนื่องจากความแตกต่างหลายอย่างของระบบทำให้จำเป็นต้องมีการปรับสมการเพื่อการปรับค่าจลนพลศาสตร์พารามิเตอร์ใหม่ จากการทดสอบที่ผ่านมาแสดงให้เห็นว่าการเปลี่ยนจากกระบวนการหมักแบบครั้งคราวเป็นแบบต่อเนื่องมีผลให้จลนพลศาสตร์ของระบบเปลี่ยนไป นอกจากนี้ค่าพารามิเตอร์ที่วัดได้ หรือข้อมูลจากการหมักที่วิเคราะห์ได้จากกระบวนการหมักจริงในอุตสาหกรรม บางครั้งมีความคลาดเคลื่อนค่อนข้างมาก มีความแตกต่างของวิธีวิเคราะห์ ทำให้การทำนายโดยใช้แบบจำลองทางจลนศาสตร์ที่สร้างจากผลในห้องปฏิบัติการมีความแตกต่างจากผลที่วัดได้จากการหมักในโรงงาน

ในปัจจุบันนี้ได้มีการนำแบบจำลอง Neural Networks มาพัฒนาใช้สำหรับการทำนายกระบวนการทางด้านเคมีและชีวเคมี การใช้แบบจำลองจลนพลศาสตร์มาใช้ทำนายสำหรับระบบที่มีลักษณะไม่เป็นเชิงเส้นและมีพารามิเตอร์หลายค่ามากไม่สะดวกนักเนื่องจากใช้เวลานานและบางพารามิเตอร์ก็ต้องใช้เวลามากในการวัดค่าหรือบางครั้งก็ไม่สามารถประมาณค่าได้โดยเฉพาะพารามิเตอร์ที่เกี่ยวข้องกับกระบวนการทางชีวภาพบางพารามิเตอร์ ดังนั้นงานวิจัยนี้จึงได้ทำการพัฒนาแบบจำลองแบบข่ายงานนิวรัล (Neural Networks) ของการผลิตเอทานอลจากกากน้ำตาลโดย *Saccharomyces cerevisiae* โดยทดสอบแบบจำลองกับข้อมูลการหมักเอทานอลในระดับห้องปฏิบัติการ และข้อมูลจากกระบวนผลิตเอทานอลจากโครงการขนาดย่อม (ถังหมักขนาด 2500 ลิตร) สำหรับแบบจำลองแบบข่ายงานนิวรัลที่ใช้ในการทดสอบนี้เป็นแบบที่ขึ้นกับเวลา เป็น Dynamic Neural Network เพื่อใช้ในการทำนายของการเปลี่ยนแปลง ของความเข้มข้นเอทานอล น้ำตาล เซลล์ ยีสต์ระหว่างการหมักแบบครั้งคราว (Batch) และแบบ Fed Batch ทำการทดสอบถึงผลของพารามิเตอร์ต่างๆที่ใช้ในชุดข้อมูลป้อน ต่อการทำนายกระบวนการหมัก โดยเลือกใช้ฟังก์ชัน logarithmic-sigmoid และ pure-linear เป็น ฟังก์ชันกระตุ้น (activation function) ในชั้น hidden และ output layer ตามลำดับเนื่องจากพบว่าฟังก์ชันดังกล่าวใช้กับลักษณะไม่เป็นเชิงเส้นของระบบได้ดี โดยใช้กลไก Backpropagation แบบ Levenberg-Marquardt ในการสร้างชุดของ

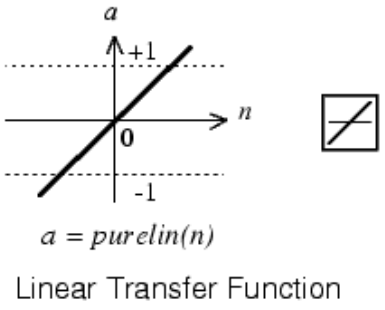
weights (W) และ biases (b) ที่เหมาะสมกับระบบ จำนวน nodes ที่ใช้ในโปรแกรมได้จากการทดสอบหาค่าที่เหมาะสม โดยลักษณะพื้นฐานทั่วไปของแบบจำลอง Neural Networksแสดงดังในรูปที่ 8.2.1, สมการ logarithmic-sigmoid และ pure-linear แสดงดังในรูปที่ 8.2.2และ 8.2.3 ตามลำดับ ลักษณะของแบบจำลอง Neural Networks ที่ใช้กับข้อมูลจากการหมักเอทานอลในงานวิจัยนี้แลดงดังในรูปที่ 8.2.4 ในกรณีที่มีการนำข้อมูลสายออก (Output) ย้อนกลับมาใช้ในการทำนายจุดต่อไป (cross combination) ทำแบบล่วงหน้า 1ขั้นเป็นไปดังแสดงที่เส้นประของรูป 8.2.4



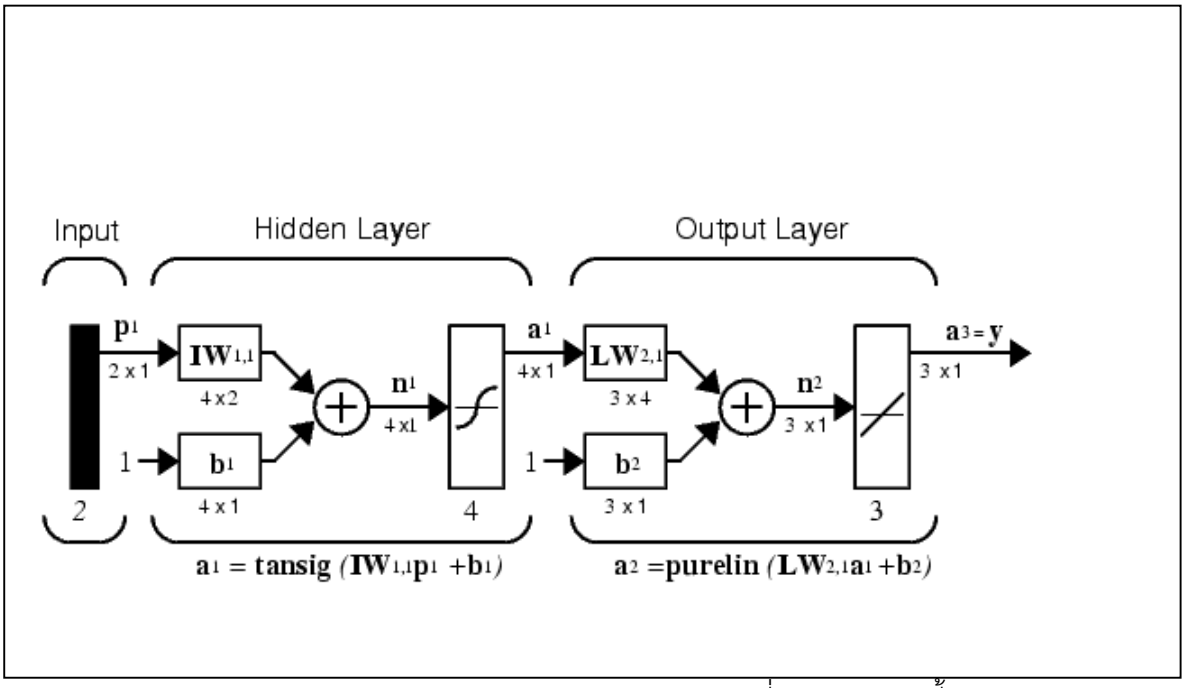
รูป 8.2.1 แสดงการกำหนดฟังก์ชันในแบบจำลอง Neural Networks พื้นฐาน



รูป 8.2.2 Log-Sigmoid Transfer function



รูป 8.2.3 Pure-linear Transfer function



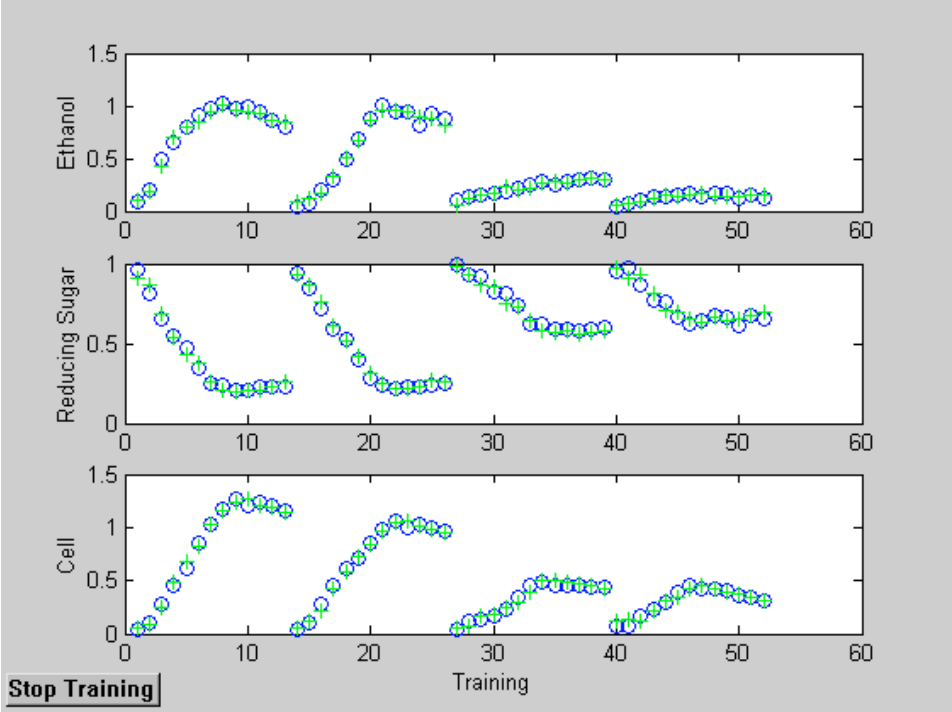
รูป 8.2.4 แสดงการกำหนดฟังก์ชันในแบบจำลอง Neural Networks ที่ใช้ในงานวิจัยนี้

ผลจากการทดสอบแบบจำลองกับข้อมูลจากห้องปฏิบัติการพบว่ามีความสอดคล้องเป็นอย่างดี (รูปที่ 8.2.5) โดยแบบจำลองสามารถทำนายการเปลี่ยนแปลงของความเข้มข้นเซลล์ น้ำตาลตาลรีดิวซ์และเอทานอลตามความเปลี่ยนแปลงของความเข้มข้นน้ำตาลเริ่มต้นและอุณหภูมิได้ดี อย่างไรก็ตามเมื่อสร้างแบบจำลองทดสอบกับชุดข้อมูลจากโรงงานพบการเบี่ยงเบนของการทำนายความเข้มข้นของเซลล์ซึ่งเทียบกับข้อมูลจำนวนนับของเซลล์ ทั้งนี้ส่วนสำคัญเนื่องมาจากความคลาดเคลื่อนอย่างมากของตัวข้อมูลจำนวนนับของเซลล์เอง อย่างไรก็ดีแบบจำลองข่ายงานนิวรัลสามารถทำนายการเปลี่ยนไปของน้ำตาลรีดิวซ์และเอทานอลได้ดีที่สภาวะควบคุมต่างๆ โดยพบว่าปัจจัยเวลาเป็นหนึ่งในปัจจัยสำคัญในการทำนายการเปลี่ยนแปลงของระบบ ผลการตรวจสอบความคลาดเคลื่อนเฉลี่ยกำลังสอง (mean square error) ของข้อมูลที่ปรับแล้ว (ให้อยู่ในช่วงประมาณ 0-1) แสดงในตารางที่ 8.2.1 โดยจำนวนที่เหมาะสมของ node คือ 13-15 ผลการเปรียบ

เทียบจากตารางแสดงให้เห็นผลของการเลือกปัจจัยในชุดข้อมูลป้อนที่มีผลต่อความถูกต้องในการทำนาย

TRAINING:
Backpropagation to update weight and bias values according to Levenberg-Marquardt optimization (13 nodes).
Input: time, temperature, [reducing sugar], [ethanol] and [cell].

Temperature =30, 35, 38, 42 ^{o}C; Initial reducing sugar=220-230 g/L



VALIDATION: Temperature =33 ^{o}C; Initial reducing sugar=222 g/L

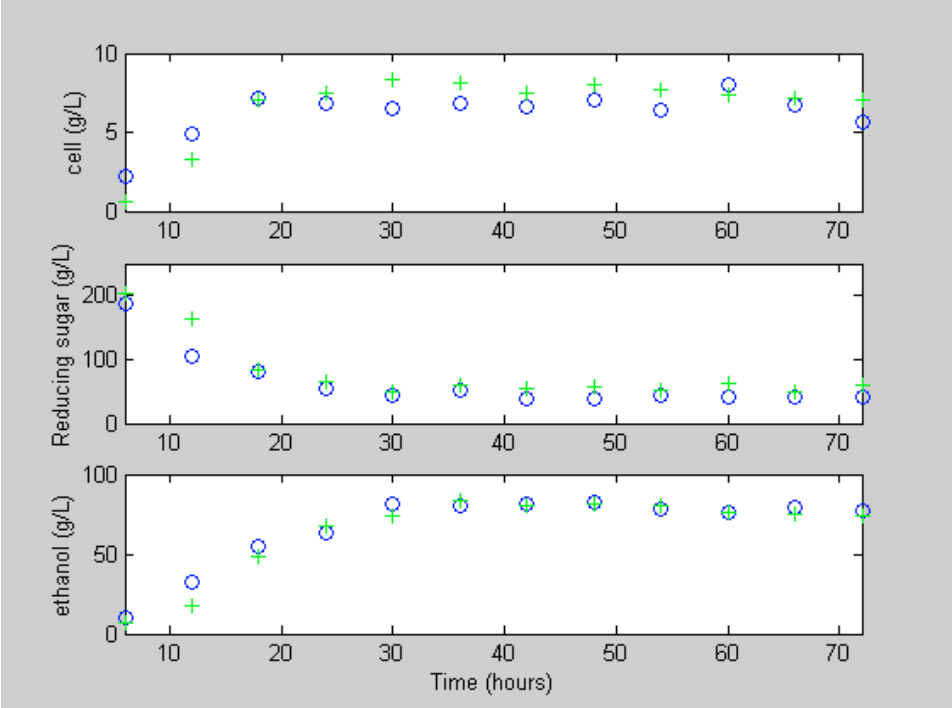
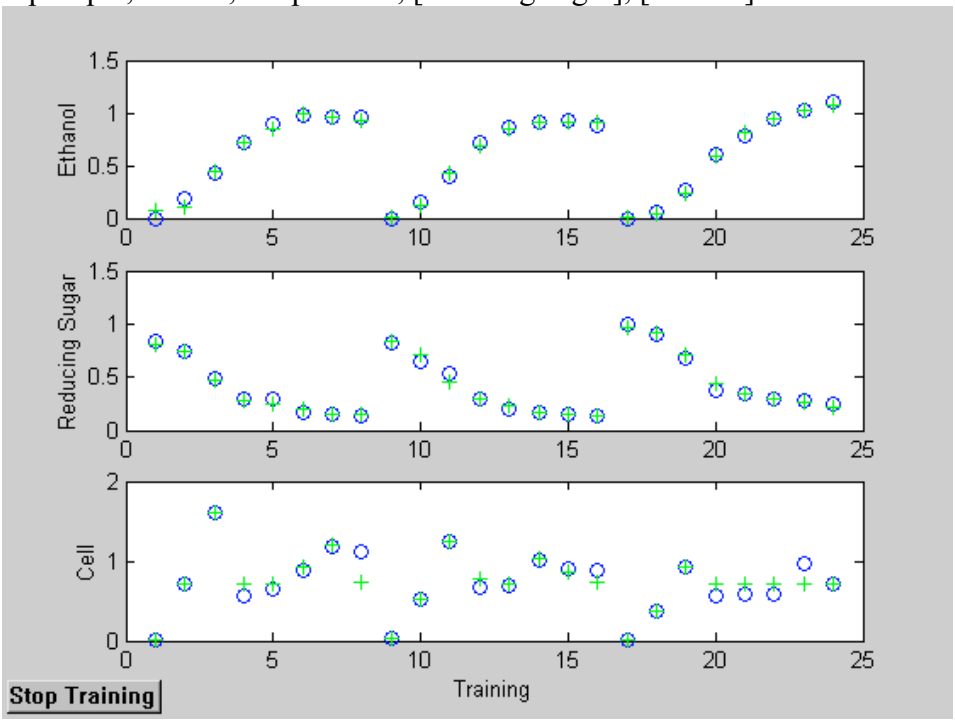


Figure 8.2.5 The Training and validation results of Dynamic Neural Network applied to experimental data from shaking flask; o = experimental data, + = simulation data.

TRAINING:
Backpropagation to update weight and bias values according to Levenberg-Marquardt optimization (13 nodes).
Input: pH, % brix, temperature, [reducing sugar], [ethanol] and cell count.



VALIDATION

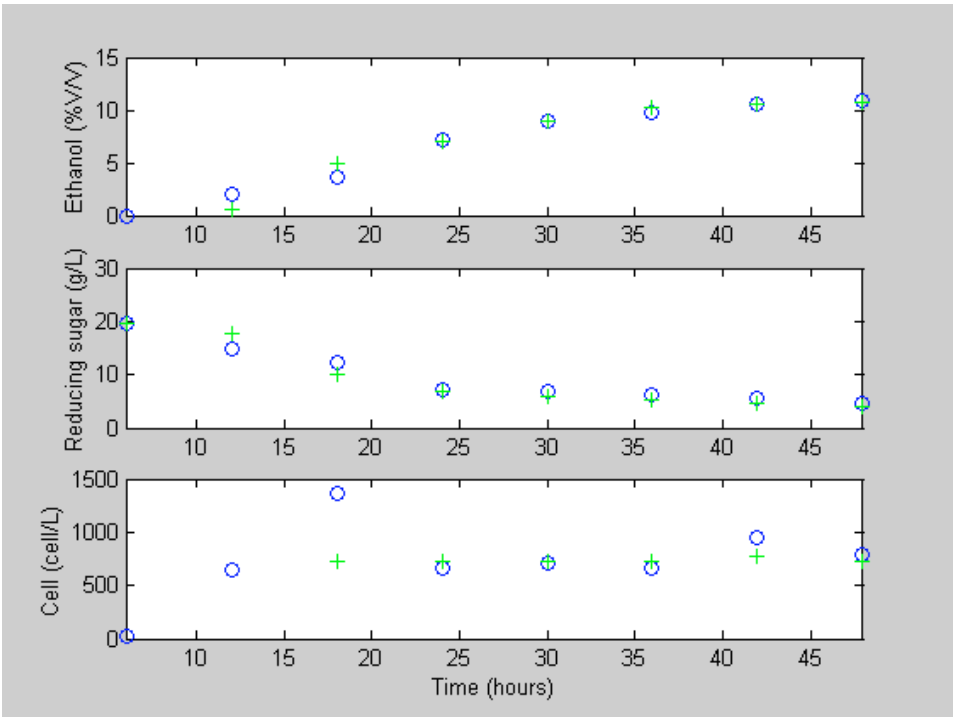
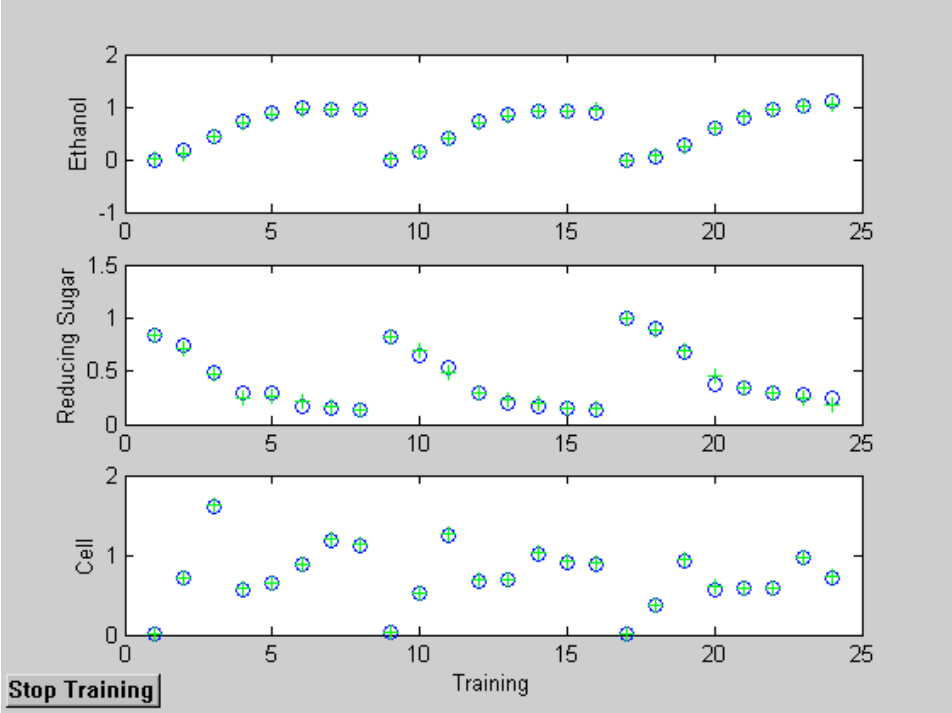


Figure 8.2.6 (a) The Training and validation results of Dynamic Neural Network applied to data from 2500 L batch bioreactor, o = experimental data, + = simulation data.

TRAINING:
Backpropagation to update weight and bias values according to Levenberg-Marquardt optimization (10 nodes).
Input: time, pH, % brix, temperature, [reducing sugar],[ethanol] and cell count.



VALIDATION

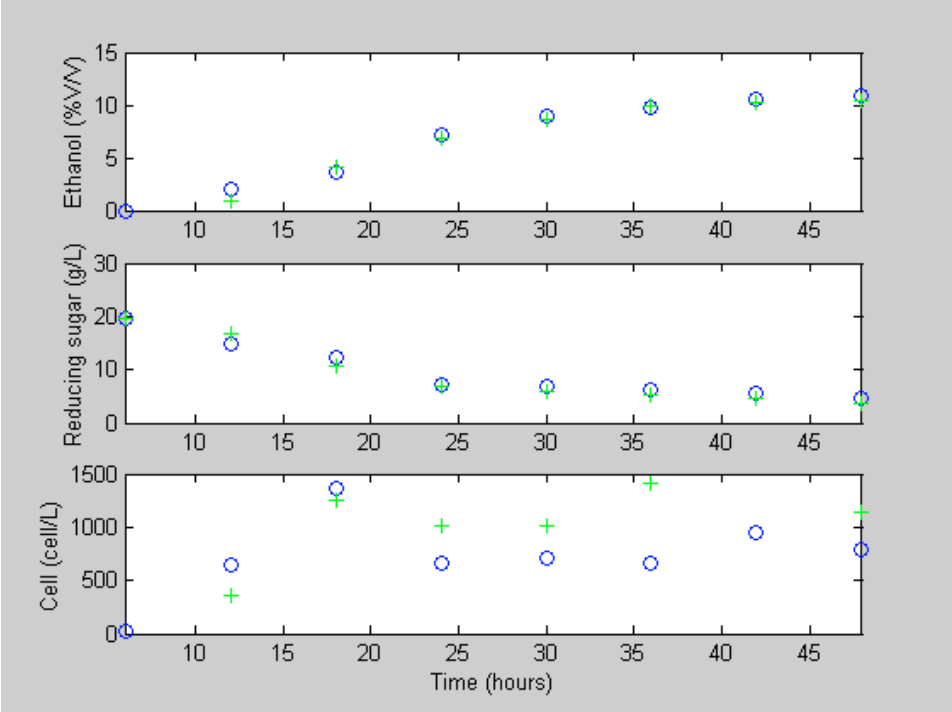
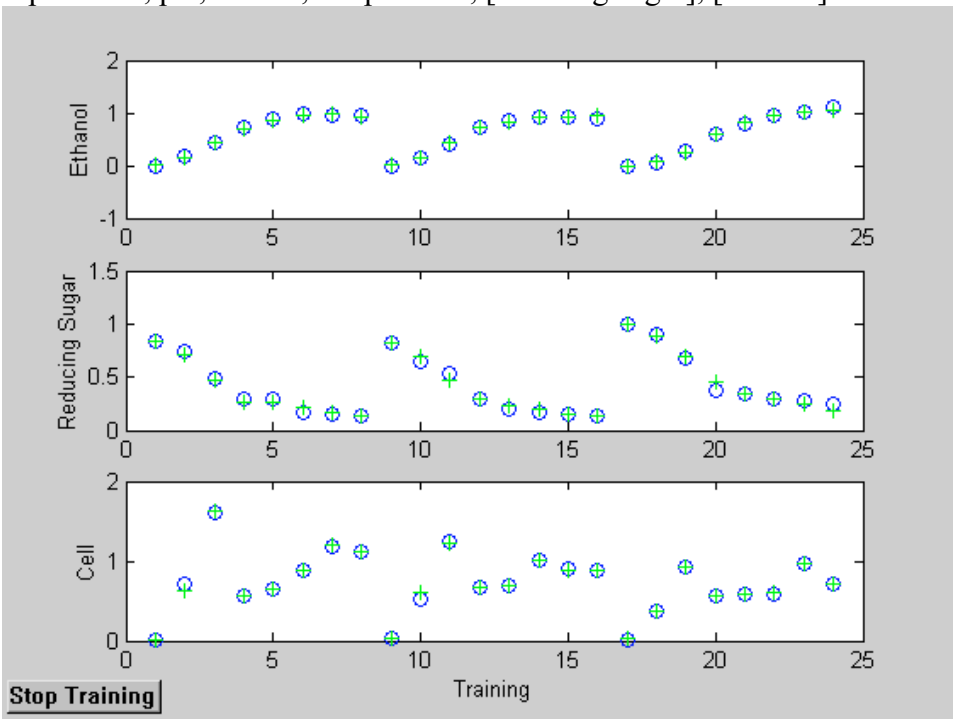


Figure 8.2.6 (b) The Training and validation results of Dynamic Neural Network applied to data from 2500 L batch bioreactor, o = experimental data, + = simulation data.

TRAINING:
Backpropagation to update weight and bias values according to Levenberg-Marquardt optimization (13 nodes).
Input: time, pH, % brix, temperature, [reducing sugar], [ethanol] and cell count.



VALIDATION

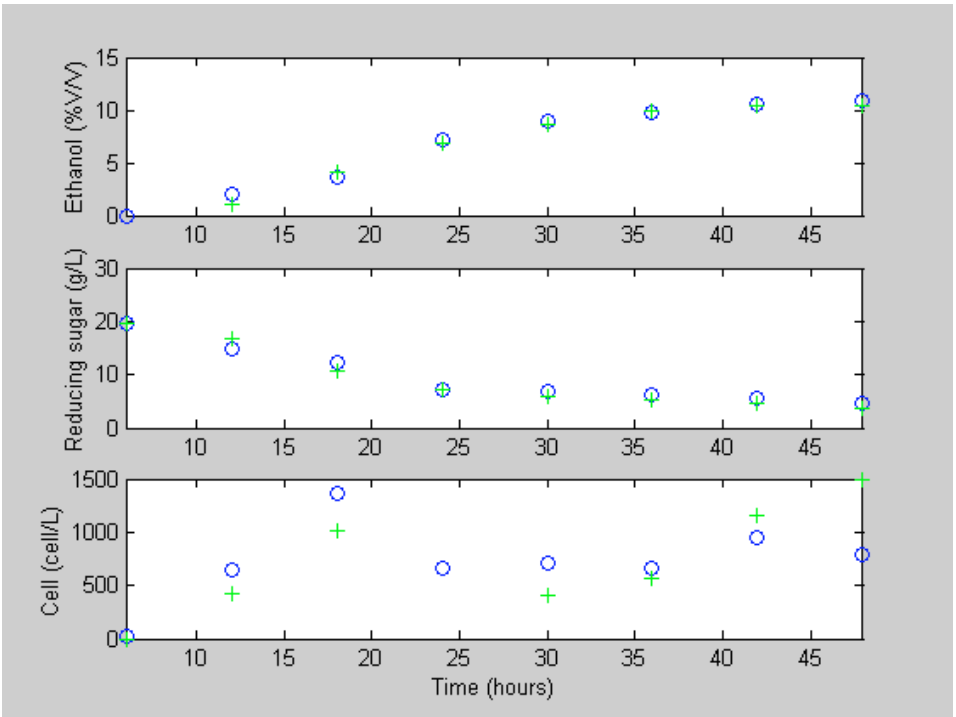
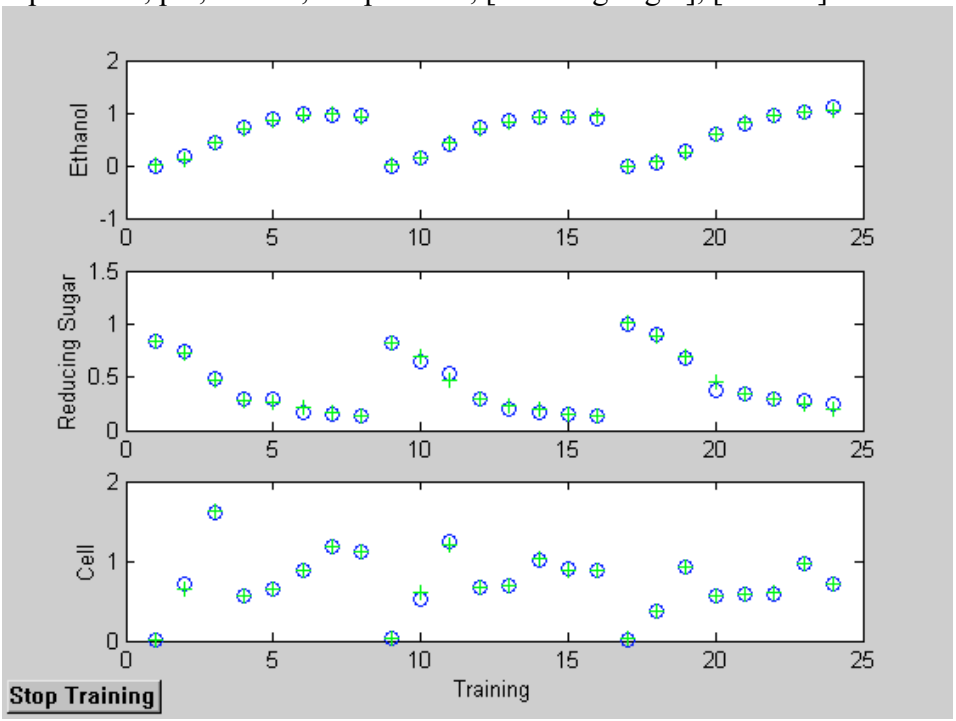


Figure 8.2.6 (c) The Training and validation results of Dynamic Neural Network applied to data from 2500 L batch bioreactor, o = experimental data, + = simulation data.

TRAINING:
Backpropagation to update weight and bias values according to Levenberg-Marquardt optimization (15 nodes).
Input: time, pH, % brix, temperature, [reducing sugar], [ethanol] and cell count.



VALIDATION

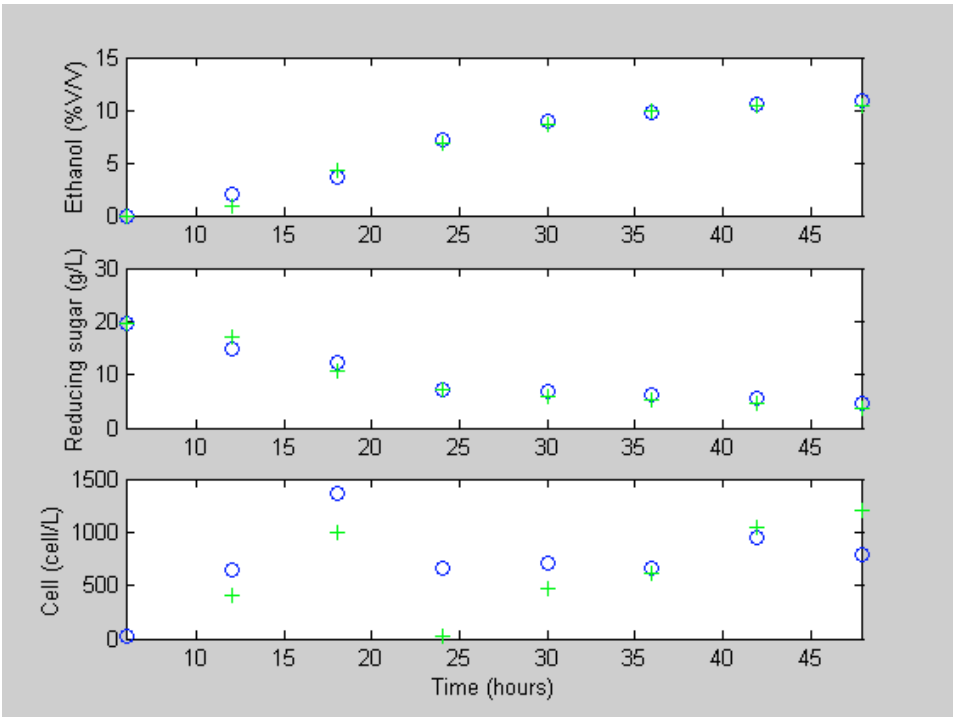
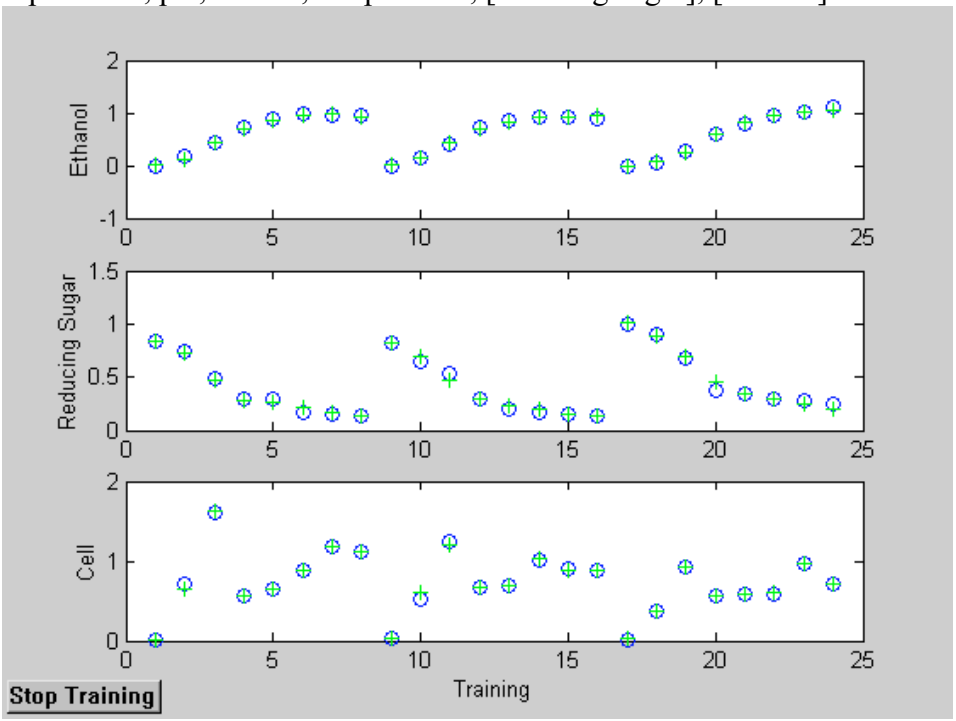


Figure 8.2.6 (d) The Training and validation results of Dynamic Neural Network applied to data from 2500 L batch bioreactor, o = experimental data, + = simulation data.

TRAINING:
Backpropagation to update weight and bias values according to Levenberg-Marquardt optimization (17 nodes).
Input: time, pH, % brix, temperature, [reducing sugar], [ethanol] and cell count.



VALIDATION

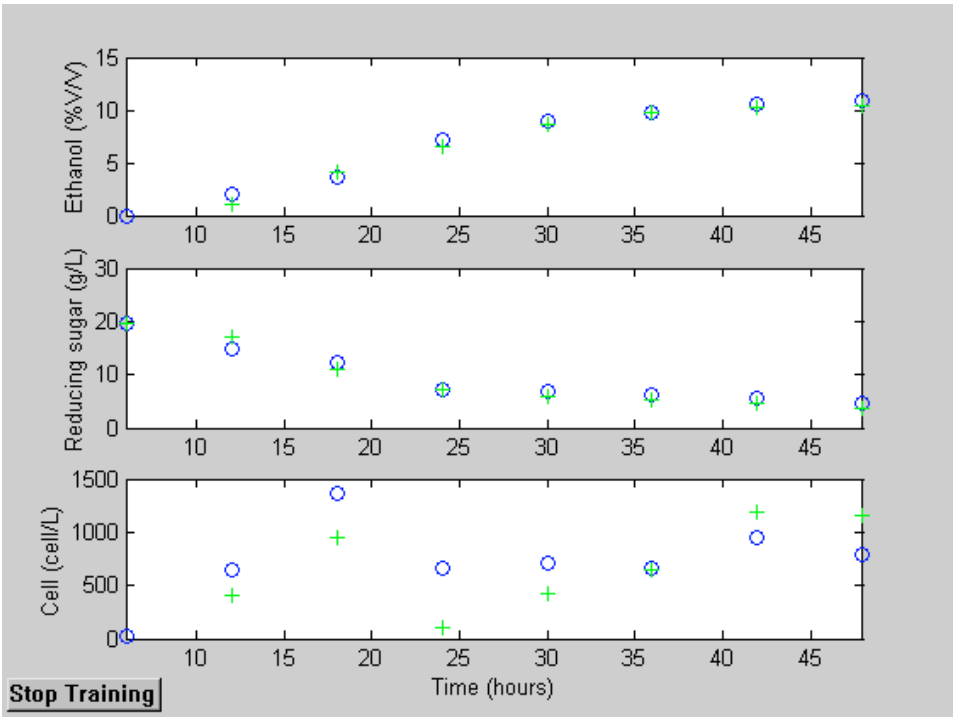


Figure 8.2.6 (e) The Training and validation results of Dynamic Neural Network applied to data from 2500 L batch bioreactor, o = experimental data, + = simulation data.

TRAINING:
Backpropagation to update weight and bias values according to
Levenberg-Marquardt optimization (15 nodes).

Data Set: 1 batch and 2 fed-batch

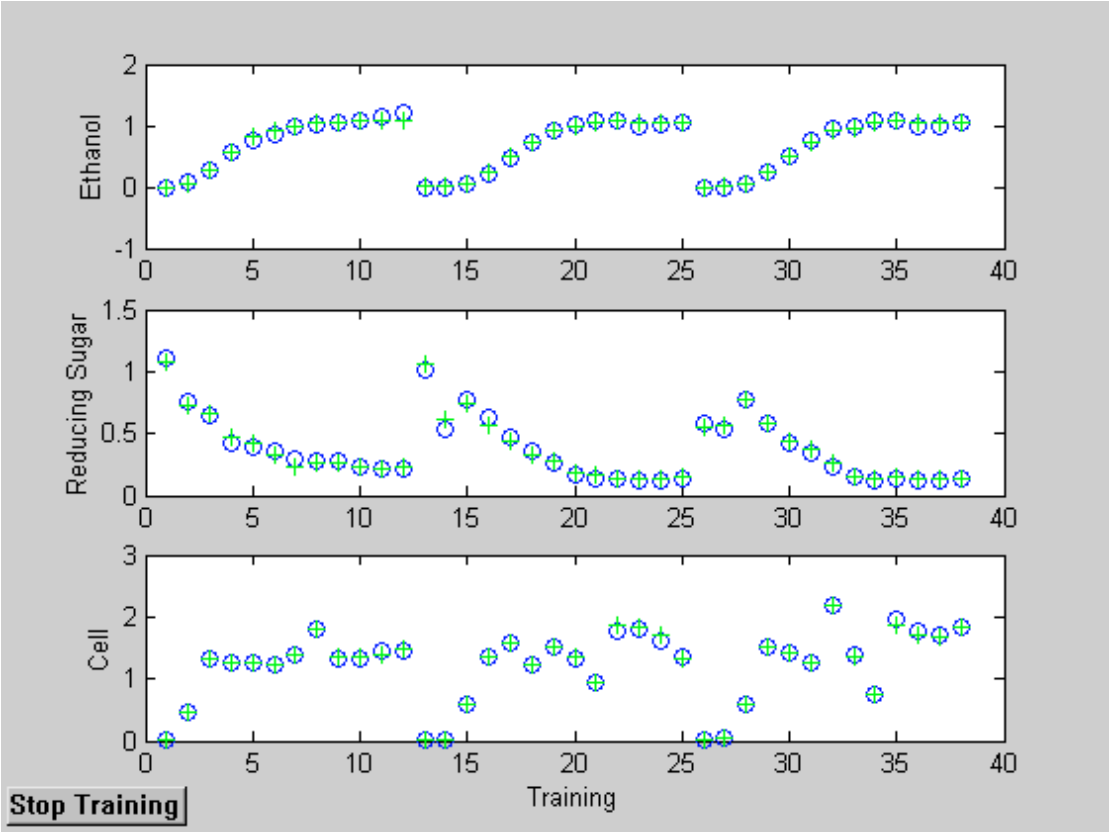
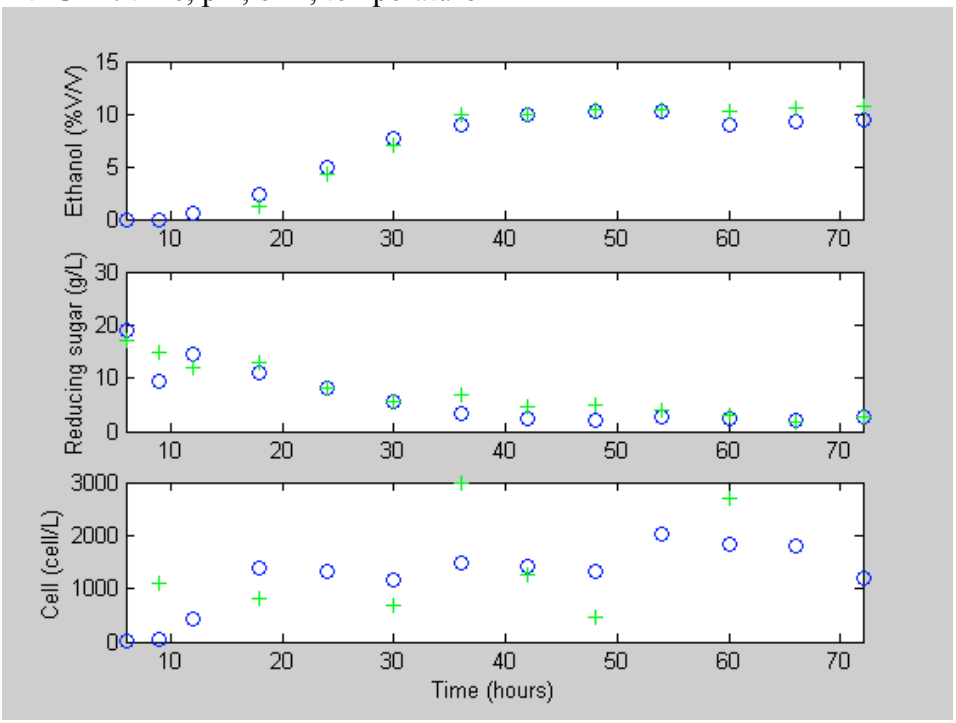


Figure 8.2.7 The Training results of Dynamic Neural Network applied to
data from 2500 L (fed-batch) bioreactor, o = experimental data, + = simulation data.

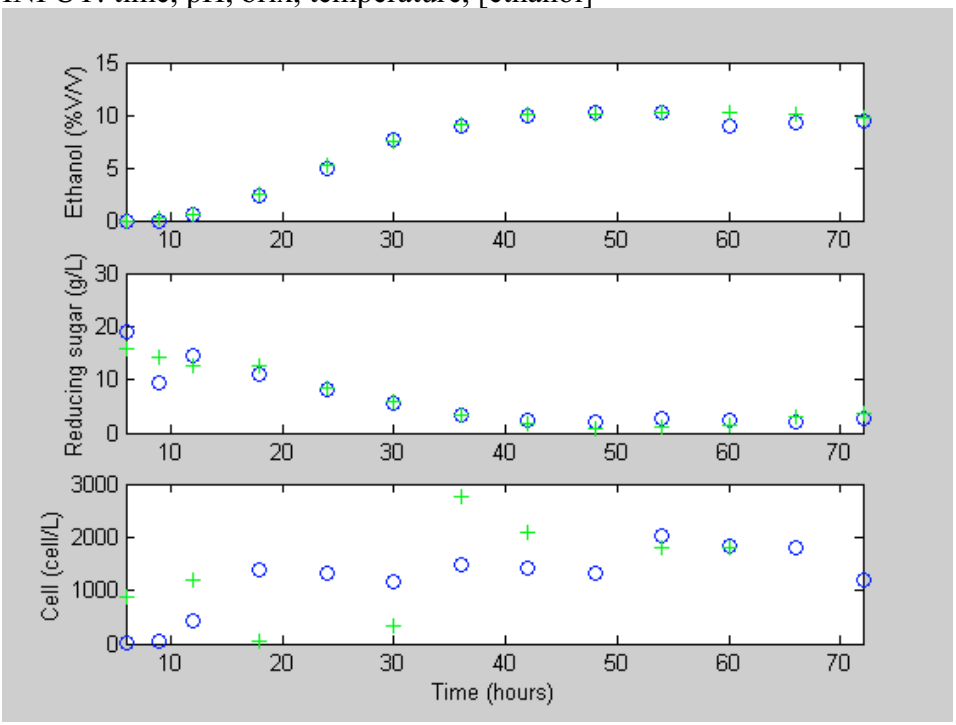
VALIDATION

INPUT : time, pH, brix, temperature



(a)

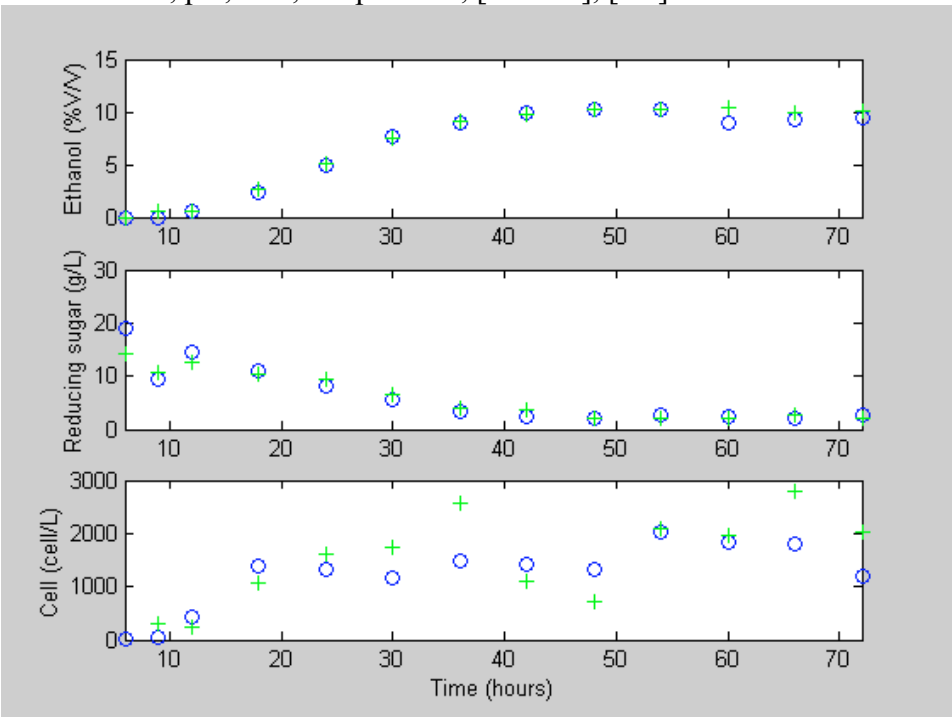
VALIDATION
INPUT: time, pH, brix, temperature, [ethanol]



(b)

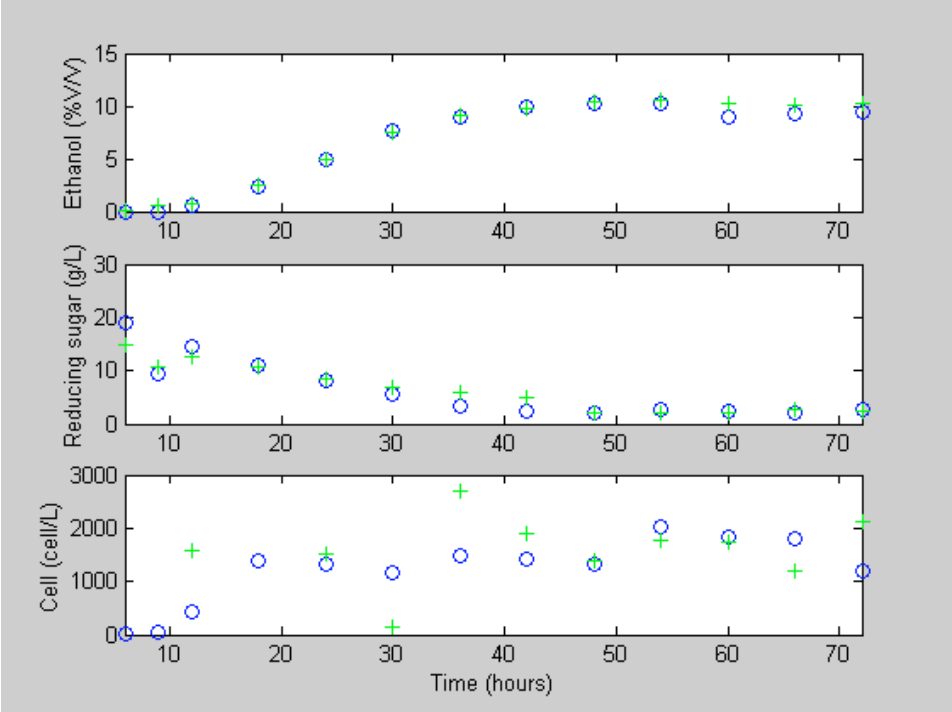
Figure 8.2.8 The validation results of Dynamic Neural Network applied to data from 2500 L (fed- batch) bioreactor, o = experimental data, + = simulation data.
(a) Input = time, pH, brix, temperature
(b) Input = time, pH, brix, temperature, [ethanol]

VALIDATION
INPUT: time, pH, brix, temperature, [ethanol], [RS]



(c)

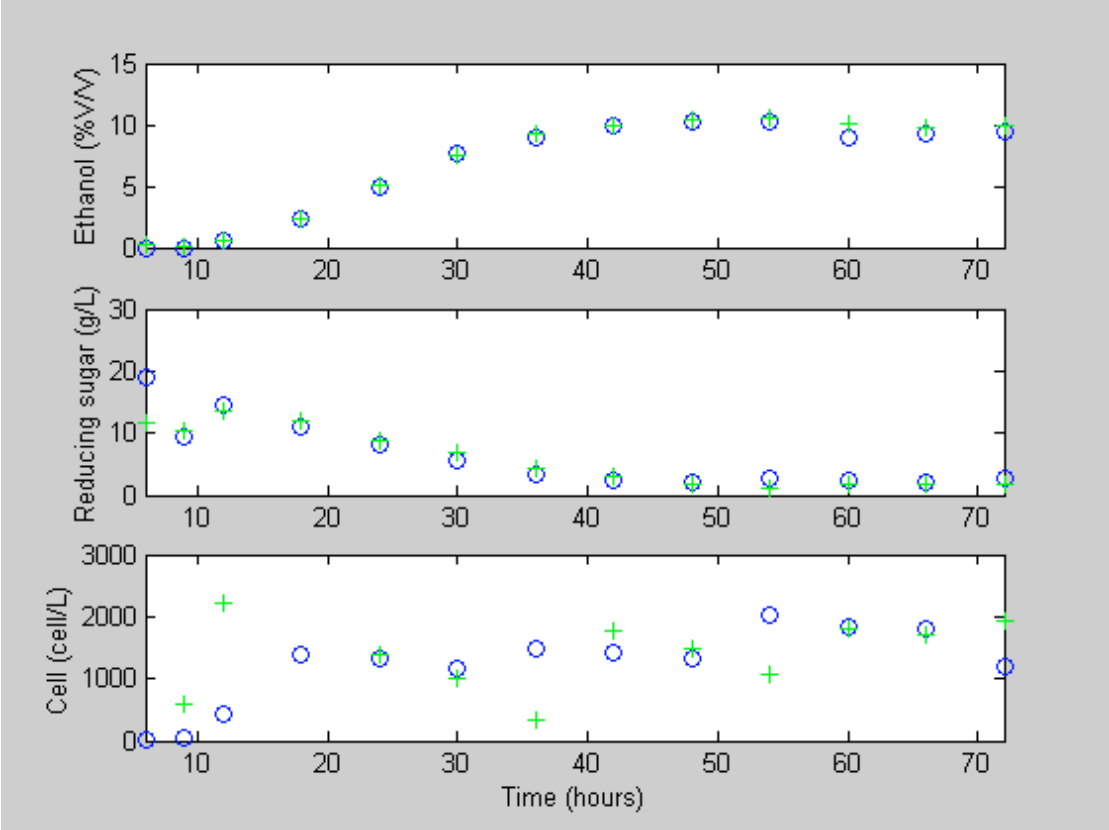
VALIDATION
INPUT : time, pH, brix, temperature, [ethanol], [RS], cell counts



(d)

Figure 8.2.8 The validation results of Dynamic Neural Network applied to data from 2500 L (fed- batch) bioreactor, o = experimental data, + = simulation data.
(c) Input = time, pH, brix, temperature, [ethanol], [RS]
(d) Input = time, pH, brix, temperature, [ethanol], [RS], cell counts

VALIDATION
INPUT: pH, brix, temperature, [ethanol], [RS], cell counts



(e)

Figure 8.2.8 The validation results of Dynamic Neural Network applied to data from 2500 L (fed- batch) bioreactor, o = experimental data, + = simulation data. (e) Input = pH, brix, temperature, [ethanol], [RS], cell counts

System	Figure	Input	# Nodes	MSE of normalized data		
				[Ethanol]	[Reducing Sugar (RS)]	[Cell]
Batch in 500 mL	8.2.5	time, temp, [ethanol], [RS], [cell],	13	0.0026	0.0093	0.0064
Batch in 2500L	8.2.6 (a)	brix, temp, pH, [ethanol], [RS], [cell count],	13	0.0051	0.0051	71.5430
	8.2.6 (b)	time, brix, temp, pH, [ethanol], [RS], [cell count]	10	0.0025	0.0036	0.1933
	8.2.6 (c)	time, brix, temp, pH, [ethanol], [RS], [cell count]	13	0.0021	0.0036	0.1585
	8.2.6 (d)	time, brix, temp, pH, [ethanol], [RS], [cell count]	15	0.0024	0.0034	0.1059
	8.2.6 (e)	time, brix, temp, pH, [ethanol], [RS], [cell count]	17	0.0026	0.0034	0.1018
Batch & Fed Batch in 2500 L	8.2.8 (a)	time, brix, temp, pH	15	0.0083	0.014	1.3442
	8.2.8 (b)	time, brix, temp, pH, [ethanol]	15	0.0019	0.0086	2.0386
	8.2.8 (c)	time, brix, temp, pH, [ethanol],[RS]	15	0.0028	0.0065	0.3189
	8.2.8 (d)	time, brix, temp, pH, [ethanol],[RS], [cell count]	15	0.0028	0.0069	0.7796
	8.2.8 (e)	brix, temp, pH, [ethanol],[RS], [cell count]	15	0.0018	0.0128	2.3380

Table 8.2.1 Mean square error (Mse) results for the validation of normalized data.

ผลที่ได้รับ (output) จากโครงการ

1. แบบจำลองจลนพลศาสตร์สำหรับทำนายกระบวนการหมักเอทานอลและอธิบายผลของปัจจัยควบคุมต่อจลนพลศาสตร์ในการหมัก
2. แบบจำลองแบบข่ายงานนิวรัล (Neural Networks) สำหรับการทำนายกระบวนการหมักในโรงงานเพื่อใช้ประโยชน์ในการควบคุม
3. ผลงานการวิจัย

   3.1 นำเสนอผลงานในที่ประชุมนานาชาติ 1 ครั้ง

   3.2 ผลงานตีพิมพ์ระดับนานาชาติ 1-2 ฉบับ (อยู่ในระหว่างการพิจารณา 1 ฉบับ ในระหว่างการจัดทำ 1 ฉบับ)

,

ภาคผนวก

Thursday , June 24, 2004

Quantitative Effect of Initial Sugar Concentration on Kinetic Parameters of Ethanol Fermentation

Muenduen Phisalaphong*, Nuttapan Srirattana, Wiwut Tanthapanichakoon
Department of Chemical Engineering
Chulalongkorn University, Bangkok 10330, Thailand

*Corresponding author: Tel: 662-218-6875
Fax: 662-218-6877
E-mail address: muenduen.p@chula.ac.th

Abstract

The quantitative effects of initial sugar concentration on the kinetic parameters of ethanol fermentation using the flocculating yeast, *Saccharomyces cerevisiae* M30, were evaluated. Molasses, one of the main feedstock for ethanol fermentation, is used as the substrate source for all experiments. An unstructured kinetic model with total biomass, substrate and ethanol concentrations as state variables and initial sugar concentration as a key parameter was developed from the batch fermentation results. Effects of the initial sugar concentration on the kinetic parameters were observed and the relations could be expressed as polynomial functions. A high initial sugar could lead to a decrease in the maximum specific growth, and increases in both the maximum ethanol production rate and the energy demand for cell maintenance. The kinetic parameters were not significantly affected by the scale-up of the

Review Copy

Thursday , June 24, 2004

fermentation process from 0.5 to 10-litre fermentors. The adopted mathematical model could explain very well the dynamics of ethanol fermentation from the beginning up to the stationary phase.

Keywords: Ethanol fermentation, *Saccharomyces cerevisiae*, Inhibitors, Kinetic parameters, Molasses, Yeast growth

1. Introduction

Thanks to limited global supply of oil, ethanol has re-emerged as an alternative to, or extender for, petroleum-based liquid fuels. In Thailand, to reduce national dependence on costly imported fuel and to assist the creation of a new domestic fuel industry, five new projects with a total ethanol production capacity of 800,000 liters/day from molasses or cassava have recently received approval. The Thai government plans to further increase the supply of ethanol to 2-mil liters/day in the next four to five years to replace the imported gasoline additive MTBE.

To effectively and efficiently operate the fermentation process, the kinetic characteristics of cell growth are required. During batch fermentation of *Saccharomyces cerevisiae*, the activities of the microorganisms closely respond to changes in the environmental conditions along with the cultivation time, which is accompanied by variations in mass transfer around and metabolic behavior of the

Review Copy

Thursday , June 24, 2004

microorganisms. To gain insight into the morphology-associated time-variant process dynamics, various kinetic models associated with key parameters for ethanol fermentation have been proposed [1-9]. Monod's model is the most widely accepted mathematical model of microbial growth. Nevertheless, Monod's model and modifications of it often fail to adequately describe the actual system in a wide range of initial glucose concentration [1, 2]. Yeast strains normally used in industrial processes have limited osmotolerance. An extremely high initial sugar concentration reportedly results in loss of sugar transport activity, causing cell death and reducing ethanol production [10]. Growth inhibition by osmotic stress occurs when the concentration of a certain solute becomes so high that a large osmotic pressure gradient is established across the cell membrane. In order to balance the osmotic pressure, yeasts produce glycerol and trehalose to stabilize the proteins and cell wall [11]. The HOG (high osmolarity glycerol) pathway mediates a significant part of the response of the yeast cells to a hyperosmotic pressure [11, 12]. Inhibition of the growth rate due to osmotic stress and variation in the ethanol yield (Y_{PS}) of multistage continuous fermentation during the production of fuel alcohol were reported **[**13**].** It is well known that an important effect of an initial reducing sugar above 30 g/L is the metabolic repression of the oxidation pathway leading to a switch from the oxidation of glucose for cell growth to ethanol fermentation [1,12]. Kinetic parameters associated with various

Review Copy

Thursday , June 24, 2004

kinetic models were investigated using data obtained in batch fermentation with immobilized *Saccharomyces cerevisiae* at 2-10% (w/v) initial glucose concentrations; however, the resulting parameter values lacked consistency in their magnitudes [1].

In order to quantify the relationship between the kinetic parameters of ethanol fermentation and the initial substrate concentration, the present study investigates the effect of the initial cane-molasses concentration, concurrently the limiting substrate, on the parameters. The results should provide a better understanding of the major factors affecting cell activities. The adopted model will be useful for the optimization of the ethanol fermentation process.

2. Material and Methods

2.1. Yeast strains

Saccharomyces cerevisiae M.30, a flocculated yeast selected on the basis of its high efficiency in ethanol fermentation from molasses at high temperature, was used in this study. *S. cerevisiae* M.30 was kindly provided by the laboratory of Dr. Savithree Limthong (Department of Microbiology, Kasetsart University, Bangkok).

Review Copy

Thursday , June 24, 2004

2.2. Culture media

Stock cultures were stored in a PDA agar slant. Starter cultures were prepared by transferring a loop of the stock culture to 100 mL of medium and incubated at 33 °C for 20 hours before each starter culture was transferred to the main culture. The medium for the starter culture contained 0.05% ammonium sulfate and 5% inverse sugar from molasses mash at pH 5.0. The prepared medium was sterilized at 121°C for 20 min.

2.3. Batch fermentation

Batch ethanol fermentation experiments were carried out in duplicate with 3-25% w/v of the initial reducing sugar solution from cane-molasses mash as sole carbon source for *S. cerevisiae*. Experiments were performed in 500 mL Erlenmeyer flasks, with 250 mL total liquid volume. Experiments were initiated by transferring 5% of the starter culture to the prepared medium. Fermentation flasks were then shaken at 150 rpm and kept at 33°C. Fermentation was monitored for 3 days by removing 6 mL samples every 6 hours for cell, sugar and ethanol analyses.

2.4. Analytical methods

Cell concentration was determined by two independent methods. For cell dry weight determination, a 5-mL sample of the fermentation broth was centrifuged at 3,000 rpm for 10 min. The cell pellet was

Review Copy

Thursday , June 24, 2004

resuspended in 0.1-N HCl and washed twice with distilled water and then dried at 90°C for 48 hr and then weighed. Cell concentrations were also measured at 600-nm wavelength with a spectrophotometer after the samples were diluted in order to work in a linear range. Concentrations of ethanol were determined by a gas chromatography system using a Shimadzu Model GC $7A_G$ equipped with a flame ionization detector. A column (0.125 cm id., 2 m, SS) packed with Porapak Q 80-100 mesh was used with N_2 as carrier gas. The injector temperature was 280 °C, and the detector temperature was 300°C. To measure the amount of sugar in the sample, a 0.2-mL of the sample solution was hydrolyzed in 33% HCl at 100°C for 10 minutes and neutralized with NaOH solution. Then the reducing sugar content in the sample solution was determined by Lane and Eynon's method.

2.5. Mathematical modeling

In general, kinetics should be taken into consideration for the globally observed behavior of the culture. To construct a mathematical model that could describe the dynamic process of ethanol fermentation by yeast *Saccharomyces cerevisiae* M30, a comprehensive kinetic model for the cell activities responding to changes in the environmental conditions was developed for three main factors: substrate limiting, substrate inhibition and product inhibition. The ethanol production and cell growth

Review Copy

Thursday , June 24, 2004

usually showed saturated at high substrate concentration and the reaction rate equaled zero if no substrate was available. However, the inhibition by the substrate and product was normally observed, especially at high concentration. Therefore, in this study, the Monod kinetic was modified in both substrate and product terms and combined with death rate and cell maintenance. The mathematical model of the batch fermentation could be written as follows:

Cells: $$\frac{dC_X}{dt} = \propto C_X - K_d C_X \quad (1)$$

Ethanol: $$\frac{dC_P}{dt} = \nu C_X \quad (2)$$

Sugar: $$-\frac{dC_S}{dt} = \frac{1}{Y_{X/S}}\left(\frac{dC_X}{dt}\right) + \frac{1}{Y_{P/S}}\left(\frac{dC_P}{dt}\right) + K_{CM} C_X \quad (3)$$

The rate of cell growth, ethanol production and substrate consumption were closely related to the cell concentration (C_X), ethanol concentration (C_P) and substrate concentration (C_S) where μ, ν, K_d and K_{CM} represent the specific growth rate, specific production rate, specific death rate and maintenance constant, respectively. $Y_{X/S}$ and $Y_{P/S}$ represent the yield coefficients for the cell and for the ethanol on the substrate, respectively. The μ and ν were controlled by substrate limiting effect and inhibitory effects of the substrate and ethanol as follows:

Review Copy

Thursday , June 24, 2004

$$\propto = \propto_m \left(\frac{C_S}{K_S + C_S + \frac{C_S^{\,2}}{K_{SS}}} \right) \left(1 - \frac{C_P}{P_m} \right) \tag{4}$$

$$\nu = \nu_m \left(\frac{C_S}{K_{SP} + C_S + \frac{C_S^{\,2}}{K_{SSP}}} \right) \left(1 - \frac{C_P}{P'_m} \right) \tag{5}$$

In order to investigate the effect of initial sugar concentration (C_{SO}), the following 10 kinetic parameters: μ_m, ν_m, K_S, K_{SS}, P_m, K_d, K_{SP}, K_{SSP}, $Y_{P/S}$ *and* K_{CM} were allowed to vary with respect to C_{SO} in each experiment. The initial parameter values were tentatively estimated from the experimental data by using the initial rate method before running the program iterations. The best-fit value of the parameter was estimated using the least-squares method to minimize the sum of squared errors between the predicted and experimental data. From the developed mathematical model, the numerical solution was obtained using functions in MATLAB.

Results and discussions

Batch fermentation in shake flasks for ethanol production was carried out in duplicate for 72 hours with various initial reducing sugar concentrations from 3 to 25 %W/V. The morphology of the yeast was found to change remarkably along with the initial sugar concentrations

Review Copy

Thursday , June 24, 2004

which reflected variations in the metabolic behavior of the cells. Photographs of cells grown under different initial sugar concentrations were taken after 48 hours cultivation as shown in Figure 1. In order to describe the process dynamics in a simple way, an unstructured model was exploited. In this case, the experimental data indicated that the applied model was affected by the substrate and product concentrations, which could be classified into two types: limiting and inhibiting.

There are many proposed kinetic model for the case of substrate limiting such as Monod, Moser, and Teissier. Apart from substrate limitation, inhibition by either the substrate (sugar) or the product (ethanol) was observed in the present study. Both aspects were examined by numerous authors [1-9]. To be able to predict zero growth for a finite substrate and ethanol concentration, inhibition expressions for the substrate and product were proposed as shown in equations (4) and (5). Figure 2 presents the predicted and experimental data of the cell, substrate and ethanol concentrations at different initial substrate concentrations. Excellent agreement was found between the experimental data and the model simulation. The model well described the growth, substrate utilization and ethanol production from the beginning up to the stationary phase.

In order to investigate the relationship between the kinetic parameters and the initial sugar concentration, the model parameters

Review Copy

Thursday , June 24, 2004

obtained at each different initial sugar concentrations were estimated using the least-squares curve-fitting method (Table 1). As expected, the effects of the initial sugar concentrations on the model parameters were evidently clear. For the batch fermentation, the maximum specific growth rate of cells (μ_m) decreased appreciably whereas the maximum specific production rate of cells (ν_m) increased considerably as the initial sugar concentration increased (Fig. 3.a). The findings were consistent with the reported initial sugar concentration effect on yeast metabolism [1, 12]. At a higher initial reducing sugar concentration, more repression of the oxidation of glucose for cell growth and more stimulation for ethanol fermentation were observed. In addition, the inhibitory effect of the initial sugar concentration above 12 %(w/v) was hereby observed in terms of the inhibition factors of the substrate on cell growth (K_{SS}^{-1}) and on ethanol production (K_{SSP}^{-1}), the values of which increased considerably when the initial sugar concentration increased (Fig. 3.b). As for the cause of the inhibition effect, an excessively high initial sugar concentration could result in the loss of sugar transport activity, which reduced ethanol production and cell growth [10, 16]. Similarly, toxic inhibition effect on the specific growth rate of 24 selected yeast strains in corn stover hydrolysate fermentation was reported [14].

Figure 4 illustrates the influence of the initial sugar concentrations on the maintenance constant, the ethanol inhibition terms and the ethanol

Review Copy

Thursday , June 24, 2004

yield, respectively. The biomass and ethanol yields obtained in this study were 0.5 and 0.35-0.45, respectively, which were comparable to those reported elsewhere [3, 8, 9 and 15]. The ethanol yield on the substrate and the cell maintenance parameter were found to increase as sigmoid curves with respect to the initial sugar concentration whereas the specific death rate and the yield coefficient of cells on the substrate were found to be essentially constant. The ethanol inhibition terms for the specific cell growth (P_m) and for the specific ethanol production rate (P'_m) were of the same magnitude and increased linearly with respect to the initial sugar concentration (Figure 4.b). In fact the increase in the ethanol inhibition terms with respect to the initial sugar concentration has been reported before but their reported values differed in the order of magnitude [1]. In this study, the effects of the initial substrate concentrations on all kinetic parameters could be expressed as polynomial equations.

The above effects of the initial sugar concentration on the kinetic parameters were consistent with a number of previously reported observations. An excessively high initial sugar concentration resulted in the loss of sugar transport activity, causing cell death and reducing ethanol production [10]. However, inhibition tests on many strains of *S. cerevisiae* with certain inhibitors revealed that inhibition by osmotic stress occurred when the concentration of the solutes (initially present or by-products produced during fermentation) became so high that a large osmotic

Review Copy

Thursday , June 24, 2004

pressure gradient was established across the cell membrane. In order to balance the osmotic pressure, the yeast reacted by producing glycerol and trehalose to stabilize the proteins and cell wall [11, 12]. For solutes such as glucose that could cross the cell membrane, the yeast protected their cells by pumping the solutes out of the cell into the medium against a concentration gradient, which resulted in increased ethanol production at the expenses of a lower growth rate and less biomass production [12]. Therefore, in order to balance the increased osmotic pressure, the energy demand for cell maintenance at a higher initial sugar concentration increased. In this study, the required substrate for cell maintenance (K_{CM}) and ethanol yield ($Y_{P/S}$) were found to increase as the initial sugar concentration in the system increased (Figure 4.a and 4.c). The findings were again consistent with those previously reported [1,12].

The investigation of various kinetic models existing in the literature for freely suspended systems reveals that no single kinetic model can describe the batch fermentation of yeast cells throughout the entire range of the initial substrate concentration [1]. Monod and Hinshelwood models were found to be appropriate for describing the batch growth but only in a narrow specific range of the initial glucose concentration of 2-4% and 8-10%, respectively. These models could not adequately handle the situation in which the cells were responding to changes in the initial substrate concentration. The present study indicates that the assumption

Review Copy

Thursday , June 24, 2004

that ethanol kinetic parameters were constant despite changes in the initial substrate concentration is not truly correct.

The assertion that the present combined model system worked well was supported by the verification results for 22% w/v initial sugar concentration (Fig. 5). To the best of our knowledge, this study is the first to quantify the effect of the initial substrate concentration on the kinetic parameters for batch ethanol fermentation.

Scale-up effect on the kinetic parameters

In order to investigate possible scale-up effects on the kinetic parameters, batch ethanol fermentation experiments were carried out in duplicate with 22% w/v of the initial reducing sugar in a 10-litre fermentor. The fermentation was carried out at 33°C and 300 rpm agitation. The kinetic parameters were estimated as described earlier and compared with those obtained from the shaking flask experiments (Table 1). The comparison reveals that the estimated kinetic parameters for the 10-litre fermentor did not significantly differed from those of the 0.5-litre fermentor. There were only some minor deviations on K_{ss}, K_{ssp} *and* K_{CM}. The observed major differences might be attributed to variations in the aeration rate and shear rate caused by differences in the geometry and mixing intensity between the two systems. Though omitted here, it should

Review Copy

Thursday , June 24, 2004

be pointed out that the scale-up case did not result in lack of fit between the model to the experimental data.

Conclusion

The experimentally obtained batch kinetic data at different initial sugar concentrations, 3 – 25 %(w/v), were used to develop an unstructured mathematical model for batch ethanol fermentation. The model parameters were estimated using a non-linear regression technique assisted by a computer program to minimize the sum-of-squares deviation between the model predictions and experimental data. The relationships between the initial sugar concentrations and the kinetic model parameters were obtained as polynomial equations. The p[resent study demonstrated that the mathematical model could adequately predict the dynamics of ethanol fermentation from the beginning up to the stationary phase at different initial sugar concentrations. In the scale-up test, the majority of the kinetic parameters remained unchanged. Therefore the results of this study could provide a better understanding of the effect of the environmental conditions on cell activities and should serve as a tool for the optimum design and operation of ethanol fermentation processes.

Review Copy

Thursday , June 24, 2004

Acknowledgements

M.P. and N.S. thank the Thailand Research Fund (TRF), Project TRG 4580047, for financial support and S. Limthong for provision of the cell cultures. W,T. also received support from Research Team Award, TRF.

Nomenclature

K_{CM} Maintenance constant [h^{-1}]
K_d Specific cell death rate [h^{-1}]
K_S Saturation growth constant [g/L]
K_{SP} Saturation production constant [g/L]
K_{SS} Substrate growth inhibition term [$(g/L)^]$
K_{SSP} Substrate production inhibition term [$(g/L)^]$
P_m Ethanol inhibition term [g/L]
C_X Cell concentration [g/L]
C_P Ethanol concentration [g/L]
C_S Substrate concentration [g/L]
C_{X0} Initial cell concentration [g/L]
C_{P0} Initial ethanol concentration [g/L]
C_{S0} Initial substrate concentration [g/L]
$Y_{P/S}$ Yield coefficient for product on substrate [g product/ g substrate]
$Y_{X/S}$ Yield coefficient for cells on substrate [g cell/ g substrate]

Greek Symbols

$\propto$ Specific growth rate [h^{-1}]
$\propto_m$ Maximum specific growth rate [h^{-1}]
ν Specific production rate [h^{-1}]
ν_m Maximum specific production rate [h^{-1}]

Derivatives

d C_X/dt Cell growth rate [g/(L.h)]
d C_S/dt Rate of change in substrate [g/(L.h)]
d C_P/dt Ethanol production rate [g/(L.h)]

Review Copy

Thursday , June 24, 2004

References

[1] Birol, G., Doruker, P., Kirdar, B., Önsan, Z.İ., and Ülgen K., Mathematical description of ethanol fermentation by immobilized *Saccharomyces cerevisiae*. Process Biochemistry, 1998, **33** 763-771.

[2] Fu, W., and Mathews, A.P., Lactic acid production from lactose by *Lactobacillus plantarum*: kinetic model and effects of pH, substrate, and oxygen. Biochemical Engineering Journal, 1999, **3** 163-170.

[3] Ghaly, A. E., and El-Taweel, A.A., Kinetic modeling of continuous production of ethanol from cheese whey. Biomass and Bioenergy, 1997, **12** 461-472.

[4] Tyagi, R.D., and Ghose, T.K., Batch and multistage continuous ethanol fermentation of cellulose hydrolysate and optimum design of fermentor by graphical analysis. Biotechnology and Bioengineering, 1980, **22** 1907-1928.

[5] Sainz, J., Francisco, Pizarro, P., Eduardo, A., Modeling of yeast metabolism and process dynamics in batch fermentation. Biotechnology and Bioengineering, 2003, **81** 818-828.

[6] Andrés-Toro, B., Giron-Sierra, J.M.A., Kinetic model for beer production under industrial operational conditions. Mathematics and Computers in Simulation, 1998, **48** 65-74.

[7] Rehm, H.J., Reed, G., Pühler, A., and Stadler, P. Biotechnology: a multi-volume comprehensive treatise: Measuring modeling and control. McGraw-Hill, New York, 2001.

[8] Aiba, S., Shoda, M., and Nagatani, M., Kinetics of product inhibition in alcohol fermentation. Biotechnology and Bioengineering, 2000, **67** 671-690.

[9] Oliveira, S.C., De Castro, H.F., Visconti, A.E.S., Giudici, R., Continuous ethanol fermentation in a tower reactor with flocculating yeast recycle: scale-up effects on process performance, kinetic parameters and model predictions. Bioprocess Engineering, 1999, **20** 525-530.

[10] Sree, N.K., Sridhar, M., Suresh, K., Banat, IM., Rao, L.V., High alcohol production by repeated batch fermentation using an immobilized osmotolerant *Saccharomyces cerevisiae*. J. Industrial Microbiology & Biotechnology, 2000, **24** 222-226.

[11] Tamas, M.J, Rep, M., Thevelein, J.M., Hohmann, S., Simulation of the yeast high osmolarity glycerol (HOG) pathway: evidence for a signal generated by a change in turgor rather than by water stress. FEBS letters, 2000, **472** 159-165.

[12] Helle, S., Cameron, D., Lam, J., White, B., Duff, S., Effect of inhibitory compounds found in biomass hydrolysates on growth and xylose fermentation by a genetically engineered strain of

Elsevier

Thursday , June 24, 2004

Saccharomyces cerevisiae. Enzyme and Microbial technology, 2003, **33** 786-792.

[13] Bayrock, D.P., Michael Ingledew, W., Application of multistage continuous fermentation for production of fuel alcohol by very-high-gravity fermentation technology. J. Industrial Microbiology & Biotechnology, 2001, **27** 87-93.

[14] Evans, K., Mohagheghi, A., Hamilton, J., and Zhang, M., Effect of corn stover hydrolysate and temperature on fermentation performance of selected yeast strains. Paper presented at 24th Biotechnology for Fuels and Chemicals Symposium, Gatlinburg, TN USA, April 2002.

[15] Najafpour, G., Younesi, H., Ku Syahidah, K.I., Ethanol fermentation in an immobilized cell reactor using *Saccharomyces cerevisiae.* Bioresource Technology (in press).

[16] Salmon, J.M., Maurico, J.C., Relationship between sugar uptake kinetics and total sugar sonsumption in different industrial Saccharomyces cerevisiae strains during alcohol fermentation. Biotechnol Letters, 1994, **16** 89-94.

Review Copy

Thursday , June 24, 2004

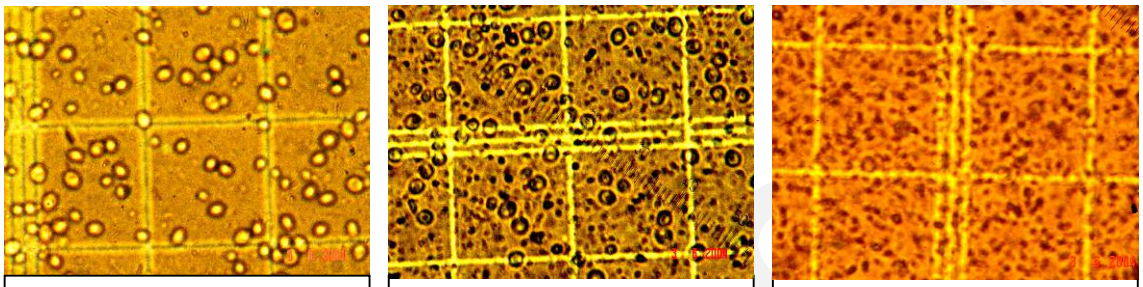
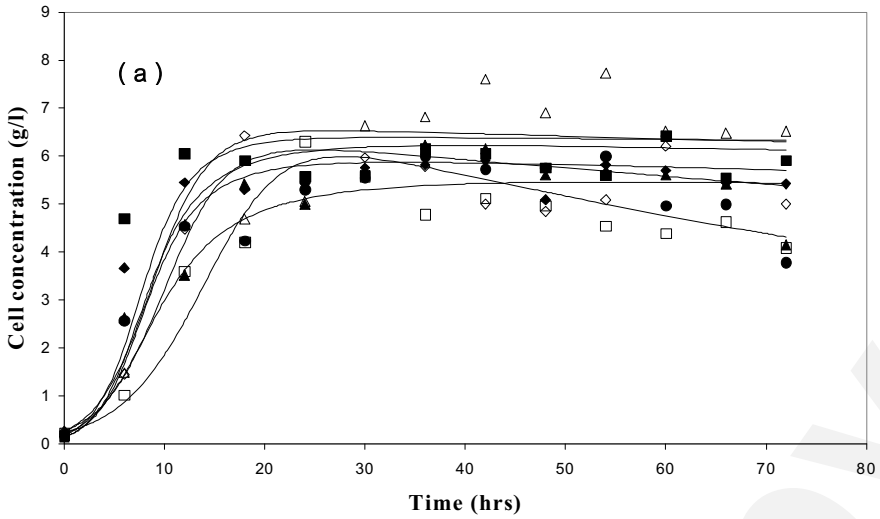
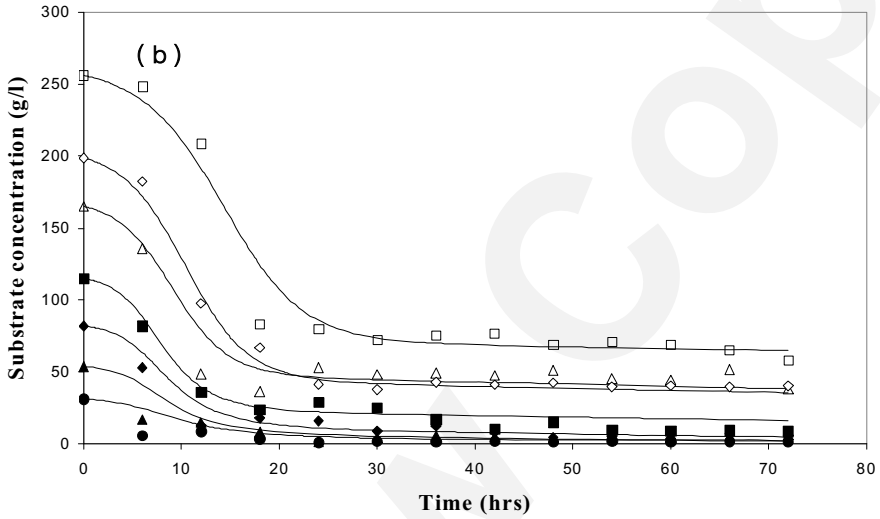


Fig.1 Photographs of *Saccharomyces cerevisiae* M30 after 24 hours cultivation under different initial reducing sugar concentrations. (a) 30 g/L; (b) 110 g/L; and (c) 220 g/L

Review Copy

Thursday , June 24, 2004





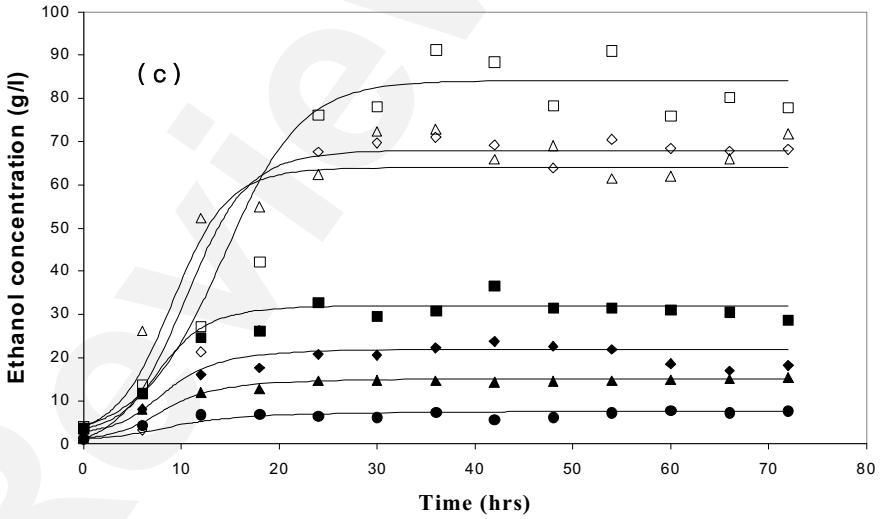


Fig. 2 Predicted and experimental data of (a) cell, (b) substrate and (c) ethanol concentrations at 3 to 25%w/v initial reducing sugar concentrations. Lines correspond to predicted data while dots correspond to experimental data.
(● 3%, ▲ 5%, ◆8%, ■ 11%, Δ17%, ◇20%, and □25%)

Review Copy

Thursday , June 24, 2004

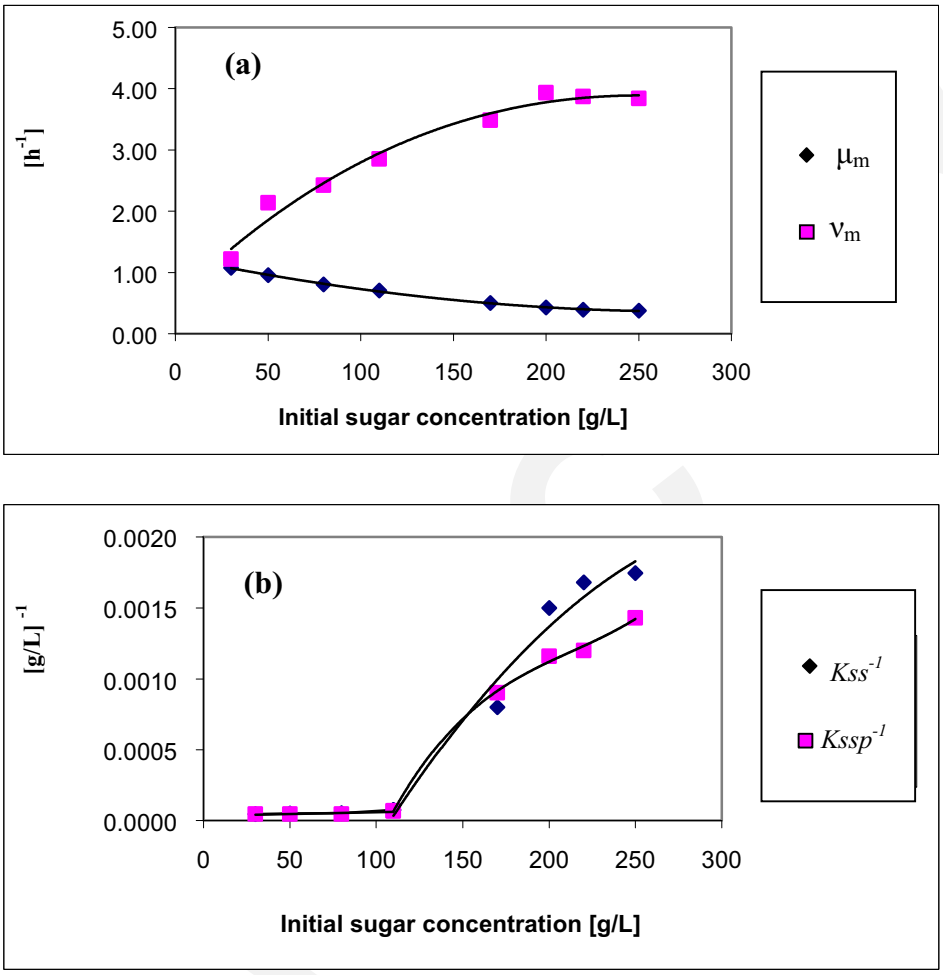


Fig.3 Effects of initial reducing sugar concentration on (a) maximum specific growth rate (μ_m) and maximum specific production rate (ν_m), and (b) substrate inhibition (Kss^{-1}) and production inhibition ($Kssp^{-1}$).

Elsevier

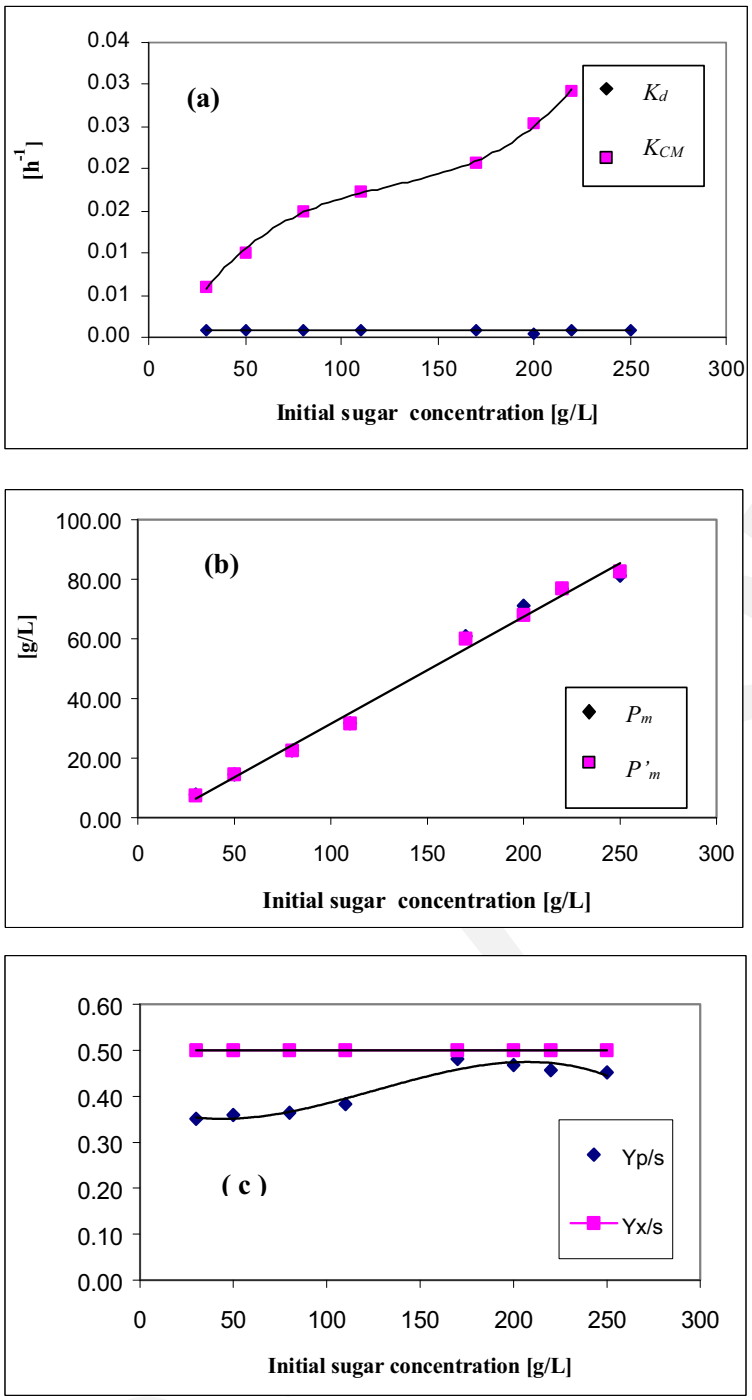


Fig. 4 Effects of initial reducing sugar concentration on (a) specific death rate (K_d) and maintenance constant (K_{CM}), (b) inhibition factor of ethanol on cell growth (P_m) and on ethanol production (P'_{mp}), and (c) ethanol yield (Yp/s) and biomass yield (Yx/s).

Reprint Copy

Thursday , June 24, 2004

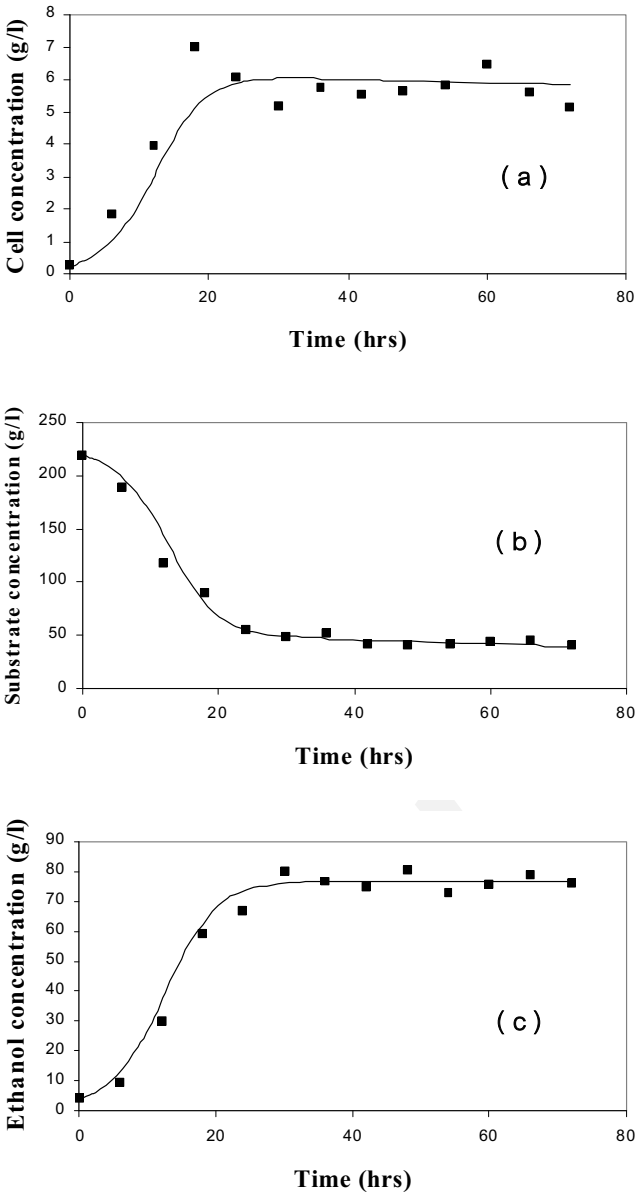


Fig. 5 Time courses of (a) cell, (b) substrate and (c) ethanol concentrations from the model validation at 22% (w/v) initial reducing sugar concentration. Lines correspond to model prediction while dots correspond to experimental data.

Review Copy

Thursday , June 24, 2004

Table 1. Kinetic parameter values obtained from experimental data with various initial reducing sugar concentration.

Initial sugar [g/L]	μ_m [h^{-1}]	K_S [g/L]	K_{SS} [g/L]	P_m [g/L]	K_d [-]	ν_m [h^{-1}]	K_{SP} [g/L]	K_{SSP} [g/L]	P'_m [g/L]	K_{CM} [-]	$Y_{P/S}$ [-]	$Y_{X/S}$ [-]
30	1.077	45.48	22500	7.70	7.50E-04	1.215	47.51	22500	7.40	0.0060	0.351	0.500
50	0.956	45.31	20500	14.51	7.50E-04	2.135	39.48	22000	14.50	0.0101	0.359	0.500
80	0.802	43.27	19000	22.53	9.50E-04	2.425	38.03	21800	22.50	0.0150	0.364	0.500
110	0.703	40.92	13300	31.62	9.05E-04	2.854	29.86	15000	31.60	0.0172	0.383	0.500
170	0.501	37.73	1250	60.93	8.50E-04	3.486	9.08	1110	60.00	0.0206	0.481	0.500
200	0.431	29.19	667	71.05	4.19E-04	3.936	4.81	863	67.91	0.0254	0.468	0.500
220*	0.390	29.52	595	76.74	8.90E-04	3.870	4.97	833	76.80	0.0293	0.457	0.500
250	0.377	29.15	573	81.15	8.25E-03	3.838	5.38	700	82.50	0.0215	0.452	0.500
220**	0.404	31.76	441.4	76.50	8.35E-04	3.855	5.86	857.5	77.04	0.0205	0.458	0.500

** Kinetic parameter values from a set for validation*
*** Kinetic parameter values from a 10 litre fermentor*

Review Copy

Mathematical modeling for ethanol fermentation from molasses by *Saccharomyces cerevisiae*

Nuttapan Srirattana, Jeerun Kingkaew, Muenduen Phisalaphong*, Wiwut Tanthapanichakoon
Department of Chemical Engineering, Chulalongkorn University, Bangkok 10330, Thailand

Abstract: A mathematical model is developed to explain the process dynamics of ethanol production by flocculating yeast, *Saccharomyces cerevisiae* M30. Molasses, the main feedstock for ethanol production in Thailand, is used as the substrate source for all experiments. The experiments are carried out by batch fermentation in shaking flasks with the initial reducing sugar concentrations ranging from 3 to 25% (w/V). Besides cell, substrate and ethanol concentrations, initial sugar concentration is investigated as a key parameter that controlled the metabolic activity of the yeast. Sigmoidal relationships between initial sugar concentration and both maximum specific growth rate and production rate are observed. The simulation reveals a good fit between the developed model and the experimental results. The model can be used to search for the optimum condition to improve process performance.

Keywords: Ethanol fermentation, *Saccharomyces cerevisiae*, Mathematical model, Molasses, Simulation

Introduction

In recent years, ethanol has become an appealing alternative to other engine fuels due to the uncertainty in price and supply of petroleum. In Thailand, the main feedstocks for ethanol production are cassava and molasses. The country has annual surplus of 2-4 million metric tons of cassava and hundreds of thousand of tons of molasses. As a result, the price of these commodities is depressed. By converting these materials to ethanol, not only it creates added value product but also could stabilize their prices.

In Thailand, more than 600,000 barrels of fuel is imported per day. To reduce the country's dependence on costly imported fuel and to assist in creating a new domestic fuel industry, the Thai government approved of five projects with a total ethanol production capacity of 800,000 liters per day to replace the imported gasoline additive methyl tertiary-butyl ether (MTBE). Ethanol is sold as a 10% blend with gasoline, in place of the toxic additive MTBE.

In effective fermentation process, the cell kinetic characteristics are necessary for controlling the process. Considering of the chemical and biochemical cell mechanism including transport phenomena, energy transfer, and also phase and component in fermentation [1], specification of mathematic model in fermentation must concern nearly real conditions. However, ideal situation for creating reality model is unreachable so the assumptions are made. In practice, a simply model to describe microbial metabolic processes is first used and it must be modified until the appropriated kinetic model for each fermentation is reached.

In this study, the modified unstructured model is developed to describe the metabolic process of *Saccharomyces cerevisiae*. Batch fermentation is performed with *Saccharomyces cerevisiae* M30 and initial sugar concentration is varied. To estimate the kinetic parameters, the time course of cell, substrate, and ethanol concentration curves calculated by iterated programming are compared with the experimental data. The best fit of these results can be used to construct the appropriated kinetic model which is tested against experimental data obtained from batch fermentation with different initial substrate concentrations. The results will provide more understanding of the controlled parameters on the cell activities and can be used to search for the optimum condition for ethanol production.

Material and Methods

Yeast strains

Saccharomyces cerevisiae M.30, flocculating yeast selected on the basis of high efficiency in ethanol fermentation from molasses at high temperature, are used for alcohol production experiments.

Culture media

Stock cultures are stored in PDA agar slant. Precultures are prepared by transferring a stock culture to 150 mL of medium and incubated at 33 °C for 20 hours before transferred to main culture. The medium for the inoculum contains 0.05% ammonium sulfate and 11 % inverse sugar from molass mash and are adjusted pH to 5.0. The prepared media is sterilized at 121°C for 20 minutes.

Shake flask fermentation

Shake flask fermentation is conducted in duplicate with assigned media. Erlenmeyer flasks of 500 mL capacity containing 250 mL of the prepared fermentation media with initial reducing sugar between 3 to 25% w/v are inoculated with 5% of cell suspensions. Fermentation flasks are shaken at 150 rpm and kept at 33°C. Fermentation is monitored for 3 days by removing 3.5 mL samples every 6 hours for cell, sugar and ethanol analyses.

Analytical methods

Cell concentration is determined by cell dry weight determination. A 3 mL sample of the fermentation

* Corresponding author:
E-mail address: muenduen.p@chula.ac.th

broth is centrifuged at 3,000 rpm for 10 minutes. The cell pellet is resuspended in 0.1 normal HCl and washed twice with distilled water, dried for 48 hours at 60°C, and then weighed. Concentration of each ethanol sample is determined by a gas chromatography system using a Shimadzu Model GC 7& equipped with a flame ionization detector. A 2 m x 0.125 m column packed with Porapak Q 80-100 mesh is used with N_2 as carrier gas. The injector temperature is 280 °C, and the detector temperature is 300°C. To measure the amount of sugar in sample, a 0.2 mL of sample solution is hydrolyzed in 33% HCl at 100°C for 10 minutes, neutralized with NaOH solution and determined for reducing sugar content by Lane and Eynon's method.

Mathematical methods

An unstructured kinetic model for the cell activities in the anaerobic fermentation of molasses to produce ethanol is developed under three main factors, substrate limitation, substrate inhibition, and product inhibition by controlling operating temperature, pH and shear force effect. The microbial reactions usually show saturation at high substrate concentrations, meaning that the reaction rate approaches a maximum value. On the other hand, the reaction rate equals zero if no substrate is available. Therefore, if there is no interfered influence on cell growth, cell growth can be estimated based on Monod equation which represents substrate limitation kinetics:

$$\frac{1}{C_X}\frac{dC_X}{dt} = \mu = \frac{\mu_m C_S}{K_S + C_S}$$

where symbols are defined in Nomenclature.

However, the inhibition by substrates and products is normally observed in biotechnological processes, especially at high concentration of substrates or products [2, 3, 4]. In this study, the Monod kinetic is modified in both substrate and product terms and counted with death rate and cell maintenance for biomass equation [5]. Reducing sugar from molasses is assigned as the sole limiting substrate of the fermentation. The effects of initial substrate concentrations, C_{S0}, on the kinetic parameters are investigated. The mathematical model of the microbiological ethanol synthesis can be written as follows:

$$\mu C_X = \frac{dC_X}{dt} = \frac{\mu_m(C_{S0})C_S}{K_S(C_{S0}) + C_S + \frac{C_S^{\,2}}{K_{SS}(C_{S0})}}\left(1 - \frac{C_P}{P_m(C_{S0})}\right)C_X - K_d(C_{S0})C_X - K_{CM}(C_{S0})C_X$$

$$\nu C_X = \frac{dC_P}{dt} = \frac{\nu_m(C_{S0})C_S}{K_{SP}(C_{S0}) + C_S + \frac{C_S^{\,2}}{K_{SSP}(C_{S0})}}\left(1 - \frac{C_P}{P_{mP}(C_{S0})}\right)C_X$$

The kinetic parameters, μ_m, K_S, K_{SS}, P_m, K_d, ν_m, K_{SP}, K_{SSP} and K_{CM} are allowed to vary as a function of C_{S0}. The relationship of these parameters can be obtained by fitting experimental data over a range of C_{S0} values.
The substrate consumption rate may be stated as

$$\frac{dC_S}{dt} = -\frac{1}{Y_{X/S}}\frac{dC_X}{dt} - \frac{1}{Y_{P/S}(C_{S0})}\frac{dC_P}{dt}$$

At this point, the yield coefficients, $Y_{X/S}$ and $Y_{P/S}$, are the relation between cell-substrate and product-substrate respectively. The yield of cell ($Y_{X/S}$) refers to the proportion of cell mass production to substrate utilization. Literature reported that yield of cell was around 0.5 g/g for *Saccharomyces cerevisiae* [12]. Similarly, the yield of production ($Y_{P/S}$) refers to the proportion of product accumulation to substrate utilization. Theoretically, the maximum conversion efficiency of glucose to ethanol is 51 percent on a weight basis and this alteration can be summarized by the following equation.

$$\underset{(C_6H_{12}O_6)}{\text{Glucose}} \longrightarrow \underset{(CH_3CH_2OH)}{2\,\text{Ethanol}} + \underset{(CO_2)}{2\,\text{Carbon dioxide}} + \text{Heat}$$

The best-fit values of the kinetic parameter are estimated by minimizing the sum of squared error between the predicted and experimental data. From the developed mathematical model, after applying numerical method, the solution is solved using software package MATLAB v.6.1.

Results and discussion

Experimental results

Batch fermentation experiments have been carried out in duplicate for 72 hours with *Saccharomyces cerevisiae* M30 culture using molasses as substrate. Experimental studies are performed at 33°C in shaking flasks. Media are prepared at 3% 5% 8% 11% 17% 20% and 25% of initial reducing sugar concentration from molasses mash. Experimental results show that biomass enters a stationary phase between 12 and 18 hours after inoculation in all initial substrate concentrations. Maximum ethanol concentration is obtained at 24 hours of fermentation with 25% initial substrate concentration. From Figure 1, it is clear from experiments that the high initial substrate concentration of 25%, a significant inhibition of cell growth can be observed, whereas at the lower initial substrate values from 5% to 17% the growth rates are greater. After stationary phase, substrate is depleted, ethanol is accumulated and cell growth has entered the death phase. At the end of fermentation, cells are unable to consume the unexploited substrate and the available reducing sugar remains exist.

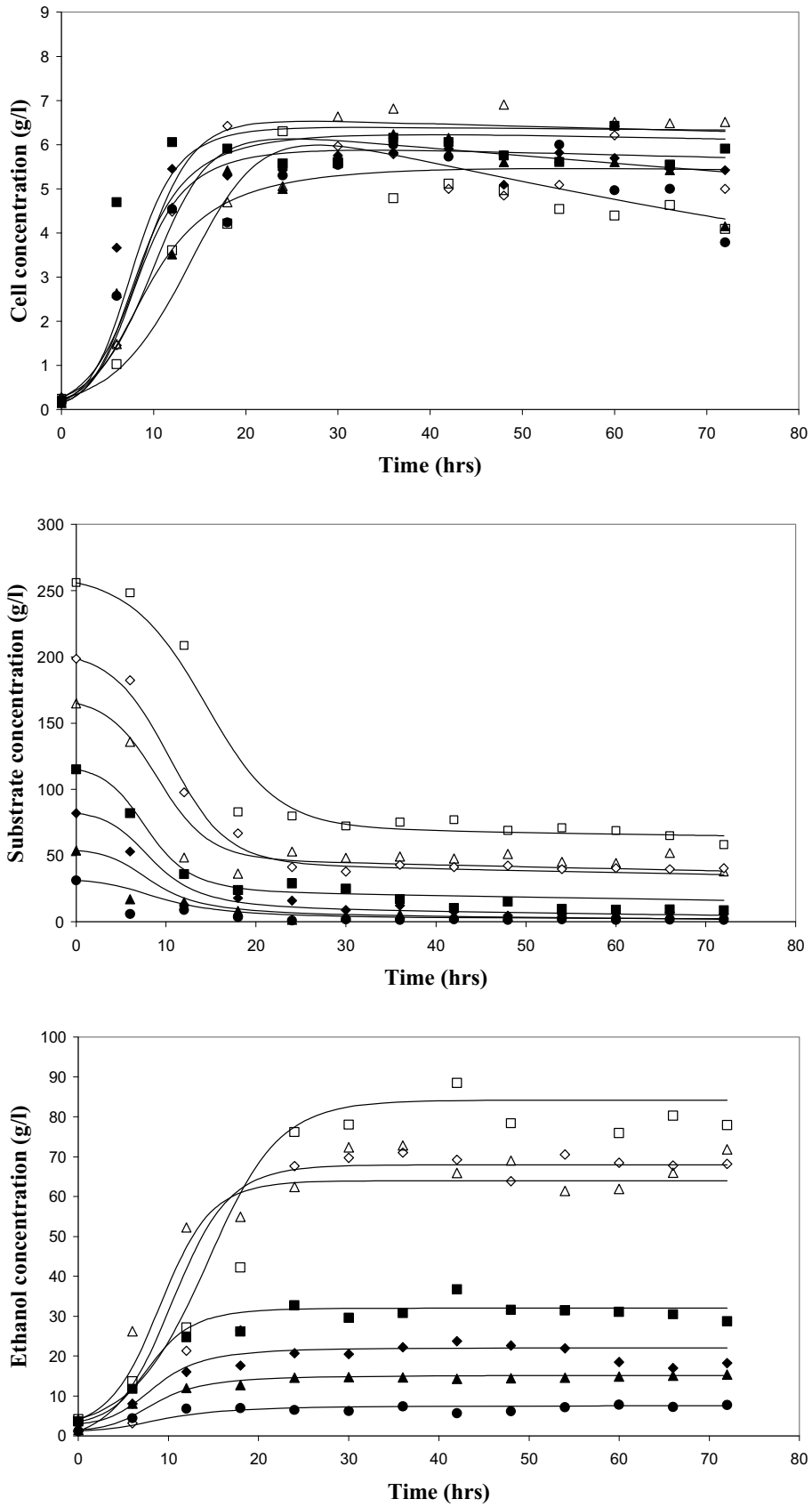


Figure 1. Experimental results and simulation of actual cell, substrate and product concentrations at different initial substrate concentrations; Lines correspond to model simulation while dots correspond to experimental data. (● 3%,▲ 5%,◆ 8%,■ 11%, △ 17%,◇ 20%, and □ 25%)

Table 1. Kinetic parameters

Substrate concentration [g/L]	μ_m [h^{-1}]	K_S [g/L]	K_{SS} [g/L]	P_m [g/L]	K_d [h^{-1}]	ν_m [h^{-1}]	K_{SP} [g/L]	K_{SSP} [g/L]	K_{CM} [h^{-1}]	$Y_{P/S}$ [-]
30	1.077	45.48	22500	7.70	7.50E-04	1.215	47.51	22500	0.0060	0.351
50	0.956	45.31	20500	14.51	7.50E-04	2.135	39.48	22000	0.0101	0.359
80	0.802	43.27	19000	22.53	9.50E-04	2.425	38.03	21800	0.0150	0.364
110	0.703	40.92	13300	31.62	9.05E-04	2.854	29.86	15000	0.0172	0.383
170	0.501	37.73	1250	60.93	8.50E-04	3.486	9.08	1110	0.0206	0.481
200	0.431	29.19	667	71.05	4.19E-04	3.936	4.81	863	0.0254	0.468
250	0.377	29.15	573	81.15	8.25E-03	3.838	5.38	700	0.0215	0.452

$Y_{X/S}$ = 0.5 g/g

Kinetic model and parameter estimation

Fermentation data are used in the pre-calculation for the initial guesses of parameter values. The maximum specific growth rate (μ_m), saturation constant of substrate (K_S), maximum specific production rate (ν_m) and saturation constant of product (K_{SP}) are calculated by using the initial method. The growth yield is assumed to be 0.5 g/g for *Saccharomyces cerevisiae* [12], thus the product yield can be calculated by the overall mass balance of substrates. These initial guesses of the parameter values are used in the first loop of numerical method solving (data not show). Afterward, the parameter values are re-calculated by MATLAB programming until they reach minimum error with nonnegative value constrains. The effects of initial sugar concentration on model parameters are shown in Table 1. The batch experiments point up that the values of maximum specific growth rate of the cell decreases appreciably and maximum specific production rate increases considerably with the increasing of initial sugar concentration. The results relate to the observation of initial sugar concentration effect on yeast metabolism. It is well known that an important effect of initial reducing sugar above 30 g/L is metabolize repression of oxidation pathway leading to switch from the oxidation of glucose for cell growth to ethanol production by fermentation [2]. The inhibitory effects of high initial sugar concentration are also observed in terms of substrate growth inhibition factor ($1/K_{SS}$) and substrate production inhibition factor ($1/K_{SSP}$), which increase considerably with the increasing of initial sugar concentration. The toxic inhibition effect on the maximum specific growth rate of 24 selected yeast strains in corn stover hydrolysate fermentation was reported [7]. Figure 1 presents the predicted and experimental data of cell, substrate and ethanol concentrations at different initial substrate concentrations. The production yields estimated in this study is 0.35-0.45, which is relevant to that from other reports [3, 8]. The functions of initial substrate concentration on the kinetic parameters can be given by polynomial equations similar to the functions proposed for a kinetic model for beer production by Andrés-Toro's in 1998. The correlation of initial substrate concentration on specific growth rate and specific production rate are illustrated in Figure 2. The results of mathematical modeling iteration have shown good fittings between the model prediction and the experimental data for all initial sugar concentrations.

Model validation

The proposed mathematical model is used to test against experimental data obtained in another batch fermentation with 22% initial sugar concentration at the same operating condition. The batch performance curves predicted by the models are compared with the experimental data as shown in Figure 3. The validation reveals a good agreement between the model predictions and the experiment results.

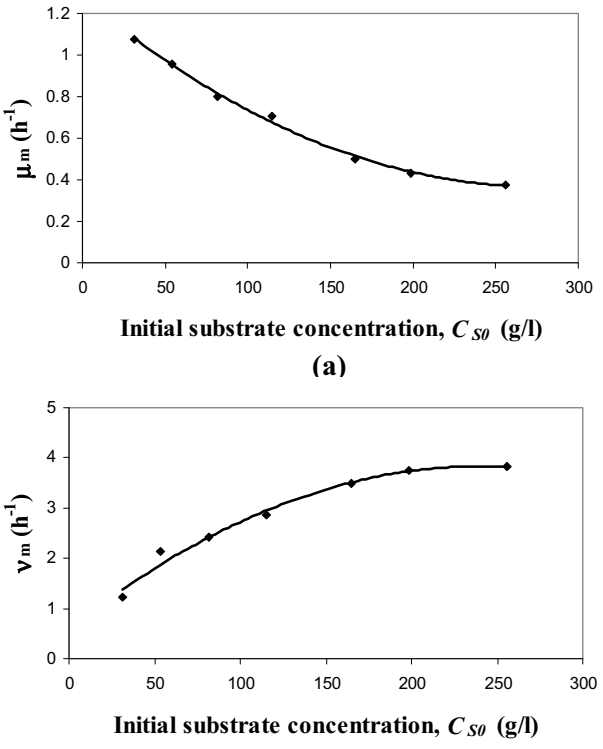
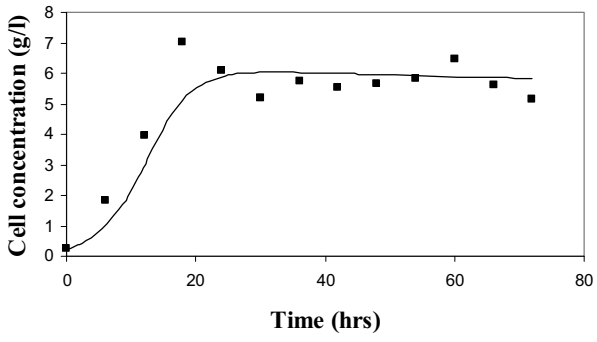
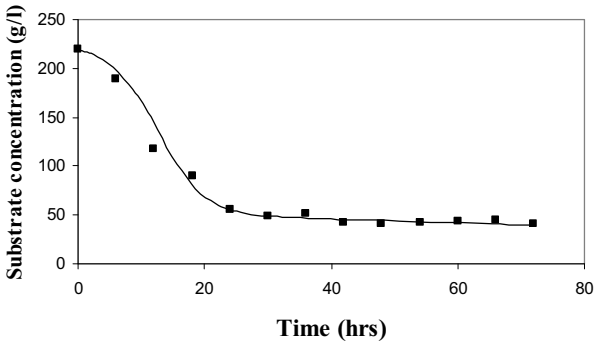


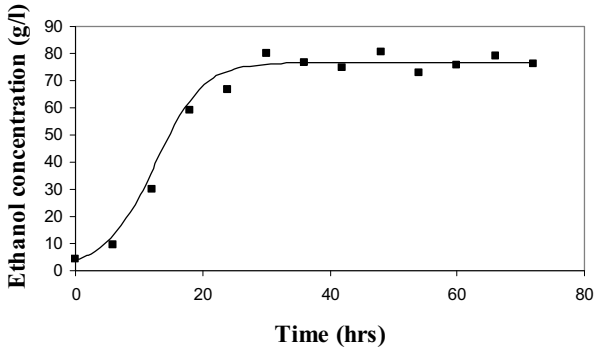
Figure 2. The relationships between: (a) the initial sugar concentration (C_{S0}) and the maximum specific growth rate (μ_m); (b) the initial sugar concentration (C_{S0}) and the maximum production rate (ν_m).



(a)



(b)



(c)

Figure 3. Model predictions at 22% initial substrate concentration. (a) cell growth; (b) substrate utilization; and (c) ethanol production. Lines correspond to model simulation while dots correspond to experimental data.

Conclusion

Batch fermentations of molasses to ethanol are carried out by using *Saccharomyces cerevisiae* M30 in shaking flasks. The mathematical model is developed from Monod kinetics with modified both substrate and product terms and counts with death rate and cell maintenance. The inhibitory effect of high ethanol concentration and high sugar concentration on yeast metabolism are assigned in quantitative terms added into the growth and metabolism functions. The effects of initial sugar concentration on kinetic parameters are investigated and the functions can be given by polynomial equations. Sigmoidal relationships between the initial substrate concentration and the specific growth and production rate are observed. The simulation reveals a good fit between the developed model and the experimental results for the initial sugar concentration ranging from 3% w/v to 25% w/v. The model can be used to search for the optimum condition to improve the process performance.

Acknowledgements

The authors acknowledge the support from The Thailand Research Fund.

References

[1] Fu, W., and Mathews, A.P. Lactic acid production from lactose by *Lactobacillus plantarum*: kinetic model and effects of pH, substrate, and oxygen. Biochemical Engineering Journal 3 (1999): 163-170.

[2] Birol, G., Doruker, P., Kirdar, B., Önsan, Z.İ., and Ülgen K. Mathematical description of ethanol fermentation by immobilized *Saccharomyces cerevisiae*. Process Biochemistry 33 (1998): 763-771.

[3] Ghaly, A. E., and El-Taweel, A.A. Kinetic modeling of continuous production of ethanol from cheese whey. Biomass and Bioenergy 12 (1997): 461-472.

[4] Tyagi, R.D., and Ghose, T.K. Batch and multistage continuous ethanol fermentation of cellulose hydrolysate and optimum design of fermentor by graphical analysis. Biotechnol. Bioengng. 22 (1980): 1907-1928.

[5] Sainz, J., et. al. Modeling of yeast metabolism and process dynamics in batch fermentation. Biotechnol. Bioengng. 81 (2003): 818-828.

[6] Andrés-Toro, B., et. al. A kinetic model for beer production under industrial operational conditions. Mathematics and Computers in Simulation 48 (1998): 65-74.

[7] Evans, K., Mohagheghi, A., Hamilton, J., and Zhang, M., Effect of corn stover hydrolysate and temperature on fermentation performance of selected yeast strains, 24th Symp. on Biotechnology For Fuels and Chemicals, Gatlinburg, TN USA, April 2002: 2-13.

[8] Aiba, S., Shoda, M., and Nagatani, M., Kinetics of product inhibition in alcohol fermentation: Biotechnol. Bioengng. 67(2000): 671-690.

[9] Bailey, J.E. Biochemical engineering fundamentals. 2nd ed. McGraw-Hill, New York, 1986.

[10] Panikov, N.S. Microbial Growth Kinetics. 1st ed. London: Chapman & Hall, 1995

[11] Rehm, H.J., Reed, G., Pühler, A., and Stadler, P. Biotechnology: a multi-volume comprehensive treatise: Measuring modelling and control. 4 Vols. 2nd ed. Weinheim: McGraw-Hill, 2001

[12] Shuler, M.L., and Kargi, F. Bioprocess Engineering. 2nd ed. New Jersey: Prentice Hall, 2001

Nomenclature

K_{CM} Maintenance constant [h^{-1}]
K_d Specific cell death rate [h^{-1}]
K_S Saturation growth constant [g/L]
K_{SP} Saturation production constant [g/L]
K_{SS} Substrate growth inhibition term [g/L]
K_{SSP} Substrate production inhibition term [g/L]
P_m Ethanol inhibition term [g/L]
C_X Cell concentration [g/L]
C_P Ethanol concentration [g/L]
C_S Substrate concentration [g/L]
C_{X0} Initial cell concentration [g/L]
C_{P0} Initial ethanol concentration [g/L]
C_{S0} Initial substrate concentration [g/L]
$Y_{P/S}$ Yield coefficient for product on substrate
$Y_{X/S}$ Yield coefficient for cells on substrate

Greek Symbols

μ Specific growth rate [h^{-1}]
μ_m Maximum specific growth rate [h^{-1}]
ν Specific production rate [h^{-1}]
ν_m Maximum specific production rate [h^{-1}]

Derivatives

$\mathrm{d}C_X/\mathrm{d}t$ Cell growth rate [g/(L.h)]
$\mathrm{d}C_S/\mathrm{d}t$ Rate of change in substrate [g/(L.h)]
$\mathrm{d}C_P/\mathrm{d}t$ Ethanol production rate [g/(L.h)]