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Kinetic models for batch and continuous ethanol fermentation from sweet sorghum juice by yeast immobilized on sweet sorghum stalks

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ABSTRACT

Kinetic models for batch and continuous ethanol fermentation from sweet sorghum juice by *Saccharomyces cerevisiae* NP 01 immobilized on unpeeled sweet sorghum stalk pieces were developed. The models accounted for substrate limitation, substrate inhibition, ethanol inhibition and cell death. Batch ethanol fermentations were done from juice containing various initial sugar concentrations (120–280 g/L). The estimated values of the maximum specific growth rate (μ_{max}) and Monod constant (K_s) were found to be 0.313 h^{-1} and 47.51 g/L, respectively, using a Lineweaver–Burk plot. These data were used to develop models for batch and continuous ethanol fermentation. For the batch fermentation, it was found that the models could be used to satisfactorily fit the experimental data for initial sugar concentrations ranging from 130 to 225 g/L. However, for the continuous fermentation, only the data for substrate consumption and ethanol production were well fitted by the developed models.

1. Introduction

Due to the world's volatile energy market and environmental concerns, alternative fuels such as bioethanol have received much attention as potential replacements for fossil fuels [1]. In Thailand, a government 15-year plan (2008–2022) is in place to increase bioethanol production capacity to 9 million liters day^{-1} by 2022 [2]. Currently, the most widely used substrates for bioethanol production in Thailand are cassava and sugarcane molasses. Increasing the production of bioethanol will eventually result in shortages of these materials. It is therefore necessary to identify promising alternative materials when these substrates are fully utilized. Sweet sorghum (*Sorghum biocolor* (L.) Moench) is potentially such a substrate. It yields high amounts of biomass and sugar. The stalk of sweet sorghum contains large amounts of soluble sugars (glucose and sucrose) and insoluble carbohydrates (holocellulose). Also, its juice contains many trace elements that are essential for microbial growth and ethanol production [3].

In many studies, batch, repeated-batch, fed-batch and continuous fermentation processes were used to produce ethanol from sugars by free yeast cells [4–6]. However, the disadvantages of using free cells for ethanol fermentation are substrate and product inhibition [7], extra time needed for inoculum preparation and cleaning the reactor between batches resulting in longer turnover times. To overcome these limitations, cell immobilization was introduced in fermentation processes. Cell immobilization is the limitation of cell mobility by isolation within a carrier. Commercially available materials, *e.g.*, alginate and carrageen, are widely used for this purpose. However, these materials are costly and much research has been directed at finding other low cost alternative natural materials. These include sorghum bagasse [8], sugarcane pieces [9], corn cobs [10], thin-shell silk cocoons [11] and sweet sorghum stalks [12].

Study of fermentation kinetic parameters is important for understanding the impacts of environmental factors on ethanol production. These factors include temperature [13] and substrate concentration [14]. Moreover, kinetic parameters coupled with mathematical models can be used to predict the dynamics of cell concentration, substrate utilization and ethanol production rate [15,16]. In many cases, optimal conditions for product formation can be predicted using mathematical models without experimentation [17]. However, preliminary studies revealed that most research on kinetic and mathematical models for ethanol production

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Nomenclature

D	dilution rate, h^{-1}
K_{IP}	substrate inhibition constant for ethanol formation, g/L
K_{IS}	substrate inhibition constant for growth, g/L
K_S	Monod constant for growth, g/L
K_{SP}	ethanol saturation constant, g/L
m	cell maintenance coefficient, h^{-1}
P_E	ethanol concentration, g/L
P_i	initial ethanol concentration, g/L
$P_{P.\max}$	maximum ethanol concentration for ethanol fermentation, g/L
$P_{X.\max}$	maximum ethanol concentration for growth, g/L
Q_P	ethanol productivity, g/L h
q_P	specific ethanol production rate, g/g h
$q_{\max}$	maximum specific ethanol production rate, g/g h
S	substrate concentration, g/L
S_C	sugar consumption (%)
S_i	initial substrate concentration, g/L
t	time, h
t_0	incubation time at the commencement of the log phase, h
t_L	incubation time at the end of the log phase, h
V	working volume, L
X	total cell concentration, g/L
X_0	cell concentration at the commencement of the log phase, g/L
X_L	cell concentration at the end of the log phase, g/L
X_f	free cell concentration in broth, g/L
X_i	initial total cell concentration, g/L
X_{im}	cell concentration in carrier, g/L
$Y_{P/S}$	ethanol yield, g/g
$Y_{X/S}$	biomass yield, g/g
y_A	actual data
y_{av}	average actual data
y_P	predicted data
μ	specific growth rate, h^{-1}
$\mu_{\max}$	maximum specific growth rate, h^{-1}
α, β	ethanol inhibition constant, g/L

focused on free cell systems [13,18,19]. Little information is available on the ethanol production by immobilized cells [15,20,21].

The aim of this research was to study the kinetics of ethanol production from sweet sorghum juice using yeast immobilized on sweet sorghum stalk pieces under batch and continuous fermentation. Then mathematical models were developed to predict ethanol production. For this purpose, a modified Monod's equation was used to account for substrate and product inhibition.

2. Materials and methods

2.1. Microorganism and inoculum preparation

S. cerevisiae NP 01 was isolated from a dried starter culture for making Thai rice wine [22]. It was inoculated into a 250-mL Erlenmeyer flask containing 150 mL of yeast extract malt extract (YM) medium [12]. The flask was incubated on a rotating shaker at 200 rpm, 30 °C for 18 h. A 10% inoculum (v/v) of the culture was added into 350 mL of sweet sorghum juice containing 100 g/L of total sugar to yield an initial cell concentration of $\sim 5 \times 10^6$ cells/mL. After being further incubated for 18 h, the cells were harvested by centrifugation at 6000 rpm for 10 min, and used for cell immobilization.

2.2. Raw material

Sweet sorghum juice extracted from its stalks (cv. KKU 40) was obtained from the Division of Agronomy, Faculty of Agriculture, Khon Kaen University, Thailand. The juice initially had total soluble solids of 17°Brix. Then it was concentrated to 65°Brix and stored at 4 °C until use.

2.3. Ethanol production medium

Ethanol production (EP) medium was prepared by diluting the concentrated juice with distilled water to total sugar concentrations of 120–280 g/L. Then, it was supplemented with 6 g/L of yeast extract and autoclaved at 110 °C for 28 min [3]. The medium was left at room temperature to cool prior to use.

2.4. Cell immobilization on sweet sorghum stalk

Cell immobilization was conducted by adsorption of cells on sterile sweet sorghum stalk (SSS) pieces (6–20 mm in diameter and 6 mm in thickness). SSS pieces were transferred into sweet sorghum juice containing 100 g/L of total sugar with active yeast cells at a concentration of $\sim 1 \times 10^8$ cells/mL and incubated at 30 °C for 18 h. After that, the SSS pieces were washed with sterile EP medium before use as an inoculum (immobilized cells) for ethanol production [23].

2.5. Batch fermentation

The SSS pieces containing immobilized yeast cells at 50% of working volume (350 mL) were transferred into sterile EP medium in a 500-mL air-locked Erlenmeyer flask. The fermentation was carried out at 30 °C for 72 h under static conditions to prevent the detachment of the immobilized cells from the carriers. Samples were taken for analysis at regular time intervals.

2.6. Continuous fermentation

The immobilized yeast cells were packed into a single-tubular packed bed bioreactor (working volume of 0.78 L) at 50% of the column height [23]. The process was started as a batch fermentation at 30 °C. When the sugar concentration in the broth decreased to approximately 20% of its initial value, a continuous system was started by feeding sterile sweet sorghum juice into the bottom of the bioreactor at a dilution rate of 0.023 h^{-1}. During the fermentation, samples were taken for analyses.

2.7. Analytical methods

Ten grams of SSS pieces containing immobilized yeast cells were blended with 90 mL of a 0.85% NaCl solution [24]. The suspension was subjected to serial dilution. Cell concentration in the suspension was determined by a direct counting method using a hemacytometer and a methylene blue staining technique. The dry weight of viable cells and the SSS pieces were measured using a gravimetric method [23]. Correlation between viable cell concentration and dry cell weight were determined by linear regression. Then, the viable cells in the SSS pieces were determined in terms of cells/g dry weight of SSS pieces. Sugar, ethanol (P_E) and viable cell concentrations were determined, and the batch ethanol production efficiencies in terms of ethanol yield ($Y_{P/S}$) and ethanol productivity (Q_P) were calculated as described by Laopaiboon et al. [3]. Additionally, the Q_P of continuous ethanol fermentation was estimated by the of P value multiplied by its dilution rate (D).