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Research article

Kinetic modeling of the simultaneous production of ethanol and fructose by *Saccharomyces cerevisiae*

Ashraf K. Sulieman [a], Meilana Dharma Putra [b,*], Ahmed E. Abasaeed [a], Mohamed H. Gaily [a], Saeed M. Al-Zahrani [a], Mohamed A. Zeinelabdeen [a]

[a] Department of Chemical Engineering, College of Engineering, King Saud University, P.O. Box 800, Riyadh 11421, Saudi Arabia
[b] Chemical Engineering Department, Faculty of Engineering, Lambung Mangkurat University, Banjarmasin 70123, Indonesia

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ABSTRACT

Background: Ethanol and fructose are two important industrial products that enjoy many uses. In this contribution, their production via selective fermentation of date extract using *Saccharomyces cerevisiae* was studied. Scaling up the process for possible commercialization was investigated in three fermentors with working volume ratio of 1:40:400.
Results: Higher ethanol concentration was obtained in the larger fermentor due to conversion of fructose. Fructose yields in the 0.5-L, 7.5-L and 80-L fermentors were 99, 92 and 90%, respectively. Good fitting was obtained with the modified Monod kinetics; however, a better fit of cell mass was obtained with the modified Ghose–Tyagi model which accounts for ethanol inhibition.
Conclusions: The modified Gompertz model was expanded to facilitate prediction of products' formation and fructose fractions in all three fermentors. Such expansion will be beneficial in industrial applications.

1. Introduction

Ethanol is one of the most potential products used in various applications. It is used as a solvent, a fuel, a chemical reagent, and a raw material for many important chemicals [1]. In North and South America, bioethanol was primarily sourced from starch, sugars and molasses [2]; on the other hand, USA and Brazil are the largest ethanol producer [3]. However, the demand for ethanol is steadily increasing especially as an energy source [4,5]. It is because the annual worldwide production of oil fuel is projected to reduce from 25 billion barrels in 2002 to about 5 billion barrels in 2050 [6]. Compared to fuel of gasoline, ethanol is renewable, non-toxic, easy to handle, safe to store, and sulfur-free; thus less contribution to global warming and air pollution [7,8].

Fructose, the sweetest natural sugar, is commercially used for foods, confectionery and beverages industries [9]. The fructose is 1.73 times sweeter than sucrose and about twice the sweetness of glucose; thus lesser amounts (subsequently calories) are needed. Fructose is recommended to use rather than other sweeteners because it is difficult to crystallize from an aqueous solution, faster to absorb moisture and slower to release it to the environment [10,11].

Over 8 million tons of date fruits have been produced in 2010 worldwide [12]. Unfortunately, almost half production of date is still unutilized [13]. About half of the date content is fructose; thereby providing a large opportunity for its production. Hydrolysis of starch followed by enzymatic isomerization process has been widely used in industry to convert glucose to fructose; unfortunately only about 42% HFS (high fructose syrup) was obtained due to equilibrium limitations [14,15]. On the other hand, 90% HFS can be produced via multistage chromatographic process [16], membrane technology [17], and ionic liquids [18]; such methods suffer from high production cost [19]. The development of various methods are still continuously researched such as the usage of nanofiltration [20], microalgae [21], and beverage waste [22] and inulin [23,24]. A very promising technique (still in its infancy) for the production of fructose from sugar mixtures is selective fermentation of glucose and other sugars (except fructose) to bioethanol [25].

Compared to other microbes, the utilization of *Saccharomyces cerevisiae* has been shown to suppress the high consumption of fructose and the formation of by-products such as sorbitol [26]. The performance of *S. cerevisiae* ATCC 36858 in media composed of sucrose, beet molasses and date syrup has been utilized [25,27]. Therefore, it is

* Corresponding author.
E-mail address: mdputra@unlam.ac.id (M.D. Putra).
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imperative to study the scale up of selective fermentation before launching an industrial production [28]. Kinetic models are valuable tools in understanding the behavior of the fermentation process that paves the way for further process development or industrial application. The classical Monod equation [29] has been proposed to elucidate the yeast performance during fermentation. However, it is interesting to study a kinetic model that describes the fermentation process in a medium containing a mixture of glucose, fructose and sucrose, such as date syrup.

In this study, the effect of scale up on the performance of *S. cerevisiae* will be investigated using three fermentors (0.5 L, 7.5 L and 80 L). Kinetic models, such as the modified Monod and Ghose–Tyagi, will be employed to study the effect on ethanol inhibition on the selective fermentation process. The modified Gompertz model will be expanded to enable the predictions of simultaneous fructose and ethanol production as well as fructose fraction in sugar.

2. Experimental

2.1. Yeast and propagation

The yeast of *S. cerevisiae* with the typical culture of ATCC 36858 was obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). The microbe was then revived in accordance with the procedure of ATCC. It was further incubated in an agar slant. The yeast colony grown in the agar was then transferred to a sterilized liquid medium in a 500-mL flask and allowed to propagate for 36 h at 30°C and 120 rpm in a water bath shaker (Julabo SW23, Allentwon, USA). The agar and liquid medium contained 10-g dextrose, 3-g malt extract, 3-g yeast extract, 5-g peptone and de-ionized water (up to 1 L)

For yeast cultivation, 2 L of liquid medium containing 6-g malt extract, 6-g yeast extract, and 10-g peptone was prepared and poured into 7.5-L fermentor. The fermentor was then sterilized in an autoclave (Astell AMB230N, Sidcup, Kent, UK) at 121°C for 15 min. The cooled yeast broth in the fermentor was then aerated with air 1 vvm and operated at 30°C and 200 rpm. About 2-L water containing 20-g glucose was then fed-batch to the fermentor as the glucose concentration in the solution was kept at less than 0.05 g/L to avoid ethanol production. The final cultivated yeast concentration obtained through this process was about 1.9 g/L.

2.2. Raw materials

Sugars (fructose and glucose) were initially extracted from the dates using deionized water at 50°C for 2 h. The weight ratio of water to the dates was 2.5. To remove the fibers, the syrups were then centrifuged for 6 min at 6500 rpm. Before used in the fermentation process, the final syrup was further sterilized at 121°C for 15 min in an autoclave (Astell AMB230N, Kent, UK)

2.3. Fermentation process

The final syrup sterilized (85%) and the liquid propagation medium containing high yeast (15%) were aseptically mixed to gain a syrup concentration about 130 g/L. The fermentation processes were experimentally conducted out in 500-mL Erlenmeyer flask, 7.5-L fermentor with 2 impellers, 6 blades each and 80-L fermentor with a 4-blade impeller with effective working volumes of 0.1, 4 and 40 L, respectively. Table 1 presents the major features and dimensions of bioreactors for 7.5 and 80 L. The flask was placed in the Julabo water bath shaker. The experiments were conducted at 33°C and 120 rpm. Detailed description of experiments and cultivation of cell mass is given in previous publications [11,25,30]

Table 1
Major dimensions of the fermentors.

Fermentor volume (L)	7.5	80.0
Height (H), m	0.32	0.64
Inside diameter (D), m	0.18	0.42
Working volume, L	4	40
Inoculum volume, L	0.6	6
Impeller diameter, m	0.06	0.21
Shaft length, m	0.30	0.60
Rotation speed, rpm	120	120
Temperature, °C	33	33

2.4. Sample analysis

A portion of the sample withdrawn from the fermentor was centrifuged at 15000 rpm to remove the cell mass from the solution. The supernatant containing the sugars and ethanol was analyzed by using high performance liquid chromatography (HPLC-Agilent 1200 Infinitely series, DE, USA) equipped with an Aminex-® column and RID detector (150 × 7.8 mm, BIO-RAD®, California, USA). The column was kept at temperature of 40°C, and the mobile phase was 0.1-mM sulfuric acid. The cell mass concentration was determined from the other portion of the withdrawn sample by using NucleoCounter® YC-100TM system (NucleoCounter YC-100, Enfield, CT, USA). The dry weight method was used to calibrate the NucleoCounter® and to occasionally double check the amount of cell mass.

2.5. Parameters calculation

The following definitions have been used in this work
Fructose yield:

$$Y_F = \frac{F_0 - F_T}{F_0} \times 100\% \qquad \text{[Equation 1]}$$

Y_F is the fructose yield (%). F_0 is the fructose concentration before fermentation (g/L), and F_T is the fructose concentration at the end of fermentation (g/L).

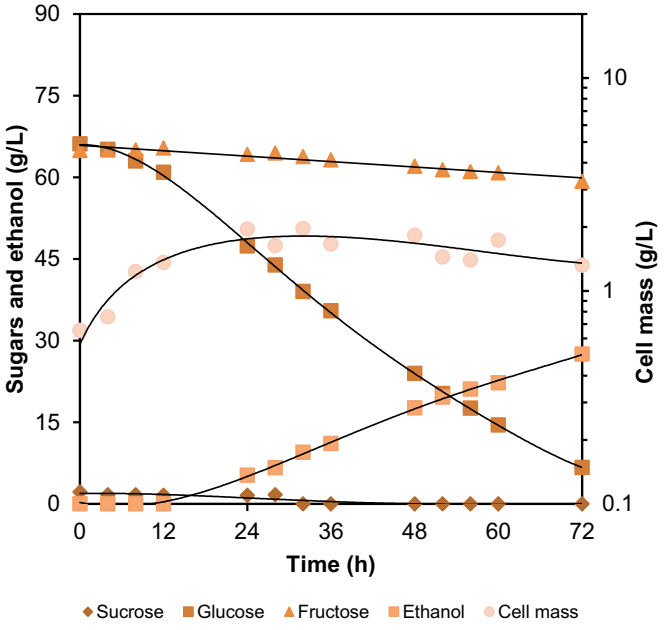

Fig. 1. The kinetic profile of sugars, ethanol and biomass for selective fermentation of date extract in 80-L fermentor.

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