Growth Kinetics and Ethanol Productivity of
Saccharomyces cerevisiae PTCC 24860 on Various Carbon Sources

H. Shafigh, G.D. Najafpour, P.S. Rezaei and M. Sharifzadeh

1School of Chemical Engineering, Nooshirvan University of Technology, Babol, Iran
2Islamic Azad University, Ayatollah Amoli Branch, Amol, Iran

Abstract: In the present study, the effect of various carbon sources such as glucose, fructose and sucrose on growth kinetics and ethanol productivity of Saccharomyces cerevisiae strain PTCC 24860 was investigated. The growth kinetic parameters were determined with Malthus, Monod and Logistic rate equations. The computed maximum specific growth rates by Monod kinetic model were highest. Then, Monod model was used to fit the growth kinetic data. The obtained maximum specific growth rates, \( \mu_{\text{max}} \) and the Monod constants, \( K_c \) for various substrates of glucose, fructose and sucrose were 0.65, 1.35, 1.85 h\(^{-1}\) and 11.39, 39.19, 97.82 g/l, respectively. Fermentation with media contained fructose, a maximum ethanol concentration of 14.45 g/l was obtained. The maximum ethanol productivity using fructose was 1.22 and 1.38-folds higher than glucose and sucrose, respectively.

Key words: Saccharomyces cerevisiae • Fermentation • Cell growth • Ethanol • Kinetic parameters

INTRODUCTION

Due to the diminishing fossil fuel reserves, global warming caused by greenhouse effect and their environmental pollutions, bio-ethanol has been considered as an alternative fuel [1-4]. To reduce the emission of carbon monoxide and unburned hydrocarbons from gasoline combustion, ethanol has widely been used as alternative fuel and fuel additives [5]. Ethanol has extensive applications in food industry, brewing process, medical and clinical applications and pharmaceutical processes [6]. Ethanol is an essential chemical which is often used as a raw material for a vast range of applications including fine chemicals and fuel (bio-ethanol) [7, 8]. Ethanol can be produced by anaerobic fermentation of sugars using yeast and other microorganisms [9]. Among the yeast kingdom, S. cerevisiae is one of the well-known ethanol producers [1, 3, 10]. Saccharomyces cerevisiae (Baker's yeast) is the most popular industrial microorganism because it utilizes cheap raw materials for growth and production and it is able to ferment many types of sugars [11]. It has been used in the fermentation of various kinds of alcohol production [9].

The Monod kinetic model used for ethanol production described as follows:

\[
\mu = \frac{\mu_{\text{max}} S}{K_c + S}
\]  (1)

Where \( \mu \) is the specific growth rate (h\(^{-1}\)), \( S \) is substrate concentration (g/l) and the terms \( K_c \) and \( \mu_{\text{max}} \) are defined as Monod constant (g/l) and maximum specific growth rate, respectively. The most active part of the cell growth curve is exponential (log) phase which is used for the determination of kinetic parameters. The log phase is a period of balanced growth, in which all components of a cell grow at the same rate [11]. Malthus model projection was used for the cell growth behavior. The derivatives for biomass generation with respect to time, is related to specific growth rate which is defined as follows [7, 8]:

\[
\frac{dX}{dt} = \mu X
\]  (2)

Where \( X \) is cell mass concentration (g/l) and \( t \) is time (h). By separation of variables and integrating Eq. 2 yields:

\[
Ln \frac{X}{X_0} = \mu t
\]  (3)

Where \( X \) is biomass concentration with respect to time and \( X_0 \) is the biomass concentration at initial time.

Corresponding Author: Dr. G.D. Najafpour, School of Chemical Engineering, Nooshirvan University of Technology, Babol, Iran E-mail: najafpour8@gmail.com
The substrate and product inhibitory effect on cell growth has been investigated in the literature [7, 8]. The cell growth rate was evaluated based on growth kinetics. Logistic equation was a suitable kinetic model for the prediction of growth curve. The specific growth rate predicted by Logistic model presented by equation 4 [8].

$$\mu = \mu_{max}(1 - \frac{x}{x_{max}})$$  \hspace{1cm} (4)

Where $x_{max}$ is the maximum cell dry weight concentration (g/l). By substitution of equation 4 into equation 2 and performing integration, the following equation for the cell concentration was obtained [8]:

$$x = \frac{x_{r} \exp(\mu \cdot t)}{1 - (\frac{x_{r}}{x_{max}}) \exp(\mu \cdot t)}$$  \hspace{1cm} (5)

The above equation was used to predict the cell growth in batch experiments. In this research, inoculation volumes were kept constant for batch experiments. The logistic model was a good approximation of the growth curve. Matlab (V 7.1) computer software was used to define logistic growth kinetic parameters.

The purpose of present research was to investigate the effect of various carbon sources such as glucose, fructose and sucrose on ethanol production. Kinetic parameters for the cell growth were determined.

**MATERIALS AND METHODS**

The microorganism for ethanol production was from Persian type culture collection (PTCC), provided by Iranian Research Organization for Science and Technology (IROST). The media used for growing *Saccharomyces cerevisiae* PTCC 24860 contained glucose, yeast extract, NH₄Cl, peptone, KH₂PO₄, NaCl, MgCl₂.6H₂O and CaCl₂.2H₂O: 15, 3, 2, 1, 0.1, 0.08, 0.07 and 0.01 g/l, respectively. The media initially had pH of 5, autoclaved at 121°C and 15 psig for 20 min. The inoculated culture was cultivated in an incubator shaker (Stuart, UK) at 30°C and agitation rate of 190 rpm for duration of 24 h.

The medium for production of alcohol consisted of: Sugar, yeast extract, NH₄Cl, KH₂PO₄, NaCl, MgCl₂.6H₂O and CaCl₂.2H₂O: 35, 1, 2, 0.1, 0.08, 0.07 and 0.01 g/l, respectively. The prepared medium was autoclaved at 121°C and 15 psig for 20 min. The supplements and sugar components of the medium were separately autoclaved.

Batch fermentation was carried out in a 250 ml shaking flasks on the incubator shaker at 30°C. The prepared media was inoculated with seed culture and incubated for 24 h. The samples were periodically taken from the culture to monitor optical density and ethanol productivity. The optical density was measured at 620 nm using a spectrophotometer (Unico, USA). The cell dry weight was determined based on the developed calibration curve. For carbohydrate concentration and ethanol analysis, 3 ml samples were collected and the cells were settled by centrifugation at 7000rpm for 7 min by a micro centrifuge made by Hermle, model: Z 233 M-2 (Germany). The concentration of the carbohydrates in the media was determined by DNS method [12].

Gas chromatograph instrument (Philips PU4400, UK) equipped with flame ionization detector (FID) and data acquisition system with computer software (Clarity lite 4.2, Data Apex, Czech Republic) was used to analyze ethanol concentration. The installed column was PEG 20 M (glass column) 1.5 m and 1/8 mm (Philips, USA). Temperature programming was implemented for the liquid sample analysis. During the analysis, the column temperature was initially maintained at 120°C. After 2 min, the oven temperature was increased at a rate of 10°C/min until it reached to 150°C. The injector and detector temperatures were 150 and 200°C, respectively. The flow rate of carrier gas (Nitrogen) was set at 30 ml/min. A solution of 2-Methyl-1-Butanol (1%, v/v) was used as an internal standard with concentration of 50 µl/ml of sample. The injection sample volume was 1 µl. In each set of experiments, the data points were repeated in triplicates and the mean value was reported.

The kinetics of growth was quantified with Malthus, Monod and Logistic rate equations. Experiment was conducted to determine bio-kinetics coefficients. The concentrations of carbohydrates, biomass and ethanol were measured as samples withdrawn in every 4 h. The course of fermentation was last for 24 h. Kinetic models were discussed, the related plots were illustrated and the coefficients obtained.

**RESULTS AND DISCUSSION**

Growth of *S. cerevisiae* was determined with respect to consumption of various carbon sources (glucose, fructose and sucrose). The relative selectivity of microorganism for the carbon sources as energy was glucose, fructose and sucrose, respectively. The carbohydrate consumption and sugar concentration profiles along with biomass generation are shown in Figure 1. The trend for biomass production presents
Fig. 1: Substrate consumption and cell growth of *S. cerevisiae* in media contained single substrate (glucose, fructose or sucrose)

Fig. 2: Cell growth and ethanol production in media contained single substrate

the growth curve. After 20 hrs of incubation time, the maximum cell concentration was achieved. The maximum biomass yield was obtained and the values are summarized in Table 1. The exponential phase for

all various carbon sources occurred in the period of 4-16 h.

Figure 2 shows ethanol concentrations and cell dry weights for three types of carbohydrates (glucose, fructose and sucrose). As the cell concentration increased the progressive ethanol synthesis along with cell density was observed. The cell growth for all carbon sources had almost the same trends; however the microorganism shows slight preference to glucose. At 20 h incubation time, maximum ethanol productions for glucose and fructose were 12 and 14.7 g/l, respectively. After 20 hours, the ethanol concentration was gradually decreased that was probably due to substrate depletion, ethanol oxidation and organic acid production [13, 14]. For each experiment, the maximum ethanol production yield was determined and the results are presented in Table 1. High ethanol production yield (theoretical yield) 0.81 g/g, in the media contained fructose made this substrate more suitable than the other two carbohydrates.

Figure 3 corresponds to equation 3 which represents the variation of the logarithm of cell concentration with respect to incubation time. The experimental data fitted
ethanol production due to the deactivation of alcohol productive enzymes [15, 16]. Figure 5 depicts the exponential growth incorporated with inhibition was projected by Logistic model for the determination of cell growth. The experimental data were fitted well and followed the model. The maximum specific growth rate for Logistic model is summarized in Table 1.

CONCLUSION

*S. cerevisiae* showed high growth rate in glucose-based medium as compared to the media contained fructose or sucrose. Also, the maximum specific growth rate estimated by the Monod kinetic model was high in the fructose-based medium. All media showed maximum ethanol production in a fermentation time of 20 h. The ethanol productivity with fructose as carbon source was 1.22-folds higher than glucose and about 1.38-folds more for sucrose. Highest ethanol production yield was achieved (81% of theoretical yield) in fructose-based medium.

REFERENCES


