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Non-Structured Kinetic Model for the Cell Growth of *Saccharomyces cerevisiae* in a Batch Culture

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Abstract: *Saccharomyces cerevisiae* is known as the most widely used eukaryotic microorganism for biological studies. In present study, cell growth profile of *Saccharomyces cerevisiae* in a batch submerged culture was evaluated with three different non-structured kinetic models. Fitness assessment of experimental data on cell growth by models was performed using the curve-fitting tool in Excel and Mat-lab software. Obtained results showed Verhulst kinetic model with R^2 equal to 0.97 was the most appropriate to describe the biomass growth rate of *Saccharomyces cerevisiae*. Maximum specific cell growth rate by Verhulst model was 0.59 h^{-1}. Other kinetic constants were also determined for all the studied models.

Key words: Cell growth · Verhulst model · Non-structured kinetic models · *Saccharomyces cerevisiae*

INTRODUCTION

The most well-known and commercially significant yeasts are the related species and strains of *Saccharomyces cerevisiae* [1]. *S. cerevisiae* is budding yeast known as a baker yeast or brewer yeast [2, 3]. Aeration is an essential factor for *S. cerevisiae* fermentation even though yeast has the ability to grow under anaerobic conditions [4]. However, *S. cerevisiae* is rather exceptional yeast since it is one of the few organisms that are able to grow anaerobic [5]. The maximum specific growth rate (μ_{max}) of *S. cerevisiae* is quite similar under aerobic and anaerobic conditions [6]. It utilizes sucrose, glucose, fructose and maltose as carbon sources to produce alcohol under anaerobic conditions [7]. *S. cerevisiae* is glucose sensitive yeast, also termed Crabtree-positive, exhibiting aerobic ethanol production in the presence of excess glucose [8, 9]. Based on a developed mathematical model for the aerobic growth of *S. cerevisiae* in batch and continuous culture, transport into and out of the mitochondrion was of the major importance in the overall metabolism of *S. cerevisiae* and was subject to long term adaptation [10]. In glucose-grown batch cultures of *S. cerevisiae*, ethanol is produced under aerobic conditions and the rate of alcoholic fermentation is barely influenced by a change to anaerobic conditions [11]. Kinetic parameters of fungi like *S. cerevisiae* may vary significantly according to culture conditions, such as oxygen and fermentation media [12]. In previous researches, nutrient uptake kinetics of filamentous microorganisms such as *Aspergillus* and *Penicillium* strains were investigated [13-19]. But, these studies were less reported for non filamentous fungi or yeast such as *S. cerevisiae*.

In this article, biomass growth rate of the *S. cerevisiae* and its fitness with three different kinetic models was assessed. This investigation was conducted with experimental data of glucose and biomass concentration in batch culture medium by three kinetic equations Monod, Moser and Verhulst. In each case, kinetic parameters were determined; the shape of statistical parameters and the fitness of the yeast growth behavior with the stated equation was evaluated using Mat Lab software.

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MATERIALS AND METHODS

Preparation of Stock Culture and Cell Suspension: The stock culture of *S. cerevisiae* was prepared on potato dextrose agar (PDA) slants. In order to prepare inoculums, yeast cells were transferred to PDA plates and incubated at 27°C for 3 days. Cell growth was observed after 5 hours on the surface of plates. Then, the cultures were transferred to a refrigerator at 4°C.

Fermentation process was performed in a laboratory shake flask as a batch submerged culture. In order to use yeast cells in the fermentation process, fresh cultures of these cells were prepared on plates contained of PDA. Linear cultivate of cells was conducted on the surface of the plates in appropriate condition under laminar flow hood and near the flame. Cultivated plates were incubated at 27°C for three days. After appear the white colonies of *S. cerevisiae* that fully covered the plate surface, the cell suspension was prepared with sterile distilled water which was used as inoculums for fermentation process.

Culture Preparation: The main culture media for batch fermentation process in 250 mL shake flasks was composed of (g. L^{-1}) glucose, 20; $MnSO_4$, 0.1; KH_2PO_4, 0.5; ammonium sulfate, 1; yeast extract, 2; and (mg. L^{-1}) $ZnSO_4$, 0.28; $FeSO_4$, 6.57; $CuSO_4$, 1.65; $MnSO_4$ 1.02. The medium pH was adjusted on 5.5 using a solution of 2 M of NaOH or 2N HCl. Then, the medium was autoclaved at 121°C for 15 min. Each of the culture components was separately autoclaved and after reach to the ambient temperature, was combined with each other under the laminar flow hood at sterile conditions.

Batch Submerged Fermentation: A 0.5 mL of spore suspension was inoculated to 100 mL prepared and sterilized medium presented in each shake flask and the flasks were put in an incubator shaker at 27°C with 200 rpm agitation speed for 30 hours. At this period, cell growth was visible with the turbidity of the medium.

Sampling and Sample Preparation: Sampling from flasks was continuously performed at two hours time intervals. At each step, 10 mL of contained broth of a shake flask was taken by a sterile syringe next to flame under laminar flow hood as a sample. Then, the sampled flask was returned to incubator shaker to extend fermentation process.

Table 1: Kinetic equation of investigated models for fitting the growth rate of *S. cerevisiae*

Model	Equation
Monod	$\mu \equiv \mu_{\max} \frac{S}{K_S + S}$
Moser	$\mu = \mu_{\max} \frac{S^n}{k_S + S^n}$
Verhulst	$\mu = \mu_{\max} \left[1 - \frac{X}{X_m} \right]$

Analytical Methods: A colorimetric method by a 1% di-nitro salicylic acid solution with 0.5 g. L^{-1} Na_2SO_4, 10 g. L^{-1} NaOH and 2 g. L^{-1} phenols was used to determine glucose concentration. In this method, a spectrophotometer (Unico 2100, USA) at a wavelength of 540 nm was used. Cell dry weight was measured using a turbidity measurement method by spectrophotometer. Cell dry weight and glucose measurements were repeated three times for each sample.

Kinetic Models: Equation of each Kinetic model examined in this study is listed in Table 1. Monod and Moser kinetic models are two unstructured growth models that are substrate concentration dependent. Verhulst kinetic model is an unstructured model depends on biomass concentration. In equations and relations of kinetic models, μ and μ_{max} in terms of 1/h are the specific growth rate and the maximum specific growth rate of yeast, respectively. Where, S is limiting substrate (glucose) concentration in term of g. L^{-1}, K_s is the semi-saturated coefficient in term of g. L^{-1} and X_m is the maximum biomass concentration in term of g. L^{-1}.

RESULTS AND DISCUSSION

The incubation time of the fermentation process was prolonged for 30 hours. *S. cerevisiae* growth in the shake flasks was observed for approximately 3 hours after inoculation as medium turbidity. At 23 hours of incubation, cell dry weight was reached to its maximum value and then yeast growth was entered to the stationary phase. The stationary phase of *S. cerevisiae* was happened in the time interval of 24 to 27 hours of incubation. After 27 hours, the cell population gradually entered to death phase, of course it cannot be realized by its appearance because it does not change a lot. The only remarkable issue in death phase was its un-favorite smell. During the fermentative process of *S. cerevisiae* in shake flask, required samples have been taken at appropriate time intervals and were prepared for next assessments.

Table 2: Experimental data on cell growth and nutrient utilization and calculated values for specific growth rate in exponential growth phase

Time (h)	X (g. L^{-1})	S_{ave}(g.L^{-1})	μ(1/h)
7	1.6484	9.35	0.633081
9	3.096	7.55	0.527105
11	6.06358	5.9	0.479353
13	8.783	4.495	0.420534
15	12.402	3.295	0.379198
17	14.7285	2.13	0.337307
19	16.09855	1.16	0.300703
21	17.4169	0.54	0.271664
23	18.3992	0.265	0.247241

The majority of glucose in the medium was consumed in the first 13 hours of process which consisted of lag and a part of exponential growth phases of yeast. With the beginning of the stationary phase, the change profile of glucose concentration in the medium was reduced significantly and glucose concentration almost reached near to zero until the end of fermentation process. In all kinetic investigation cases, experimental data on glucose concentration and cell dry weight were used to determine an appropriate kinetic model for *S. cerevisiae* growth in batch culture.

The kinetic constants and parameters (X_{max}, K_s, μ_{max}) were determined based on the curve fitting method. The values of specific growth rate (μ) was calculated according to the cell dry weight as biomass concentration (X) and glucose concentration as limiting substrate concentration (S) during the exponential growth phase. Experimental and calculated values are presented in Table 2. Based on the experimental data, X_0 and t_0 were considered 0.13 g. L^{-1} and 3 hours, respectively.

Investigation on curve fitting of cell growth with Monod model did not show acceptable fitness (Fig. 1). Based on the software analysis, μ_{max} and K_s with Monod kinetic model were evaluated as 0.377 h^{-1} and 0.157 g. L^{-1}, respectively. Also, in this case, R^2 was fitted on 0.81 that it does not seem so desirable. According to the results, Monod kinetic model is not seemed to be a suitable model to express the kinetic behavior of this strain.

Investigation on curve fitting of cell growth with Moser model have shown that despite suitable R^2 and μ_{max}, the behavior of this yeast does not have acceptable consistency with the described model due to illogical K_s (Fig. 2). In this case, R^2 and μ_{max} were obtained 0.97 and 0.502 h^{-1}, respectively. R^2 with Moser is more than Monod that demonstrated the better fitness of the experimental data of *S. cerevisiae* growth and substrate utilization with the theoretical base of the Moser kinetic

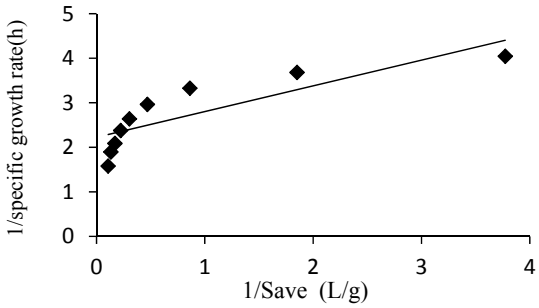

Fig. 1: The Lineweaver-Burk linear plot to fitting the experimental data on substrate utilization and cell growth to Monod kinetic model

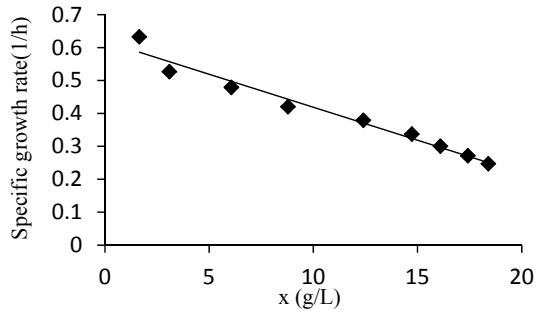

Fig. 2: The Lineweaver-Burk power plot to fitting the experimental data on substrate utilization and cell growth to Moser kinetic model

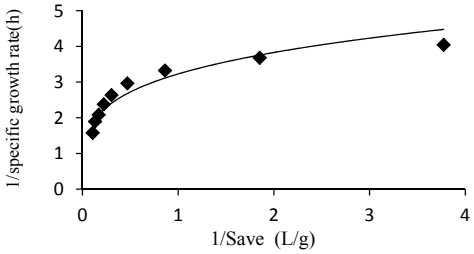

Fig. 3: The linear plot to fitting the experimental data on substrate utilization and cell growth to Verhulst kinetic model

Model. Also, in Moser case, a greater μ_{max} was obtained in comparison to the Monod. But, the obtained K_s with the Moser kinetic model was not satisfactory.

As can be seen in Figure 3, to evaluate kinetic behavior fitness of *S. cerevisiae* with Verhulst model, linear curve fitting method was used on μ based on X curve. Results showed that the experimental data of the cell growth and substrate consumption in batch submerged culture did not have a good fitness with Verhulst model by the regression of 0.97. In this regard, the maximum specific growth rate (μ_{max}) and the maximum biomass concentration (X_m), was 0.59 h^{-1} and 32.43 g. L^{-1}, respectively.

Table 3: A comparison survey of kinetic parameters of *S. cerevisiae* growth and substrate utilization with three different kinetic models

Kinetic model	RMSE	SSE	R^2	K_s (g. L^{-1})	μ_{max} (h^{-1})	X_m (g. L^{-1})
Monod	0.5721	3.273	0.81	0.157	0.377	-
Moser	0.2361	0.502	0.97	1.003	0.502	-
Verhulst	0.1103	0.121	0.97	-	0.593	32.43

Table 3 represents a comparison of the kinetic parameters obtained by fitting the kinetic models examined in this study.

CONCLUSION

This is the first report on the cell growth and substrate utilization kinetic of *S. cerevisiae* with respect to Monod, Moser and Verhulst kinetic models. The experimental data on cell growth and nutrient utilization in submerged batch fermentation process were interpreted using Monod, Moser and Verhulst kinetic models as unstructured models based on substrate concentration (Monod and Moser models) and biomass concentration (Verhulst model). Based on the results, the Verhulst kinetic model was the most appropriate to describe the biomass growth rate of *S. cerevisiae*. Maximum specific cell growth rate by Verhulst model was 0.59 h^{-1}.

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Persian Abstract

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چکیده

ساکارومایسس سرویسیه به عنوان پرکاربردترین میکروارگانیسم یوکاریوتی در مطالعات زیستی شناخته شده است. در تحقیق حاضر پروفیل رشد *ساکارومایسس سرویسیه* در یک محیط کشت غوطه ور ناپیوسته با سه مدل سینتیکی غیر ساختاری متفاوت مورد بررسی قرار گرفته است. بررسی برازش داده های آزمایشگاهی رشد سلول با سه مدل سینتیکی با استفاده از ابزار برازش منحنی در نرم افزارهای اکسل و مت لب انجام شده است. نتایج حاصله نشان داده که مدل سینتیکی ورهالست با رگرسیون ۰/۹۷ مناسب ترین مدل برای بیان رفتار سینتیکی رشد *ساکارومایسس سرویسیه* بوده است. بیشینه شدت رشد ویژه سلول بر اساس این مدل برابر با ۰/۵۹ بر ساعت بوده است. سایر پارامترهای سینتیکی نیز برای هر سه مدل مورد بررسی تعیین گردیده است.