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Survey of the Nutrient Utilization and Cell Growth Kinetic with Verhulst, Contois and Exponential Models for *Penicillium brevicompactum* ATCC 16024 in Batch Bioreactor

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Abstract: In the most cases of the practical fermentation processes with filamentous microorganisms, direct monitoring of the cell morphology and biomass distribution in the culture medium is not easy possible. *Penicillium brevicompactum* is one of the morphologically complex filamentous fungi with different structural forms. In this research, nutrient uptake and cell growth kinetics was investigated compare to Verhulst, Contois and Exponential models using batch submerged fermentation in a bench-scale stirred tank bioreactor. The compatibility of the experimental data fitted with Verhulst, Contois and Exponential models with the regression values as 0.85, 0.86 and 0.90, respectively. In the case of Verhulst, the maximum specific growth rate and maximum cell dry weight were determined as 0.06 h^{-1} and 33.2 g. L^{-1}, respectively. For Contois study, the maximum specific growth rate and the half-saturation coefficient were obtained as 0.04 h^{-1} and 0.31 g. L^{-1}, respectively. However, in Experimental model, the maximum specific growth rate was determined as 0.01 h^{-1}. Thus, although Verhulst and Contois are proper kinetic models to describe substrate utilization and cell growth behavior of *Penicillium brevicompactum* in submerged batch bioreactor culture, the Exponential model is a better kinetic model for this purpose.

Key words: *Penicillium brevicompactum* · Verhulst kinetic · Contois kinetic · Exponential kinetic · Maximum specific growth rate · Submerged batch bioreactor

INTRODUCTION

Filamentous fungi are used for production of many valuable biological products such as antibiotics, drugs, industrial enzymes, pharmaceutical proteins and also in biotransformation processes. On the other hand, these organisms have been used as appropriate hosts in recombinant DNA technology, has applied in the discovery of new drugs, commercial recombinant enzymes and other useful new products. Fungal secondary metabolites such as antibiotics, immunosuppressive agents, hypercholesterolemia agents, anti-tumor agents, mycotoxins, pigments and polyunsaturated fatty acids are very important to human health and nutrition with abundant economic impacts [1]. Filamentous fungi are morphologically complex organisms exhibiting pellet or mycelial forms [2]. Their morphology is depending on physical culture conditions, such as agitation rate, aeration, oxygen and heat transfer rates, growth rate, cultivation mode, temperature and pH [3].

The *Penicillium* species as well as *P. brevicompactum* identified as a main group of filamentous fungi belong to the most important known mycotoxin producers strains [4]. These organisms are widely found on the natural solid surfaces such as bread, fruits and vegetables. Twenty-five *Penicillium* species and their mycotoxins were found in food waste from private households [5]. *P. brevicompactum* is belong to Eukaryota, Fungi/ Metazoa group, Fungi, Dikarya, Ascomycota, Pezizomycotina, Eurotiomycetes, Eurotiomycetidae, Eurotiales, Trichocomaceae, Mitosporic Trichocomaceae, Penicillium family [6]. *P. brevicompactum* is known as a beneficial mould, able to produce some valuable products such as mycophenolic acid, brevianamide A, asperphenamate and ergosterol [7].

Fungi kinetics may significantly vary with the changes in culture conditions. Maximum growth rate (μ_{max}) must be determined as an input for process optimization, modeling and scale-up. Growth kinetics of filamentous microorganisms has been studied in a few previous

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researches [8]. Some researches consider a classic kinetics for filamentous fungi like other organisms including a lag and then, an exponentially growth phase [9, 10]. However, some others believe that the growth kinetics of filamentous fungi is fitted to cubic model [11].

In some cases, Verhulst kinetic has been used for the demonstration of growth characteristics of the cell population [12]. This model, contains two parameters, the maximum specific growth rate (μ_{max}) and maximum cell dry weight (X_m), define the relationship between cell growth and substrate utilization, to describe kinetic behaviors of a microbial cell population [13, 14]. The Verhulst model is still used very often in environmental and industrial microbiology studies [15, 16, 17]. In our knowledge, the Verhulst kinetic has been not studied until now in the case of filamentous fungi. The Contois model is one of the common models used to describe microbial cell growth and substrate uptake kinetics [18, 19]. The Exponential model was applied in some studies to investigate cell population kinetics [11, 20, 21, 22].

Experimental data on nutrient utilization and cell growth of *P. brevicompactum* ATCC 16024 were compared with Verhulst, Contois and Exponential models in batch submerged fermentation using a bench-scale stirred tank bioreactor. As well as, such kinetic parameters including maximum growth rate, half saturation coefficient and maximum cell dry weight were determined.

MATERIALS AND METHODS

Microorganism and Culture Conditions: In this study, *P. brevicompactum* ATCC 16024 was used. The culture was maintained on the potato dextrose agar (PDA) slants. In order to inoculum preparation, spores were transferred to PDA plates and incubated at 27°C for 3 days. Then, three days spores were suspended in sterile distilled water and their numbers were counted and adjusted to 10^7-10^8 per mL^{-1} using a Thoma lam. The spore suspension was used as an inoculum for subsequent fermentation study carried out in the bioreactor [23].

The synthetic medium composition was given in Table 1. Trace elements mixture was included (g. L^{-1}) $FeSO_4 \cdot 7H_2O$, 2.2; $CuSO_4 \cdot 5H_2O$, 0.3; $ZnSO_4 \cdot 7H_2O$, 2.4; $MnSO_4 \cdot 4H_2O$, 0.16 and $KMoO_4$, 0.2. In order to perpetrate culture medium, all medium components except for glycine, methionine and the trace elements, were strilled by autoclaving separately at 121°C for 15 min (Keyhan Lab autoclave, Iran). other components were sterilized using a 0.2 µm filter (Millipore, USA).

Table 1: Composition of the experimental medium culture

Constituent	Concentration (g. L^{-1})
Glucose	80
Glycine	15
Enzymatically hydrolyzed casein	30
Methionine	2.5
KH_2PO_4	5
$MgSO_4 \cdot 7H_2O$	1
Trace elements mixture	1 ml. L^{-1}

Experiments and Analytical Procedures: Submerged batch fermentation was performed for 350 hours using a 5 L bench-scale stirred tank bioreactor (INFORS HT, Switzerland). Initially, the bioreactor was autoclaved at 121°C for 1 hour. Then, 2.5 liter of the prepared sterile medium was transferred to the bioreactor and inoculated with 10 mL of the spore suspension. Operational conditions containing temperature, agitation speed and pH were adjusted to 27°C, 700 rpm and 6.0±0.1, respectively. Aeration rate and dissolved oxygen were maintained as 1 vvm and 20% of saturation point, respectively [23].

In the process duration, at determined time intervals, a silicon tube connected to a vacuum pump was used for sampling (Millipore, USA). After filtration (a 0.2 µm filter) and centrifugation at 6000 rpm for 10 min, the supernatant was used for glucose assaying, while cell dry weight measurement was performed using the obtained biomass. Cell dry weight in the samples was determined by drying and weighting the biomass at 60-65°C until reaching constant weight. The glucose concentration was measured by a colorimetric method using the dinitrosalicylic acid (DNS) reagent and a spectrophotometer (Unico 2100, USA) at a wavelength of 540 nm. Cell dry weight and glucose measurements were repeated three times for each sample.

Kinetic Models: Verhulst equation is an unstructured kinetic model based on biomass concentration. Verhulst equation is presented in bellow form:

$$\mu = \mu_{\max}\left[1 - \frac{X}{X_m}\right] \quad (1)$$

Contios equation is an unstructured kinetic model based on substrate and biomass concentrations. Contois equation is presented as:

$$\mu = \mu_{\max}\frac{S}{k_s x + S} \quad (2)$$

Where μ is specific growth rate (h^{-1}), μ_{max} is maximum specific growth rate (h^{-1}), S is limiting substrate concentration (g. L^{-1}), X is biomass concentration (g. L^{-1}), K_s is half-saturation coefficient (g. L^{-1}) and X_m is maximum biomass concentration [24].

Also, Exponential equation is presented as:

$$S = S_0 + S_1\,[1 - \exp(\mu_{max}\,.t)] \quad (3)$$

Where S_0 is initial glucose concentration (80 g. L^{-1}), S_1 is the required substrate to produce an initial cell population (10 g. L^{-1} in this study) and t is time (h^{-1}).

RESULTS AND DISCUSSION

Experimental Data on Substrate Uptake and Cell Growth: In the batch process in stirred tank bioreactor, spore germination and growth of *P. brevicompactum* started 24 h after inoculation. Cell growth feature under the applied culture conditions such as temperature, pH and other conditions was happened as mycelial form. At the start of the experiment, agitation rate was set at 200 rpm, which resulted in mycelial clumps forming and perturbation in the control of process parameters. To overcome this, maximum agitation rate was set at 700 rpm. As mentioned in some previous researches, the formation of mycelial clumps could cause to limit mass and oxygen transfer inside the bioreactor and decrease cell growth (data not shown) [25].

Exponential growth phase was extended from about 24 to 150 h after incubation. The stationary growth phase was started at 150 h and ended at about 350 h. Most of the main substrate, glucose, in the medium was consumed after 150 h of inoculation (Fig. 1).

Kinetic Studies: In all kinetic studies cases, the experimental data on glucose and biomass concentrations during the growth phase of *P. brevicompactum* in batch bioreactor were used for the determination of kinetic parameters. These parameters ($\mu_{max,}$ $K_{s,}$ X_{max}) were determined, based on the curve-fitting procedure. Considering the cell dry weight as biomass concentration values (X) and glucose concentration as limiting substrate concentration quantities (S) during the exponential growth phase, the values for μ were calculated from Eq. 4. The experimental and calculated values are presented in Table 2. Based on experimental data (Fig. 1), X_0 and t_0 were determined as 2.6 g. L^{-1} and 24 h, respectively.

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

Time (h)	S_{ave} (g. L^{-1})	X (g. L^{-1})	μ (h^{-1})
24	66.95	2.60	-
48	58.05	9.30	0.053
60	51.26	11.20	0.040
70	46.16	12.45	0.034
80	40.97	13.60	0.029
90	31.02	16.60	0.028
100	19.45	18.24	0.026
110	13.27	19.00	0.023
120	10.52	22.10	0.022
130	9.07	22.78	0.020
140	7.92	24.13	0.019
150	6.28	24.60	0.018
160	-	24.60	0.016

$$\mu = \frac{Ln\left(X/X_0\right)}{t - t_0} \quad (4)$$

Investigation of the Compatibility of Experimental Data to Contois Model: In the case of Contois kinetic studing, Eq. 2 was lineated as:

$$\frac{1}{\mu} = \left(\frac{K_s}{\mu_{max}}\right)\left(\frac{X}{S}\right) + \frac{1}{\mu_{max}} \quad (5)$$

The Lineweaver-Burk linear plot for $\frac{1}{\mu}$ versus $\left(\frac{X}{S}\right)_{ave} = \frac{X_1 + X_2}{S_1 + S_2}$ was fitted to the experimental data using Excel software as shown in Fig. 2.

The obtained results showed a relatively acceptable compatibility to Contois kinetic model with a regression of 0.86. The observed consistency and low value of K_s demonstrates that glucose, as limiting substrate, in applied concentrations has not any inhibitory effect on the cell growth. In this study, the maximum specific growth rate and half-saturation coefficient were determined as 0.04 h^{-1} and 0.31 g. L^{-1}, respectively. Therefore, Contois kinetic model could be consider as a suitable model to describe cell growth and nutrient uptake kinetics of *P. brevicompactum* ATCC 16024 in submerged batch stirred tank bioreactor.

Investigation of the Compatibility of Experimental Data to Verhulst Model: To evaluate the fitting of *P. brevicompactum* ATCC 16024 kinetic behaviors to Verhulst model, the linear curve-fitting procedure was applied on the μ versus X curve, as presented in Fig. 3.

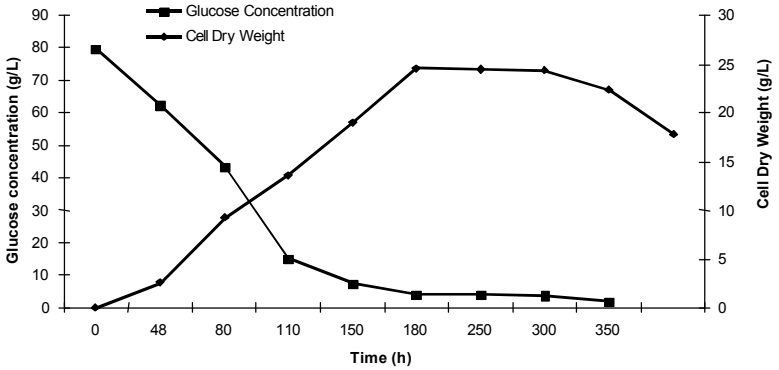

Fig. 1: Glucose and cell dry weight concentration profiles in submerged batch stirred tank bioreactor. Temperature, agitation speed, pH, aeration rate and dissolved oxygen were adjusted to 27°C, 700 rpm, 6.0±0.1, 1 vvm and 20% of saturation point, respectively

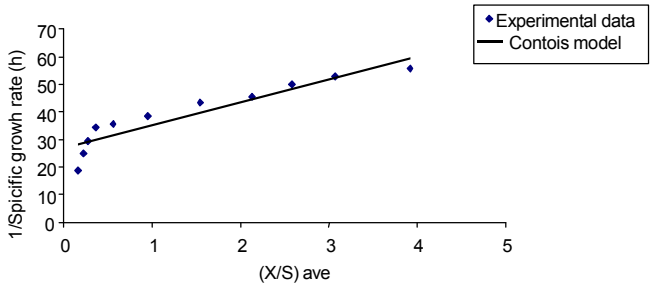

Fig. 2: The Lineweaver-Burk linear plot $\frac{1}{\mu}$ for versus $\left(\frac{X}{S}\right)_{ave}$ to fitting the experimental data on substrate utilization and cell growth to Contois kinetic model

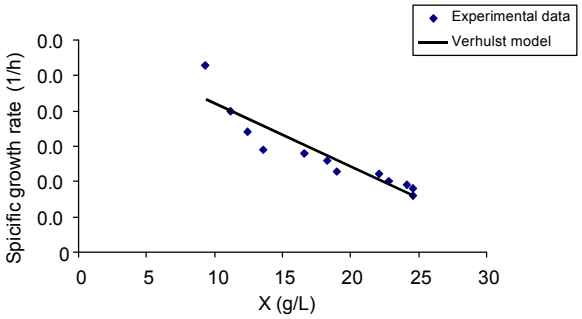

Fig. 3: The linear plot for μ versus X to fitting the experimental data on substrate utilization and cell growth to Verhulst kinetic model

Results showed that the same as Contois, experimental data on cell growth and nutrient uptake of *P. brevicompactum* in submerged batch stirred tank bioreactor were fitted to Verhulst model with a regression value of 0.86. In this case, two kinetic parameters, the maximum specific growth rate (μ_{max}) and the maximum biomass concentration (X_{max}), were determined as 0.06 h^{-1} and 33.2 g. L^{-1}, respectively. With regard to a high value μ_{max} obtained for a filamentous microorganism, Verhulst seems to not be a suitable kinetic model for *P. brevicompactum* ATCC 16024 in the applied culture condition.

Investigation of the Compatibility of Experimental Data to Exponential Model: To investigate the compatibility of experimental data with Exponential kinetic model, Eq. 6 was obtained after putting the values of S_0 and S_1 in Eq. 3:

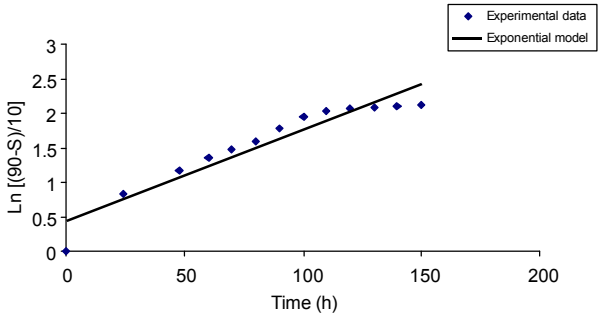

Fig. 4: The logarithmic plot applied to fitting the experimental data on substrate utilization and cell growth to Exponential kinetic model

$$Ln\left(\frac{90-S}{10}\right)=\mu_{max}\cdot t \qquad (6)$$

Based on the plotted logarithmic curve for Eq. 6, the maximum specific growth rate (μ_{max}) and the regression values obtained 0.01 h^{-1} and 0.9, respectively (Fig. 4). The lowest amount of the maximum specific growth rate as well as the best regression value compare to Contois and Verhulst models, demonstrate that the Exponential model is a better kinetic model to describe cell growth and nutrient uptake behavior of *P. brevicompactum* ATCC 16024 in the applied culture condition.

CONCLUSION

The present study is the first report on the cell growth and nutrient uptake kinetics of *P. brevicompactum* with respect to Verhulst, Contois and Exponential kinetic models. The experimental data on cell growth and substrate utilization in submerged batch fermentation process were compared to Verhulst, Contois and Exponential kinetics models as unstructured models. The obtained results showed a relatively acceptable fitting of the experimental data to all three kinetic models with the regression values as 0.85, 0.86 and 0.90, respectively. The maximum specific growth rate was determined as 0.06, 0.04 and 0.01 h^{-1}, respectively for Verhulst, Contois and Experimental models. In Contois case study, the half-saturation coefficient was obtained as 0.31 g. L^{-1}. Based on obtained results, although Verhulst and Contois are proper kinetic models to describe substrate utilization and cell growth behavior of *Penicillium brevicompactum* ATCC 16024 in submerged batch bioreactor culture, the Exponential model seem to be a better kinetic model for this purpose.

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