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# Non-Structured Kinetic Model of *Aspergillus niger* Growth and Substrate Uptake in a Batch Submerged Culture

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***Authors' contributions***

*This work was carried out in collaboration between both authors. Authors FA and RK contributed in this study. The first author designed the research subject, work stages and procedures and wrote the manuscript. The second author was performed the experiments and kinetic investigations. Both authors read and approved the final manuscript.*

***Original Research Article***

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## ABSTRACT

**Aims:** To investigate cell growth profile of *Aspergillus niger* in a batch submerged culture medium and then evaluation of cell kinetic behavior using some different non-structured kinetic models.
**Methodology:** Experiments of cell growth and substrate utilization were conducted in batch submerged cultures with identified medium composition. Fitness assessment of experimental data on the cell growth and glucose consumption by models was performed using the curve-fitting tool in Mat Lab software. This report is the first in the kinetic investigation of *Aspergillus niger* PTCC 5010 with the studied models. This work was performed in Department of Chemical Engineering, Islamic Azad University, Qaemshahr Branch between April 2013 to September 2013.
**Results:** Based on the obtained results; Moser kinetic model with R2 equal to 0.913 and Gompertz kinetic model with R2 equal to 0.949 were the best fitted models to describe the growth behavior of *Aspergillus niger* PTCC 5010 in the applied culture condition. Maximum specific cell growth rate with Moser and Gompertz kinetic models were 0.024 and 0.003h-1, respectively. Other kinetic constants for all studied models were also

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determined at the applied culture conditions. The consistency of the experimental data with Monod, Verhulst and Contois kinetic models wasn't in an acceptable range.
**Conclusion:** In scale up biochemical projects by *Aspergillus niger* PTCC 5010 for some industrial products, Moser and Gompertz kinetic models are able to demonstrate cell growth behavior and its substrate uptake profile. In continuous processes, dilution rate could be determined based on obtained maximum specific cell growth rate equal to 0.024 h-1.

*Keywords: Aspergillus niger; batch submerged culture; non-structured kinetic models; maximum specific growth rate.*

## 1. INTRODUCTION

Filamentous fungi are morphologically complex organisms exhibiting pellet or mycelia forms [1]. Their morphology is depending on physical culture conditions, such as agitation speed, aeration rate, available or dissolved oxygen, heat transfer, growth rate, cultivation mode, temperature and pH [2]. These groups of microorganisms have been recognized as proper hosts for recombinant proteins due to their advantages in growth characteristics and protein secretion and their ability to perform post-translational processing [3].

*Aspergillus niger* is one of the most important filamentous fungi used for biotechnological purposes. It has been utilized in industrial fermentations for more than 80 years because of its ability to accumulate and secrete large quantities of metabolites, such as organic acids [4-6] as well as large amounts of heterologous and homologous proteins [7]. *Aspergillus niger* is important in industrial fermentations such as the brewing of beer, manufacture of wine, production of antibiotics, vitamins and organic acids [8]. In the food industry, also some processes such as baking and repining of cheese are depend on this saprophytic fungus [9]. This filamentous fungus is a good known organism to produce industrially 40 different commercial enzymes with applications to food, beverage, textile, pulp and paper [10-12]. For example, food-enzymes such as glucoamylase and chymosine as well as citric acid are produced by *Aspergillus niger* in commercial process [13]. Enzymes are very important commercial products with a global market growing 6.5% annually and projected global sales in 2015 of USD 7,400 million [14]. So, it is necessary to acquire sufficient knowledge about its fermentation process and kinetic behavior of cell growth. Only a few studies have been conducted in this field.

In this article, kinetic behavior of *Aspergillus niger* PTCC 5010 was investigated based on some different kinetic models. Experimental data on glucose concentration and cell dry weight in a batch culture medium were used to compare with four kinetic equations: Monod, Moser, Verhulst, Contois and Gompertz [15-17]. In each case, kinetic parameters were determined and the fitness of the cell growth and substrate uptake behavior with the noted equation was cleared using Mat Lab software in the form of statistical parameters.

## 2. MATERIALS AND METHODS

### 2.1 Microorganism and Inoculum Preparation

*Aspergillus niger* PTCC 5010 was prepared from Iranian Research Organization for Science and Technology. The stock culture of *A. niger* PTCC 5010 prepared on the potato dextrose agar (PDA) slants. To inoculum preparation, spores were transferred to PDA plates (Fig. 1) and incubated at 27ºC for 3 days and then stored in a refrigerator at 4ºC.

Fermentation process was performed in a laboratory shake flask as a batch submerged culture. In order to use fungus spores in the fermentation process, new cultures of these cells were prepared on plates contained of PDA. Linear cultivate of spores has been done on the surface of the plates in appropriate condition under laminar flow microbial hood and near the flame at sterile condition. Cultivated plates were incubated at 27ºC for three days. After appear the black spores of *A. niger* PTCC 5010 that fully covered the plate surface, the spore suspension was prepared and has been used as inoculums for fermentation process. Following growth, spores were suspended in sterile distilled water and their numbers were counted and adjusted to $10^7$–$10^8$ spores per mL using a Thoma lam [18].

## 2.2 Culture Preparation

The main culture media for batch fermentation process in 250mL shake flasks was composed of (g. $L^{-1}$) glucose, 20; $MgSO_4$, 0.36; $KH_2PO_4$, 6.44; $CaCl_2$, 0.6; and (mg. $L^{-1}$) $ZnSO_4$, 0.28; $FeSO_4$, 6.57; $CuSO_4$, 1.65; $MnSO_4$ 1.02 [8]. The medium pH was adjusted on 5.5 using a solution of 2 N of NaOH or HCl. Then the medium was autoclaved at 121ºC for 15 min. Each of the culture components was autoclaved separately and after reach to the ambient temperature, was combined with each other under sterile conditions.

## 2.3 Batch Submerged Fermentation

0.5mL of spore suspension was inoculated to 100mL prepared and sterilized medium presented in each shake flask and the flasks were put in a shaker incubator at 27ºC with 200 rpm agitation speed for 200 hours. At this period, cell growth was visible with the pellets of *A. niger* PTCC 5010. After that, flasks were exited from shaker incubator and were used in order to measure cell dry weight and glucose concentration after filtering.

## 2.4 Measurement Methods

A colorimetric method by a 1% di-nitro salicylic acid solution with 0.5 g. $L^{-1}$ $Na_2SO_4$, 10g. $L^{-1}$ NaOH and 2g. $L^{-1}$ phenols was used to determine glucose concentration [18]. In this method, a spectrophotometer (Unico 2100, USA) at a wavelength of 540 nm was used. Glucose standard curve (Fig. 1) was determined by prepared standard solutions of glucose. Glucose concentration was adjusted on 0.1 to 1g. $L^{-1}$. Cell dry weight in the samples was determined by drying and weighting the biomass at 60-65ºC until reaching constant weight. Cell dry weight and glucose measurements were repeated three times for each sample.

## 2.5 Kinetic Models

Monod and Moser equations were the non-structured kinetics models based on substrate concentration which were chosen for two parameters modeling. Monad's equation is presented as:

$$\mu \equiv \mu_{\max} \frac{S}{K_s + S} \tag{1}$$

Also, Moser's equation is presented in bellow form:

$$\mu = \mu_{\max} \frac{S^n}{k_s + S^n} \tag{2}$$

Verhulst kinetic model (Eq. 3) is a non-structured model depends on biomass concentration.

$$\mu = \mu_{\max}\left[1 - \frac{X}{X_m}\right] \tag{3}$$

Contois kinetic model is a non-structured model depends on both substrate and biomass concentration (Eq. 4).

$$\mu = \mu_{\max}\frac{S}{k_s x + S} \tag{4}$$

Gompertz curve (Eq. 5) is a suitable model for slow growth of cells such as tumors. In fact, tumors are cellular populations growing in a confined space where the availability of nutrients is limited. This model could be applied for fungi growth too.

$$X(t) = K\exp(\log(\frac{X(0)}{K})\exp(-\alpha t)) \tag{5}$$

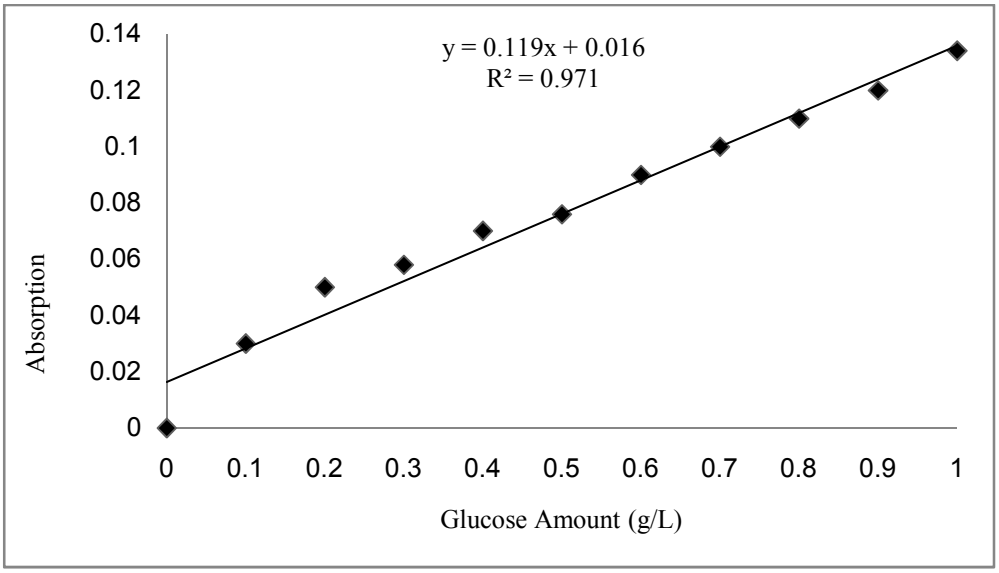


**Fig. 1. Standard curve for spectrophotometer Unico-2100 prepared using glucose standard solutions at 540nm**

In equations and relations of kinetic models, μ and $\mu_{max}$ are the specific growth rate and the maximum specific growth rate of fungi respectively in terms of $h^{-1}$, 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. $L^{-1}$. In Gompertz model, K is the carrying capacity, i.e. the maximum size that can be reached with the available nutrients and α is a constant related to the proliferative ability of the cells (same as $\mu_{max}$ in other models).

## 3. RESULTS AND DISCUSSION

*A. niger* PTCC 5010 cell growth phase was evaluated in a 200 hour process. Cell growth was observed as pellets remained until the end of exponential growth phase (150 h). At 150 hours after incubation, cell dry weight reached to its maximum value (38g. $L^{-1}$) and then fungus growth entered to the stationary phase for 20h. Then, gradually the pellet numbers have been decreased and their destruction was observed slightly in flask and death phase was started. During the fermentative process of *A. niger* PTCC 5010 in shake flask samples have been taken at proper time intervals and were prepared for analyzing of glucose concentration.

The majority of glucose content in the medium was consumed in the first 102 hours of process (more than 16 g. $L^{-1}$ consumed glucose) which consisted of lag phase and a part of exponential growth phase. While the stationary phase appeared, glucose concentration had low changes and reached from 0.7 to 0.21g. $L^{-1}$. In all kinetic investigation cases, experimental data on glucose and biomass concentrations were used to determine kinetic models during the exponential growth phase of *A. niger* PTCC 5010 in batch culture (Fig. 2). The obtained process yield and productivity were evaluated as 1.97 g produced biomass for each g consumed glucose and 0.253 g biomass. $L^{-1}$ $h^{-1}$.

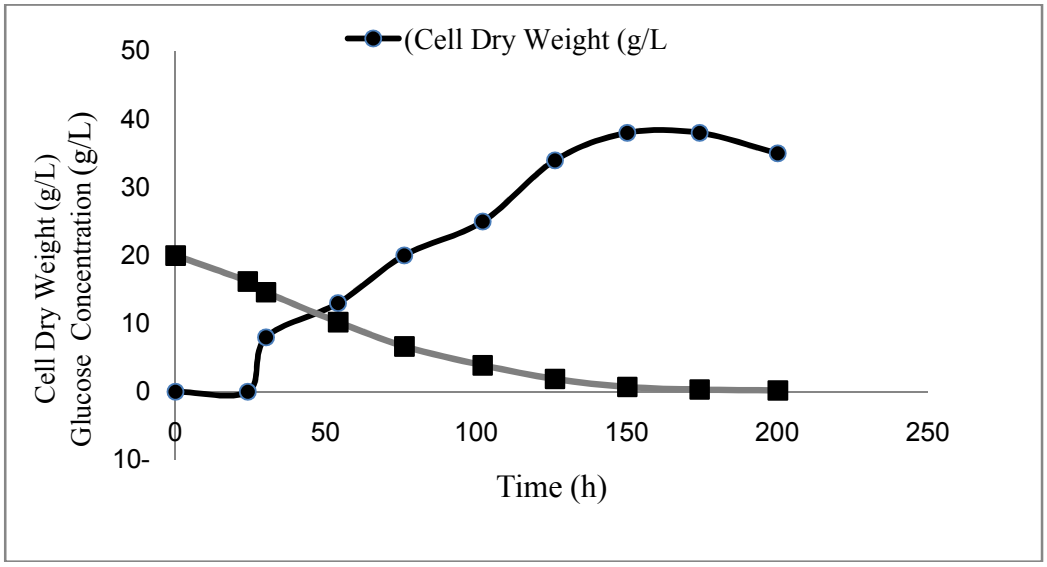


**Fig. 2. Cell dry weight and glucose concentration profile of *A. niger* PTCC 5010 growth in a batch submerged culture 27ºC with 200 rpm agitation speed for 200 hours**

These parameters ($X_{max}$, $K_s$, $\mu_{max}$) were determined using the curve fitting method. Specific cell growth rate values were calculated according to cell dry weight as biomass concentration (X) and average glucose concentration as limiting substrate concentration ($S_{ave}$) during the exponential growth phase. Experimental and calculated values are presented in Table 1. Specific cell growth rate and the average of biomass concentration to substrate concentration were calculated by equations No. 6 and 7.

$$\mu = \frac{Ln\left(\frac{X}{X_0}\right)}{t - t_0} \quad (6)$$

$$\left(\frac{X}{S}\right)_{ave} = \frac{X_1 + X_2}{S_1 + S_2} \quad (7)$$

Based on the experimental data (Fig. 2), $X_0$ and $t_0$ were considered 8 g. $L^{-1}$ and 30 hours, respectively. Curve fitting of fungus growth with Monod kinetic model didn't show an acceptable fitness (Fig. 3). In this investigated case, R-square was obtained as 0.777, indicated a non-satisfactory fitness between the experimental data on cell growth and substrate consumption and theoretical Monod kinetic equation. Maximum specific cell growth rate ($\mu_{max}$) and Monod semi-saturated coefficient ($K_s$) were evaluated as 0.019 $h^{-1}$ and 0.291 g. $L^{-1}$, respectively (Table 2).

**Table 1. Experimental data on cell growth and nutrient utilization and calculated values for specific growth rate in exponential growth phase of *A. niger* PTCC 5010 in a batch culture**

| Time (h) | X (g. $L^{-1}$) | $S_{ave}$ (g. $L^{-1}$) | $1/S_{ave}$ (L. $g^{-1}$) | μ ($h^{-1}$) | 1/μ (h) | $(X/S)_{ave}$ |
|---|---|---|---|---|---|---|
| 0 | 0 | 20 | - | - | - | 0 |
| 24 | 0 | 16.2 | - | - | - | 0.259 |
| 30 | 8 | 14.63 | 0.08 | - | - | 1.184 |
| 54 | 13 | 10.23 | 0.118 | 0.0202 | 49.505 | 1.573 |
| 65 | 16 | 7.42 | 0.135 | 0.0198 | 50.555 | 2.424 |
| 76 | 20 | 6.65 | 0.19 | 0.0199 | 50.251 | 3.707 |
| 90 | 23 | 4.42 | 0.226 | 0.0176 | 56.818 | 5.430 |
| 102 | 25 | 3.89 | 0.345 | 0.0158 | 63.291 | 8.132 |
| 115 | 29 | 2.32 | 0.431 | 0.0151 | 66.225 | 13.548 |
| 126 | 34 | 1.9 | 0.769 | 0.0150 | 66.666 | 22.152 |
| 138 | 36 | 0.98 | 1.020 | 0.0139 | 71.942 | 37.755 |
| 150 | 38 | 0.7 | 1.904 | 0.0129 | 77.519 | 72.381 |
| 174 | 38 | 0.35 | 3.571 | 0.0108 | 92.592 | 130.357 |
| 200 | 35 | 0.21 | 9.523 | 0.0086 | 116.279 | - |

Based on the curve-fitting results, Moser kinetic model was one of the best and suitable models to present *A. niger* PTCC 5010 growth and substrate utilization behavior at the applied conditions (Fig. 4). This model with R-square 0.913 showed a good consistency with experimental cell growth data. As presented in Table 2, $\mu_{max}$ and $K_s$ for Moser kinetic model were obtained as 0.024$h^{-1}$ and 1.747g. $L^{-1}$, respectively. As a positive point in comparison to Monod kinetic model, here a greater $\mu_{max}$ value was obtained,indicated better cell growth rate. Moser kinetic model has been resulted the greatest value for $\mu_{max}$ in compare with other three studied models.

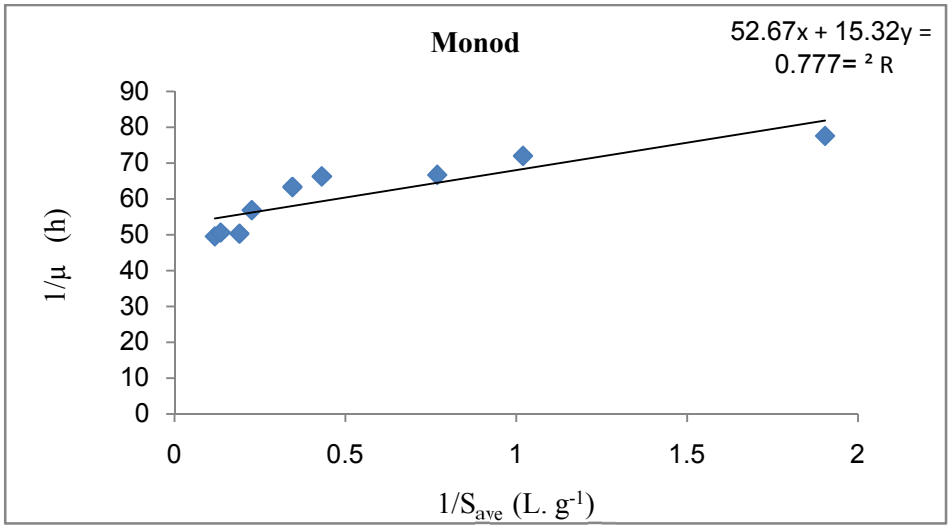


**Fig. 3. The Line weaver-Burk linear plot for $\frac{1}{\mu}$ versus $\frac{1}{s_{ave}}$ to fitting the experimental data on substrate utilization and cell growth to Monod kinetic model for *A. niger* PTCC 5010 in a submerged batch culture medium**

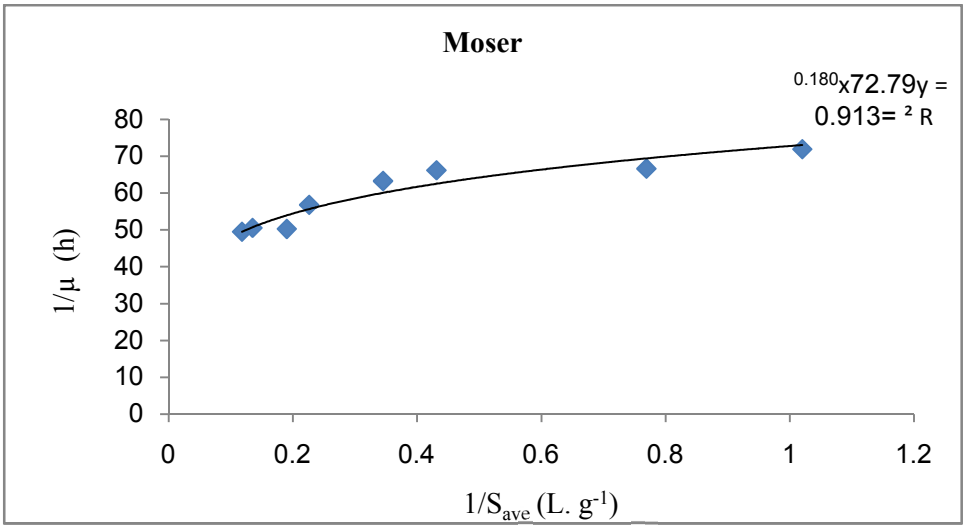


**Fig. 4. The Line weaver-Burk power plot for $\frac{1}{\mu}$ versus $\frac{1}{s_{ave}}$ to fitting the experimental data on substrate utilization and cell growth to Moser kinetic model for *A. niger* PTCC 5010 in a submerged batch culture medium**

To investigate the consistency of cell kinetic behavior with Verhulst model, linear curve fitting method (μ versus X) was used (Fig. 5). 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. In this case, $R^2$ was obtained as 0.677 and maximum specific growth rate ($\mu_{max}$) and the maximum biomass concentration ($X_m$) were 0.018 $h^{-1}$ and 6g. $L^{-1}$, respectively.

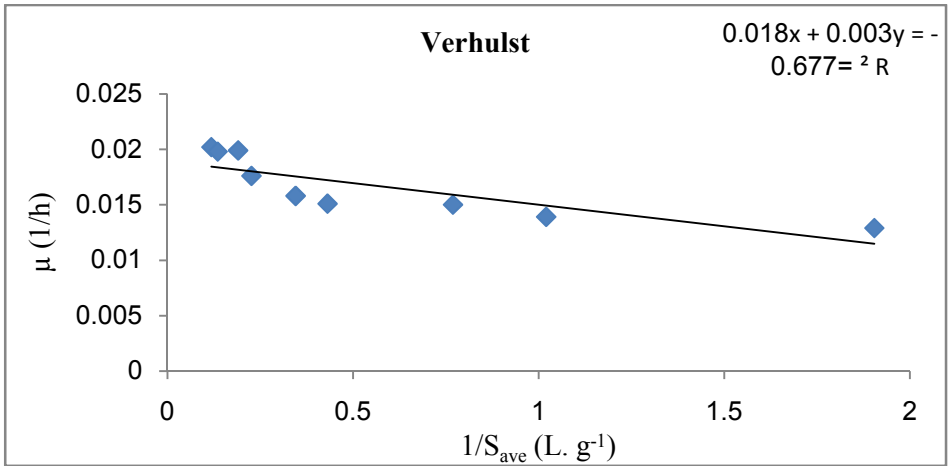


**Fig. 5. The linear plot for *μ* versus *X* to fitting the experimental data on substrate utilization and cell growth to Verhulst kinetic model for *A. niger* PTCC 5010 in a submerged batch culture medium**

Linear curve fitting results also showed that cell growth profile of *A. niger* PTCC 5010 didn't follow up of Contois kinetic model principles. For this studied theory, a 0.751 R-square value was obtained suggests not acceptable match between actual cell growth data and theoretical concepts of Contois kinetic model (Fig. 6). According to the results, maximum specific growth rate ($\mu_{max}$) and the Contois semi-saturated coefficient ($K_s$) were evaluated as 0.018$h^{-1}$ and 0.007g. $L^{-1}$, respectively (Table 2).

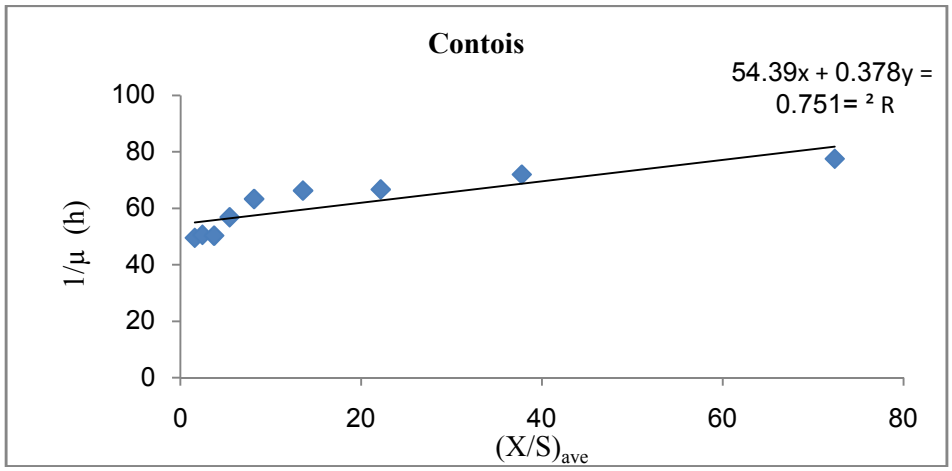


**Fig. 6. The linear plot for 1/μ versus $(X/S)_{ave}$ to fitting the experimental data on substrate utilization and cell growth to Contois kinetic model for *A. niger* PTCC 5010 in a submerged batch culture medium**

Based on the curve-fitting results, Gompertz kinetic model was the best and suitable model to present *A. niger* PTCC 5010 growth and substrate utilization behavior at the applied conditions (Fig. 7). This model with R-square 0.949 showed an excellent consistency with experimental cell growth data. As presented in Table. 2, α (equivalent with $\mu_{max}$) and K for

Gompertz kinetic model were obtained as 0.003 $h^{-1}$ and 12.5g. $L^{-1}$, respectively. This means that the ultimate cell dry weight will be 12.5g. $L^{-1}$.

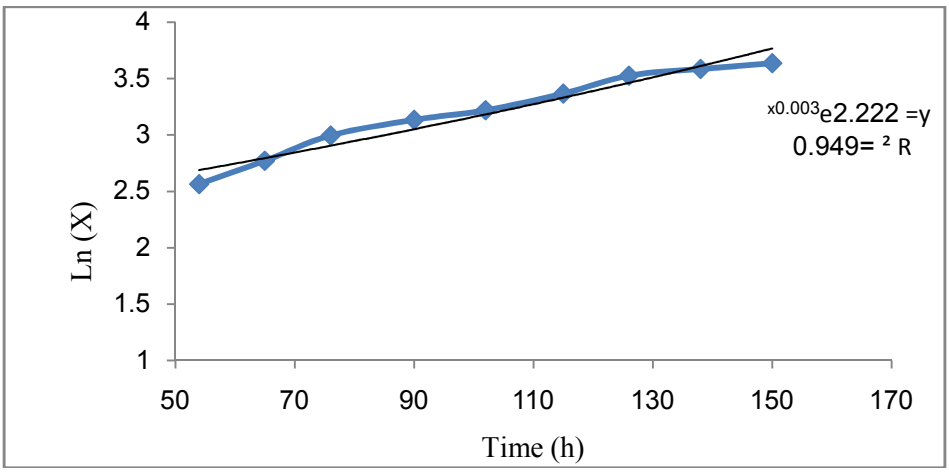


**Fig. 7. The exponential plot for Ln (X) versus t to fitting the experimental data on substrate utilization and cell growth to Gompertz kinetic model for *A. niger* PTCC 5010 in a submerged batch culture medium**

**Table 2. A comparison of kinetic parameters of *A. niger* PTCC 5010 growth and substrate utilization with five different kinetic models**

| Kinetic model | $R^2$ | $\mu_{max}$ ($h^{-1}$) | $K_s$ (g. $L^{-1}$) | $X_m$ (g. $L^{-1}$) | n | K (g. $L^{-1}$) |
|---|---|---|---|---|---|---|
| Monod | 0.777 | 0.019 | 0.291 | - | - | - |
| Moser | 0.913 | 0.024 | 1.747 | - | 0.18 | - |
| Verhulst | 0.677 | 0.018 | - | 6 | - | - |
| Contois | 0.751 | 0.018 | 0.007 | - | - | - |
| Gompertz | 0.949 | 0.003 | - | - | - | 12.5 |

## 4. CONCLUSION

This is the first report on the cell growth and substrate utilization kinetic of *A. niger* PTCC 5010 with respect to Monod, Moser, Verhulst, Contois and Gompertz kinetic models. The experimental data on cell growth and nutrient utilization in submerged batch fermentation process were interpreted using Monod, Moser, Verhulst, Contois and Gompertz kinetic models as unstructured models based on substrate concentration (Monod and Moser models), biomass concentration (Verhulst and Gompertz models) and both substrate and biomass concentrations (Contois model). Based on the results, Gompertz and Moser kinetic models were the most appropriate to describe the biomass growth rate of *A. niger* PTCC 5010. Maximum specific cell growth rate by Moser and Gompertz models were 0.024 and 0.003 $h^{-1}$, respectively.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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