Iranica Journal of Energy & Environment 2 (2): 117-121, 2011
ISSN 2079-2115
IJEE an Official Peer Reviewed Journal of Babol Noshirvani University of Technology

BUT

Investigation of the Nutrient Uptake and Cell Growth Kinetics with Monod and Moser Models for *Penicillium brevicompactum* ATCC 16024 in Batch Bioreactor

Fatemeh Ardestani

Department of Chemical Engineering, Islamic Azad University, Qaemshahr Branch, Qaemshahr, Iran

(Received: May 11, 2011; June 13, 2011)

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 easily possible. Use of a mathematical model is a suitable way to describe the substrate uptake and cell growth behavior of these microorganisms. *Penicillium brevicompactum* is one of the morphologically complex filamentous fungi with different structural forms. In this work, nutrient uptake and cell growth kinetics were investigated using Monod and Moser models through batch submerged fermentation in a bench-scale stirred tank bioreactor. The experimental data were fitted with both the Monod and the Moser kinetic with a regression value of 0.87. The maximum specific growth rate and Monad's half-saturation coefficient were determined as 0.04 h^{-1} and 9 g. L^{-1}, respectively. In Moser case, the maximum specific growth rate and K_s were obtained as 0.06 h^{-1} and 6.8 g. L^{-1}, respectively. Thus, both Monod and Moser kinetic are considered to be partially applicable models to explain kinetics behavior of *Penicillium brevicompactum* in submerged batch bioreactor culture.

Key words: *Penicillium brevicompactum* · Monod kinetic · Monod saturation constant · Moser kinetic · 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, hypocholesterolemic 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 depend 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 the main group of filamentous fungi belonging to the most important known mycotoxin producers [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* belonges 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

Corresponding Author: Fatemeh Ardestani, Department of Chemical Engineering, Islamic Azad University, Qaemshahr Branch, Qaemshahr, Iran. Tel: +98-1232253216-19, E-mail: f.ardestani@qaemshahriau.ac.ir.

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, Monod kinetic has been used for the demonstration of growth characteristics of filamentous fungi [12]. This model which contains two parameters that define the relationship between cell growth and substrate utilization, proposed by Monod more than 60 years ago to describe some of the important microbial growth's characteristics [13, 14]. Original form of Monod model is still used very often in environmental and industrial microbiology especially in biodegradation processes [15-19]. In our knowledge, Moser kinetic has been not studied until now in the case of filamentous fungi.

In the present article, the experimental data on nutrient utilization and cell growth of *P. brevicompactum* ATCC 16024 were fitted with Monod and Moser models in batch submerged fermentation using a bench-scale stirred tank bioreactor. Also, maximum growth rate and Monad's saturation constant were determined in each case.

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. Stock cultures were stored in the refrigerator at 4 °C. The spore suspension was prepared by extracting the 3 days aged spores in sterile distilled water as mentioned by Ardeatani *et al.* [20]. The spore suspension was used as the inoculum for fermentation study carried out in the bioreactor.

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 stilled by autoclaving separately at 121 °C for 15 min. Other components were sterilized using a 0.2 µm filter (Millipore, USA).

Experiments and Analytical Procedures: In the present work, 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.

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

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 including 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 at 1 vvm and 20% of saturation point, respectively [20].

During the fermentation process, 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. A colorimetric method 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 and glucose measurements were repeated three times for each sample.

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

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

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

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

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}) and K_s is half-saturation coefficient (g. L^{-1}) [21].

RESULTS AND DISCUSSION

Evaluation of Substrate Utilization and Cell Growth: In the batch fermentation, germination of spores and mycelial 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, forming of mycelial clumps could cause a limited mass and oxygen transfer inside the bioreactor and decreases cell growth (data not shown) [22].

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).

Kinetics Studies: Experimental data for glucose and biomass concentrations during the growth phase of *P. brevicompactum* in batch bioreactor were used for the determination of kinetic parameters. These parameters (μ_{max} and K_s) were determined, based on the curve-fitting procedure. Monod equation was lineared using Lineweaver-Burk plot method as:

$$\frac{1}{\mu} = \frac{K_s}{\mu_{max}}\frac{1}{S} + \frac{1}{\mu_{max}} \quad (3)$$

Moser Equation Was Lineated Similarly:

$$\frac{1}{\mu} = \left(\frac{K_s}{\mu_{max}}\right)\left(\frac{1}{S^n}\right) + \frac{1}{\mu_{max}} \quad (4)$$

Considering the cell dry weight as microorganism 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. (5). 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. The Lineweaver-Burk linear plot for $\frac{1}{\mu}$ versus $\frac{1}{S_{avg}}$ as well as power plot for $\frac{1}{\mu}$ versus $\frac{1}{S_{avg}}$ were fitted to the experimental data with the aid of Excel software as shown in Fig. 2 and Fig.3 for Monod and Moser kinetic, respectively.

$$\mu = \frac{Ln\left(X/X_0\right)}{t - t_0} \quad (5)$$

The results obtained from the model fitting with the experimental data showed a relatively acceptable compatibility between them with a regression of 0.87 in both Monod and Moser kinetic. This observed consistency could demonstrates that glucose as limiting substrate in applied concentrations has not any inhibitory effect on the cell growth. For Monod kinetic, the maximum specific growth rate and Monad's half-saturation coefficient were determined as 0.041 h^{-1} and 9 g. L^{-1}, respectively. As well as, for Moser kinetic, the maximum specific growth rate and Moser's half-saturation coefficient were determined as 0.06 h^{-1} and 6.8 g. L^{-1}, respectively. Thus, both Monod and Moser models were able to describe cell growth and nutrient uptake kinetic of *P. brevicompactum* ATCC 16024 in submerged batch stirred tank bioreactor as mentioned in previous research

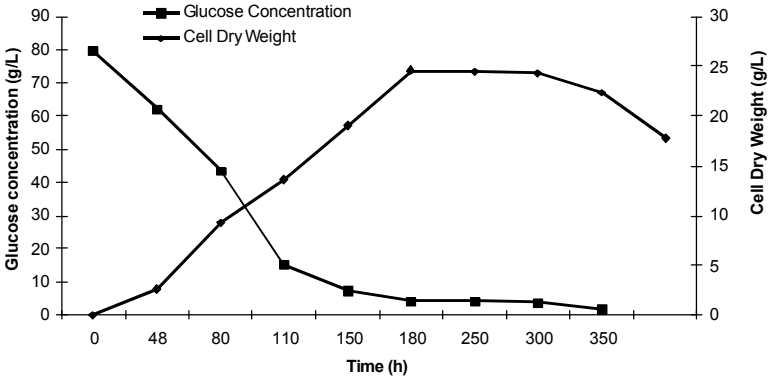

Fig. 1: Glucose and cell dry weight concentration profiles in submerged batch stirred tank bioreactor. Temperature, agitation speed, pH, aeration rate and dissolved oxygen were adjusted to 27 °C, 700 rpm, 6.0±0.1, 1 vvm and 20% of saturation point, respectively.

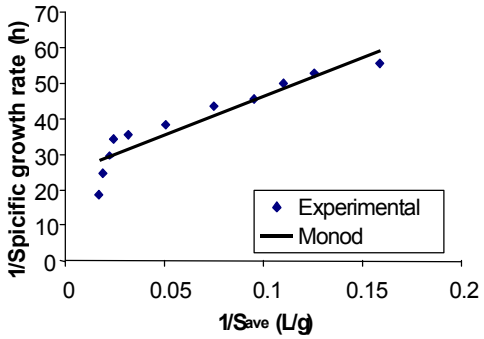

Fig. 2: The Lineweaver-Burk linear plot for $\frac{1}{\mu}$ versus $\frac{1}{S_{ave}}$ to fitting the experimental data on substrate utilization and cell growth to Monod kinetic model.

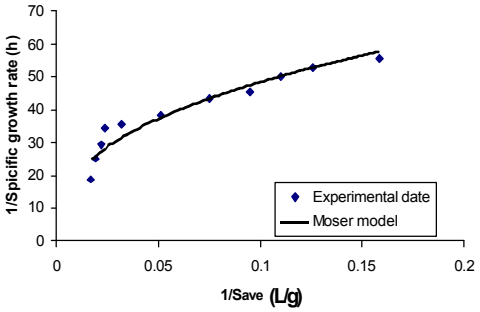

Fig. 3: The Lineweaver-Burk power plot for $\frac{1}{\mu}$ versus $\frac{1}{S_{ave}}$ to fitting the experimental data on substrate utilization and cell growth to Moser kinetic model.

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	2.6	-
48	58.05	9.3	0.053
60	51.26	11.2	0.040
70	46.16	12.45	0.034
80	40.97	13.6	0.029
90	31.02	16.6	0.028
100	19.45	18.24	0.026
110	13.27	19	0.023
120	10.52	22.1	0.022
130	9.07	22.78	0.020
140	7.92	24.13	0.019
150	6.28	24.6	0.018
160	-	24.6	0.016

on growth kinetic of filamentous fungi [12]. The half-saturation coefficients, the substrate concentration at which $\mu = \frac{1}{2}\mu_{max}$, were obtained very lower compared to previous study [12], indicating the ability of *P. brevicompactum* ATCC 16024 to grow on lower substrate concentrations.

CONCLUSION

The present study is the first report on the cell growth and nutrient uptake kinetic of *P. brevicompactum* with respect to Monod and Moser kinetic models. The experimental data on cell growth and substrate utilization in submerged batch fermentation process were interpreted using Monod and Moser kinetic models as two unstructured models based on substrate concentration. The obtained results showed a relatively acceptable fitting of the experimental data to both kinetic models with the regression values of 0.87. The maximum specific growth rate and the half-saturation coefficient were determined as 0.04 h^{-1} and 9 g. L^{-1}, respectively for Monod and 0.06 h^{-1} and 6.8 g. L^{-1}, respectively for Moser kinetic. Therefore, Monod and Moser models could be applied as suitable kinetic models to describe cell growth and substrate utilization behavior of *P. brevicompactum* ATCC 16024 in submerged batch bioreactor culture.

ACKNOWLEDGEMENTS

The present work was accomplished and supported by Isalmic Azad University, Qaemshahr Branch.

REFERENCES

1. Adrio, J.L. and A.L. Demain, 2003. Fungal biotechnology. Int. J. Microbiol., 6: 191-199.
2. Papagianni, M., 2004. Fungal morphology and metabolite production in submerged mycelial processes. Biotechnol. Adv., 22: 189-259.
3. Znidarsic, P. And A. Pavko, 2001. The morphology of filamentous fungi in submerged cultivations as a bioprocess parameter. Food. Technol. Biotechnol., 39(3): 237-252.
4. Frisvad, J.C. and U. Thrane, 1995. Mycotoxin production by foodborne fungi. Introduction to Food and Airborne Fungi, 4th ed., pp: 260-351.
5. Rundberget, T., I. Skaar and A. Flayen, 2004. The presence of *Penicillium* and *Penicillium* mycotoxins in food wastes. Int. J. Food. Microbial., 90: 181-188.
6. www.biocyc.org
7. Bird, B.A. and I.M. Campbell, 1982. Disposition of mycophenolic acid, brevianamide A, asperphenamate and ergosterol in solid cultures of *Penicillium brevicompactum*. Appl. Environ. Microbiol., 43(2): 345-348.

8. Koutinas, A.A., R. Wang, I.K. Kookos and C. Webb, 2003. Kinetic parameters of *Aspergillus awamori* in submerged cultivations on whole wheat flour under oxygen limiting conditions. Biochem. Eng. J., 16: 23-34.
9. Barclay, C.D., R.L. Legge and G.F. Farquhar, 1993. Modeling the growth kinetics of *Phanerochaete chrysosporium* in submerged static culture. Appl. Environ. Microbiol., 59(6): 1887-1892.
10. Garcia, I.G., J.L.B. Venceslada, P.R.J. Pena and E.R. Gomez, 1997. Biodegradation of phenol compounds in vinasse using *Aspergillus terreus* and *Geotrichum candidum*. Water Res., 31(8): 2005-2011.
11. Prosser, J.I. and A.J. Touch, 1991. Growth mechanisms and growth kinetics of filamentous microorganisms. Crit. Rev. Biotech., 10: 253-274.
12. Seki, K., M. Thullner and P. Baveye, 2004. Nutrient uptake kinetics of filamentous microorganisms: Comparison of Cubic, Exponential and Monod models. Ann. Microbiol., 54: 181-188.
13. Monod, J., 1949. The growth of bacterial cultures. Annu. Rev. Microbiol., 3: 371-393.
14. Dette, H.B., V. Melas, A. Pepelyshev and N. Strigul, 2005. Robust and efficient design of experiments for the Monod model. J. Theoretical Biol., 234: 537-550.
15. Taghizade Firozjaee, T., G. Nagafpour, M. Khavarpour, Z. Bakhshi, R. Pishgar and N. Mousavi, 2011. Phenol biodegradation kinetics in an anaerobic batch reactor. Iranica J. Energy and Environ., 2(1): 68-73.
16. Knightes, C.D. and C.A. Peters, 2000. Statistical analysis of nonlinear parameter estimation for Monod biodegradation kinetics using bivariate data. Biotech. Bioeng., 69: 160-170.
17. Goudar, C.T. and T.G. Ellis, 2001. Explicit oxygen concentration expression for estimating extant biodegradation kinetics from respirometric experiments. Biotech. Bioeng., 75: 74-81.
18. Kiviharju, K., K. Salonen, M. Leisola and T. Eerikainen, 2007. Kinetics of *Bifidobacterium longum* ATCC 15707 growth. Process Biochem., 42: 1140-1145.
19. Strigul, N., H. Dette and V.B. Melas, 2009. A practical guide for optimal designs of experiments in the Monod model. Environ. Modelling and Software, 24: 1019-1026.
20. Ardestani, F., S.S.A. Fatemi, B. Yakhchali, S.M. Hosseyni and G. Najafpour, 2010. Evaluation of mycophenolic acid production by *Penicillium brevicompactum* MUCL 19011 in batch and continuous submerged cultures. Biochem. Eng., 50: 99-103.
21. Najafpour, G., 2007. Biochemical engineering and biotechnology, Elsevier.
22. Xu, Z.N. and S.T. Yang, 2007. Production of mycophenolic acid by *Penicillium brevicompactum* immobilized in a rotating fibrous-bed bioreactor, Enzyme. Microb. Technol., 40: 623-628.