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Mathematical Modelling of an Endophytic Fungus *Fusarium Oxysporum* NFX06 Isolated from *Nothapodytes Foetida*

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Abstract

Industrial bioprocesses with the use of filamentous fungi embrace the production of a majority of commercially important metabolites such as antibiotics, pigments and toxins under submerged fermentation. A dynamic relationship exists between environmental conditions and the growth pattern of these modular microorganisms. In such cases a direct monitoring of the cell morphology and biomass distribution in the culture medium is potential. Hence in this research nutrient uptake and cell growth kinetics of an endophytic fungus Fusarium oxysporum NFX 06 isolated from Nothapodytes foetida possessing the ability of producing metabolites with antimicrobial and anticancerous property was studied. The three different models Contois, Verhulst and Tessier models were used to investigate the nutrient uptake and cell growth kinetics in a batch submerged fermentation process carried out in a bench-scale stirred tank bioreactor. The compatibility of the experimental data fitted with Contois, Verhulst and Tessier models with the regression (R^2) values are 0.73, 0.88 and 0.98, respectively. In the case of Verhulst, the maximum specific growth rate (μ_{max}) and maximum biomass (X_{max}) in terms of cell dry weight were determined as 1.889 day^{-1} and 26.6 g/L respectively. For Contois, μ_{max} was 1.449 $day^{-1}$and the half-saturation coefficient (K_s) was obtained as 0.105 g/L. However, in Tessier model, μ_{max} was determined to be 0.732 $day^{-1}$and K_s of 0.032 g/L. Although Verhulst and Contois are the most suitable kinetic models to describe substrate utilization and cell growth behaviour of filamentous fungi in submerged culture, the Tessier model was found best fitted with the experimental data.

1. Introduction

Filamentous fungal fermentation is widely used to commercially produce useful products such as organic acids, enzymes, antibiotics, and cholesterol lowering drugs (Papagianni, 2004). The study on endophytic fungi from medicinal plants has received much attention in recent years as they are believed to be an excellent source of biologically active compounds. Endophytes are "microbes that colonize living, internal tissues of plants without causing any immediate, overt negative effects" (Bacon and White 2000).

Growth kinetics of filamentous microorganisms has been studied by various researches (Bell, 1968; Koutinas et al, 2003; Fujikawa 2004). A classic kinetics for filamentous fungi including a lag and then, an exponential growth phase (Barclay, 1993; Garcia, 1997; Wang et al, 2011) and cubic model (Prosser, 1991) is best fitted for the growth kinetics of filamentous fungi. Contois model is one of the common models used to describe microbial cell growth and substrate uptake kinetics (Nelson et al, 2009; Juska, 2011). Verhulst kinetic model has been used for the demonstration of growth characteristics of the cell population (Wang et al, 2009) and this model has been applied mainly for environmental and industrial microbiology studies (Troncosa et al, 2007; Swart and Murrrel, 2008; Vadasz and Vadasz,2008).

This study demonstrates the comparison of Experimental data on substrate utilization and cell growth kinetics of *F. oxysporum* with Contois, Verhulst and Tesseir models in batch submerged fermentation

2. Materials and Methods

2.1 Microorganism

In this study *Fusarium oxysporum* NFX06 an endophytic fungus producing cell associated Camptothecin was isolated from the leaf of *Nothapodytes foetida* (Musavi et al, 2013). The endophytic fungus was identified based on internal transcribed spacer (ITS) Sequence analysis and the nucleotide sequence was submitted to the Genbank with an accession number KC914432.

2.2 Culture Maintenance

The culture was maintained on Potato Dextrose Agar (PDA) slants and stored at 4°C. The spore suspension was prepared by dispersing (1cm x 1cm) mycelial agar plug taken from an actively growing colony in a solution containing Tween 80 (0.1% v/v), filtered using Whatmann No.1 filter paper and counted for number of spores in colony forming unit using Haemocytometer.

2.3 Batch Fermentation

The growth kinetic studies were conducted in 3L fermenter containing 1L of optimized fermentation medium: Dextrose, 42.64 (g/L), Peptone, 9.23 (g/L), KH_2PO4, 0.60(g/L), $MgSO_4.7H_2O$, 0.26 (g/L) and $ZnSO_4.7H_2O$, 0.88 (g/L). The initial pH of the medium was adjusted to pH 5.4. The media was autoclaved at 121°C for 20 min. Fermentation was carried out at 26±2ºC, aeration rate of 1lpm and at 150 rpm. 100 µL of spore

suspension was used as inoculum for seed culture containing 100 mL of PDB liquid medium in 250mL flask, incubated in an orbital shaker at 120 r/min and 28°C for 2 days.

2.4 Biomass and Dextrose Determination

The mycelial biomass was separated from fermentation broth by filtration using a sterilized pre-weighed filter cloth. The biomass was dried in a hot air Owen at 65^{o}C till it attains a constant weight. The biomass was weighed and expressed in g/L. The dextrose concentration in the fermentation broth was determined by dinitrosalicylic acid method (Miller, 1959)

2.5 Unstructured Models for Fermentation Kinetics

2.5.1 Cell growth kinetics

In general the microbial growth kinetics is expressed in terms of specific growth rate and saturation constant for substrate. There are several mathematical expressions that could be used to describe this sigmoidal relationship between μ and S. The unstructured kinetic model tested were Contois, Verhlust and Tessier models for cell growth as given in the following equations.

$$\text{Contois, } \mu = \frac{\mu_{\max} S}{K_X X + S} \tag{1}$$

$$\text{Verhlust, } \mu = \mu_{\max}(1 - \frac{X}{X_{\max}}) \tag{2}$$

$$\text{Tessier, } \mu = \mu_{\max}(1 - e^{-K_s S}) \tag{3}$$

Where μ is the specific growth rate (day^{-1}); $\mu_{\max}$ is the maximum specific growth rate (day^{-1}); S is the concentration of the limiting substrate (g/L); K_s is the saturation constant, equal to the substrate concentration at one-half the maximum specific growth rate (g/L).

2.5.2 Substrate Utilization Kinetics

The substrate utilization kinetics is given by the following equation, which considers substrate conversion to cell mass, to product and substrate consumption for maintenance (Weiss and Ollis, 1989). The expression for substrate utilization with respect to time can be correlated by rate of change of biomass and maintenance coefficient.

$$-\frac{dS}{dt} = \frac{1}{Y_{XS}} \frac{dX}{dt} + m_S X \tag{4}$$

Where $\gamma = \frac{1}{Y_{xs}}$ inverse of yields of biomass with respect to substrate and m_s is maintenance coefficient.

3. Results and Discussion

3.1 Evaluation of Substrate Utilization and Cell Growth Kinetics

Germination of spores and mycelial growth of *F.oxysporum* in the fermenter started after 24 hrs of inoculation under above mentioned culture conditions. Unlike several fermentation processes where at lower agitation pellet morphology was observed (Ardestani, 2001), which minimizes the mass and oxygen transfer inside the bioreactor and decrease cell growth (Xu and Yang, 2007), the growth of *F.oxysporum* was found to be filamentous. As shown in Fig.1, exponential phase was observed till 7th day of fermentation where majority of the substrate (Dextrose) was utilized after which it entered into stationary phase.

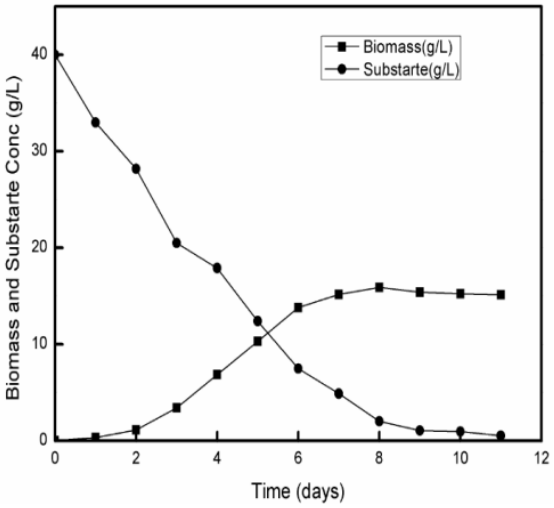

Figure 1: Substrate and Biomass concentration profiles in submerged batch stirred tank bioreactor.

3.1.1 Contois Kinetic Model

The compatibility of experimental data to contois model was determined by linearizing Eq (2) as given below in Eq (5).

$$\frac{1}{\mu} = \left[\frac{K_s}{\mu_{\max}}\right]\left[\frac{X}{S}\right] + \frac{1}{\mu_{\max}} \tag{5}$$

The lineweaver-Burk plot of $\frac{1}{\mu}$ versus $\left[\frac{X}{S}\right]_{avg} = \frac{X_1 + X_2}{S_1 + S_2}$ was fitted to the experimental data on substrate utilization and cell growth to Contois kinetic model using Origin 8.0 software as shown in Fig.2 . A relatively acceptable compatibility was observed with a regression coefficient R^2 of 0.73.

The observed consistency and low value of Ks demonstrates dextrose, as limiting substrate, in applied concentrations has not any inhibitory effect on the cell growth. In this study, the maximum specific growth rate μ_{max} = 1.449 day^{-1}and half-saturation coefficient K_s = 0.105 g/L was obtained.

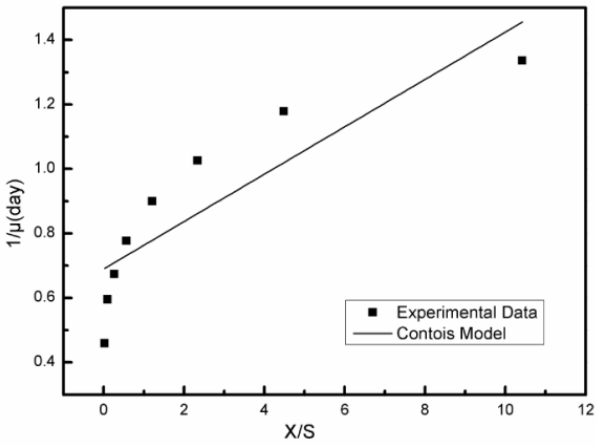

Figure 2: The Lineweaver-Burk linear plot for Cointois Kinetic Model.

3.1.2 Verhulst Kinetic Model

Linear curve fitting of the experimental data to the Verhulst kinetic model was plotted between μ and X as shown in Fig.3. It was observed that, the maximum specific growth rate (μ_{max}) and maximum biomass (X_{max}) in terms of cell dry weight were determined as 1.889 day^{-1} and 26.6 g/L respectively whereas the regression coefficient R^2 was found to be 0.88. The high value of μ_{max} found not to be a suitable model for filamentous microorganisms as reported by Fatemeh, (2010, 2012).

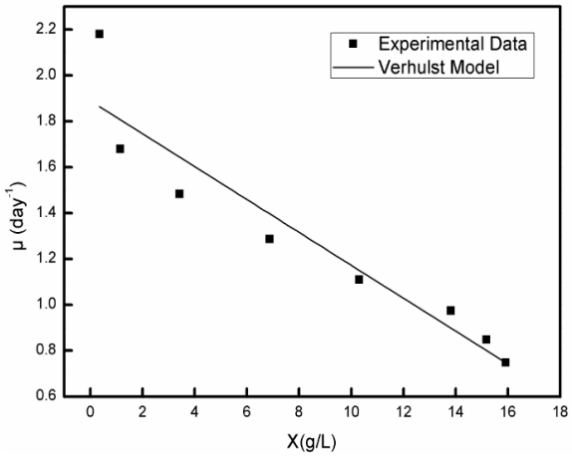

Figure 3: The linear plot for X versus μ to fitting the experimental data on substrate utilization and cell growth to Verhulst kinetic model.

3.1.3 Tessier Kinetic Model

Similarly in the case of Tessier kinetic model, a plot of S versus ln μ was fitted linearly with experimental data. In this case μ_{max} was determined to be 0.732 $day^{-1}$and K_s of 0.032 g/L with the regression coefficient R^2 of 0.9834. The lower value of μ_{max} and higher value of R^2 makes it suitable for filamentous fungal fermentation comparing to the other two models. Although Verhulst and Contois are the most suitable kinetic

models to describe substrate utilization and cell growth behavior of filamentous fungi in submerged culture, the Tessier model was found best fitted with the experimental data as shown in Fig. 4.

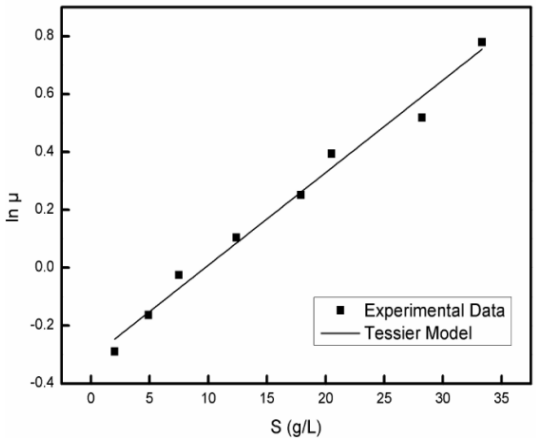

Figure 4: The linear curve fitting of S versus ln μ, fitting the experimental data on substrate utilization and cell growth to Tessier kinetic model.

4. Conclusion

The commonly used unstructured kinetic model for microbial growth is the Monod equation, which relates the specific growth rate to a single limiting substrate in a saturation form. Microbial processes may not always follow the classical kinetic model of substrate-limited growth and product formation as proposed by Monod (1949). This study is the first report on the substrate utilization and growth kinetics of an endophytic fungus *F.oxysporum* NFX 06. The experimental data on cell growth and substrate utilization in submerged batch fermentation was compared with Contois, Verhulst and Tessier models showed relatively acceptable fitting. The kinetic parameters and regression coefficient revealed that the Tessier model is the best suited model compared to Contois and Verhulst .

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