Indian J. Fish., 62(3) : 100-106, 2015

Growth kinetic profiles of *Aspergillus niger* $S_1$4 a mangrove isolate and *Aspergillus oryzae* NCIM 1212 in solid state fermentation

ASHA-AUGUSTINE, IMELDA-JOSEPH, PAUL RAJ, R. AND NEETHA S. DAVID
ICAR-Central Marine Fisheries Research Institute, Post Box 1603, Ernakulam North P. O., Kochi - 682 018 Kerala, INDIA
e-mail: dr.ashaaugustine@gmail.com

ABSTRACT

Aspergillus niger is one among the many species of fungi thriving in mangrove ecosystem with potential for biotechnological and industrial applications. Understanding the growth profile and kinetics is of use in studying dynamics of mangrove ecosystem. In the present investigations, empirical equations and growth models commonly used in elucidating the dynamic nature of growth were fitted to the data of the biomass, protein and glucosamine levels during solid state fermentation (SSF) with wheat bran as substrate using *Aspergillus niger* $S_1$4, a fungal isolate from mangrove and *Aspergillus oryzae* NCIM 1212, an industrial strain. Linear, exponential, two-phase models, logistic equations, Richards, Michaelis-Menten, Weibull, Modified Gompertz and Morgan-Mercer-Flodin models were used. Among the models, logistic equation and Michaelis-Menten model could provide an adequate fit to the biomass variation for 21 days. The protein and glucosamine contents followed a different trend compared to the direct biomass measurements which made them inept for growth kinetic studies of the two fungal strains in SSF.

Keywords: *Aspergillus*, Growth kinetic profiles, Growth models, Logistic equations, Mangrove fungi

Introduction

The primary productivity of mangroves is enormous. Bacteria and fungi are involved in recycling the detritus in this ecosystem. Recent investigations have increased our knowledge of the ecology of mangrove fungi. Marine mycology as a specialised branch of science has evolved recently. Fungus modifies the detritus by the process of fermentation. Solid state fermentation (SSF) involves the cultivation of microorganisms on moist solid substrates in the absence of free flowing water (Viccini *et al.,* 2003). SSF is used for the production of fermented food, enzymes and metabolites, vitamins, amino acids and pharmaceuticals (Pandey *et al.,* 1999). SSF has also the potential for upgrading the nutritional quality of agro industrial products and byproducts such as wheat straw, wheat bran, oil cakes and soybean meal that can be used in aqua feed and animal feed industries (Imelda-Joseph *et al.,* 2008).

Filamentous fungi are the source of about 40% of all available enzymes such as amylases, lactase, raffinase, dextranase, pectinase and cellulase and form the most prominent group of microorganisms used in SSF process owing to their physiological, enzymological and biochemical properties (Archer and Peberdy, 1997). *Asperglls oryzae* and *Aspergillus niger* are well characterised industrial microorganisms, which have obtained GRAS (Generally Regarded As Safe) status from the U.S. Food and Drug Administration (USFDA) and the World Food Organisation (WFO) of the United Nations (Oxenboll, 1994). *A. niger* has been extensively used in SSF for commercial production of many enzymes including amylase, pectinase, glucose oxidase, glucoamylase, hemicellulase, glucanases, acid proteinase, cellobiase and catalase (Bhatnagar *et al.,* 2010). Another major commodity chemically manufactured by *A. niger* fermentation is citric acid which is widely used in the food and pharmaceutical industries. *A. oryzae* has been an essential component in oriental food production for centuries and is used in the production of fermented food products like koji, soy-sauce, sake and miso. *A. oryzae* also produces many commercial enzymes, such as α- amylase, glucoamylase, lipase and protease (Oxenboll, 1994). It is also used to produce livestock probiotic feed supplements through fermentation.

Even though a variety of substrates are used for SSF, abundant agricultural residues such as wheat bran and rice bran have received a great deal of attention in SSF. Wheat bran, however, holds the key, and has been most commonly used for enzyme production (Pandey *et al.,* 1999). Measurement of microbial biomass is essential for kinetic studies on SSF. Direct determination of biomass in SSF is very difficult for processes involving fungi,

because the fungal hyphae penetrate into and bind tightly to the substrate. The most readily measured biomass component is protein as determined by the Lowry method (Raimbault and Alazard, 1980). Glucosamine is another useful compound for the estimation of fungal biomass, taking advantage of the presence of chitin, poly-N-acetyl glucosamine, in the cell walls of many fungi. The accuracy of the glucosamine method for determination of fungal biomass depends on establishing a reliable conversion factor relating glucosamine to mycelial dry weight (Muralikrishna *et al.,* 2003). Mathematical models have become essential tools in the development of rational strategies for the design and optimisation of operation of large scale bioreactors, as in traditional packed-bed bioreactors, rotating drum bioreactors, scraped drum bioreactors and stirred bioreactors (Nagel *et al.,* 2001). The objective of the present investigation was to study the growth kinetics of *Aspergillus niger* $S_1$4 isolated from mangrove swamp and *Aspergillus oryzae* NCIM 1212 by biomass measurement using wheat bran as the substrate and to fit in the growth equations and suitable mathematical models that have been applied to SSF systems to understand the growth pattern.

Materials and methods

Fungi

Aspergillus niger strain $S_1$4 isolated from the soil samples of Mangalavanam, a mangrove swamp, at Kochi (lat. 10° 03' N; long. 76° 14' E) and *Aspergillus oryzae* NCIM 1212 obtained from NCIM, Pune, India, were used for the present studies. Spore suspensions were obtained by growing the fungi in slants on potato dextrose agar (PDA) (Hi-media, Mumbai) for one week and harvesting the spores with 1 g l^{-1} Tween 80 in normal saline (Smith *et al*., 1996).

Solid- state fermentation (SSF)

SSF was carried out in 500 ml conical flasks containing 20 g wheat bran fortified with Czapek Dox medium ($NaNO_3$ - 2.5 g l^{-1}; K_2HPO_4 -1 g l^{-1}; $MgSO_4$, $7H_2O$ - 0.5 g l^{-1}; KCl - 0.5 g l^{-1}; pH - 5.0), with the moisture content adjusted to 60% and autoclaved at 121ºC at 1.034×10^5Pa for 20 min (Aikat and Bhattacharya, 2000). The substrate (20 g) was inoculated with 2×10^7 spores and incubated at 30±1ºC. Control was maintained without inoculation. All the flasks were kept stationary at an initial pH of 6.4 - 6.5 for 21 days, with sampling at every 24 h starting from day 0 to day 21.

Growth parameters

At each time interval, three flasks were removed from the incubator for biomass estimation. Direct measurement of biomass was done according to the method of Asha-Augustine *et al*. (2006) by sampling 1g fermented substrate in duplicate every 24 h and by centrifuging at 1.256×10^3 rad per sec for 15 min by addition of 5 ml sodium sulphate (150g l^{-1}). Centrifugation was repeated thrice under similar conditions to achieve complete separation of fungal mass from substrate. At the end of centrifugation, the biomass which floated was transferred to a pre weighed filter paper and dried in hot air oven for 72 h at 85±1ºC to obtain a constant weight. Glucosamine was measured in dry fermented wheat bran samples (Ramachandran *et al.,* 2005) and the results of glucosamine measurements were corrected for the amount of glucosamine already present in the wheat bran. Protein estimation was carried out according to Lowry *et al.* (1951).

Study of growth kinetics

Growth kinetics of *A. niger* was studied by applying the empirical equations to the kinetic profiles exhibited by the fungus during SSF. Growth profiles in SSF obtained by the direct and indirect methods were analysed by fitting the integrated forms of equations to the data by least square method in Microsoft Excel (Draper and Smith, 1981). Non-linear regression algorithm of SYSTAT 7.0 was used to estimate the parameters. After estimating the parameters, goodness of fit was determined to test the appropriateness of the overall model.

The different models fitted were:

Linear equation:

$$\frac{dX}{dt} = K \qquad \text{(Differentiated form)}$$

$$X = Kt + X_0 \qquad \text{(Integrated form)}$$

where, K = linear growth rate (mg dry biomass/mg initial dry substrate), t - time (h), X - biomass concentration (mg dry biomass/mg initial dry substrate) and X_o - initial biomass concentration (mg dry biomass/mg initial dry substrate).

Exponential equation:

$$\frac{dX}{dt} = \mu X \qquad \text{(Differentiated form)}$$

$$X = X_0 e^{\mu t} \qquad \text{(Integrated form)}$$

where, t - time (h), X - biomass concentration (mg dry biomass/mg initial dry substrate), X_o - initial biomass concentration (mg dry biomass/mg initial dry substrate) and μ - specific growth rate constant (h^{-1})

Logistic equation:

$$\frac{dX}{dt} = \mu X\left[1 - \frac{X}{X_m}\right] \qquad \text{(Differentiated form)}$$

$$X = \frac{X_m}{1 + \left[\frac{X_m}{X_0} - 1\right] e^{\mu t}} \qquad \text{(Integrated form)}$$

$\mu = - K X_m$

where, K - linear growth rate (mg dry biomass/mg initial dry substrate), t - time (h), X - biomass concentration (mg dry biomass mg initial dry substrate), X_{o-} initial biomass concentration (mg dry biomass/mg initial dry substrate), X_m - maximum possible biomass concentration (mg dry biomass/mg initial dry substrate), μ - specific growth rate constant (h^{-1})

Two phase model:

$$\frac{dX}{dt} = \mu X \qquad t < t_a$$

$$\frac{dX}{dt} = \mu L e^{k(t-ta)} X \qquad t > t_a$$

(Differentiated forms)

$$X = X_0 e^{\mu t} \qquad t < t_a$$

$$X = X_0 \exp \frac{\mu L}{k} (1 - e^{k(t-ta)}) \; t > t_a$$

(Integrated forms)

where, k - first order decay constant in the second phase of the two phase model (h^{-1}), L - the fraction of hyphal tips surviving the entry into the second phase in the two phase model (dimensionless), t - time (h), t_a - the time at which there is a switch from the first to the second phase in the two phase model (h), X - biomass concentration (mg dry biomass/mg initial dry substrate), X_o - initial biomass concentration (mg dry biomass/mg initial dry substrate) and μ - specific growth rate constant (h^{-1}).

Richards model:

$$X = \frac{X_\alpha}{\left[1 + \left(\frac{1}{b} e^{-ht}\right)^d\right]}$$

where, $X_{\alpha -}$ asymptote or the potential maximum of the response variable, b-constant of integration or biological constant, k - growth-rate constant and d - shape parameter or allometric constant.

Michaelis-Menten model:

$$Y = \frac{b_1 t}{t + b_2}$$

where, b_{1-} asymptote or the maximum growth rate attainable, t – time, b_{-} time at which growth occurs at half the maximum rate.

Weibull model:

$$Y = b_1 - b_2 e^{-b_3 X^{b4}}$$

where, b_1 - asymptote or the potential maximum of the response variable, b_2 - the biological constant, b_3 - parameter governing the rate at which the response variable approaches its potential maximum and b_4 - allometric constant

Modified Gompertz model **:**

$$Y = a\left(1 - e^{-be^{ct}}\right)$$

where, Y - weight at time t, a - asymptote or the potential maximum of the response variable; b - the biological constant, c - instantaneous rate of growth.

Morgan-Mercer-Flodin model:

$$Y = \frac{b_1 b_2 + b_3 X^{b_4}}{b_2 + X^{b_4}}$$

where, Y - observed weight gain of the organism, b_1 - ordinate intercept of the curve, b_2 - a constant, b_3 - asymptotic or maximum response of the organism and b_4 - apparent kinetic order of the response with respect to X as X approaches zero.

Index of fit (IF):

$$IF = 1 - \left[\frac{\sum_{i=1}^{n} (Y_i - \hat{y}_i)^2}{\sum_{i=1}^{n} (y_i - \bar{y}_i)^2}\right]$$

Models were compared using the mathematical parameter, index of fit (IF) (Rosa *et al.,* 1997). If is defined as: where, y_i - observed values, $\bar{y}_i$- mean of the observed values, $\hat{y}_i$ - estimated values of $\bar{y}i$.

Results and discussion

In the present investigation, different models were fitted and used to analyse the growth kinetic profile of *A. niger* (Fig. 1 and 2) and *A. oryzae* (Fig. 3 and 4).

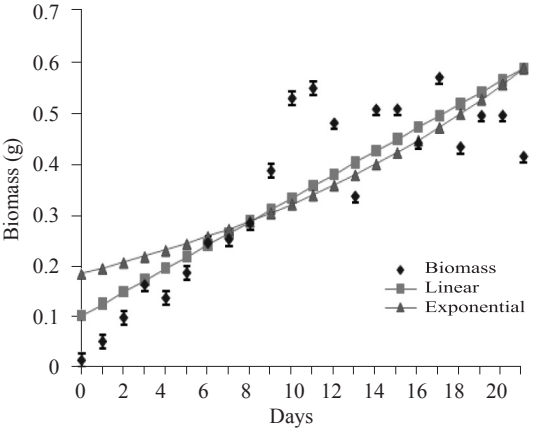

Fig. 1. Linear and exponential equations fitted to biomass of *A. niger*

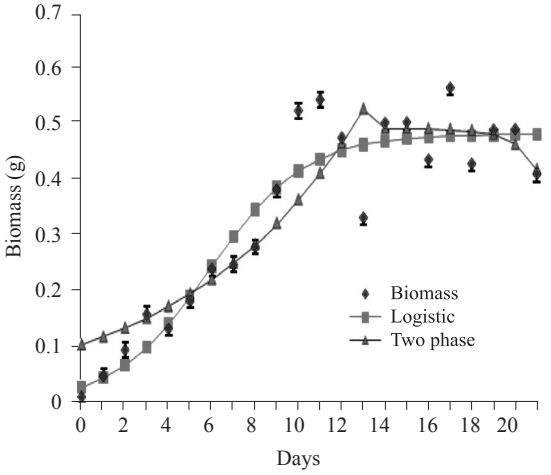

Fig. 2. Logistic equation and two phase model fitted to biomass of *A. Niger*

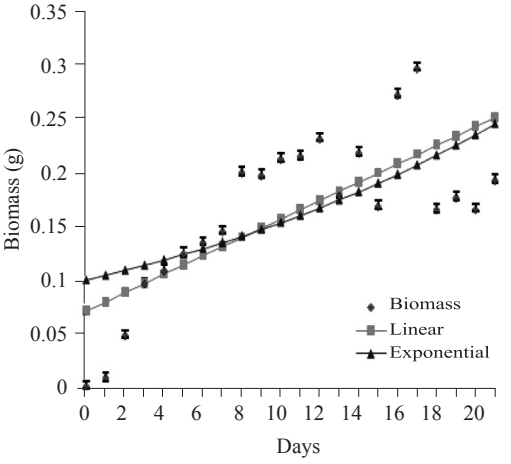

Fig. 3. Linear and exponential equations fitted to biomass of *A. oryzae.*

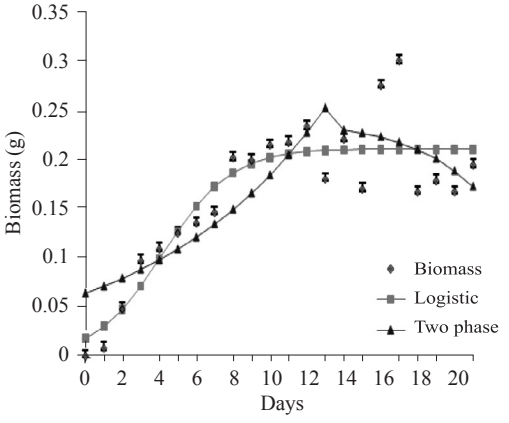

Fig. 4. Logistic equation and two phase model fitted to biomass of *A. oryzae*

Values of the parameters obtained by regression of the original experimental data, in order to obtain the growth kinetic profile of *A. niger* and *A. oryzae* are presented in Table 1. For biomass, logistic equation gave the best fit for both *A. niger* (IF=0.8895) and *A. oryzae* (IF=0.8079). It is evident in the present observation that, the two phase model gave a comparable fit (*A. niger* (IF=0.8206) and *A. oryzae* (IF=0.7053) whereas, the linear and exponential equations failed to provide an adequate fit for both the strains (Table 1). The logistic law of growth assumes that a system grows exponentially until an upper limit or carrying capacity inherent in the system is approached, where the growth rate reduces and eventually levels off, producing the characteristic sigmoid curve. The logistic equation fits well to the data collected in the first 10-45 h of fermentation, but clearly overestimates the initial biomass concentration. In the present observations, even though the logistic equation describes the data adequately, the direct biomass or measured components decreased steadily during the later stages of fermentation probably due either to death and autolysis of the biomass or endogenous maintenance metabolism (Viccini *et al.,* 2001). There is also a possibility that different cycles of growth were completed by the fungi during 21 days of SSF which could possibly result in a fluctuation in the growth curve at the later stage. It is presumed that the logistic equation did not fit well at the later stages due to the asymptotic nature of the growth curve. Two phase model had followed an exponential equation in the initial phase, but in actual case the growth itself followed a logistic equation at this stage. Therefore, a modification of logistic equation which includes the deceleration of growth at the later stages is necessary to give a perfect description of the growth kinetics of the fungi in SSF. In the logistic model, the growth curve is symmetric around the point of the maximum growth rate and has equal periods of slow and fast growth. Based on logistic equation, the various growth models *viz.,* Richards, Michaelis-Menten, Weibull, Modified Gompertz and Morgan-Mercer-Flodin models were fitted to biomass of *A. niger* (Fig. 5, 6 and 7) and *A. oryzae* (Fig. 8, 9 and10). The parameter values for the models are given in Table 1.

Michaelis-Menten model gave the maximum goodness of fit (IF = 0.9672) followed by Morgan-Mercer-Flodin (IF = 0.8812),Weibull (IF = 0.8793), Richards (IF = 0.8778) and Modified Gompertz (IF = 0.8777) models. As in the case of *A. niger,* Michaelis-Menten model gave the maximum goodness of fit (IF = 0.9596) for *A. oryzae*. Modified Gompertz model gave the next best fit (IF = 0.9036) followed by Morgan-Mercer-Flodin (IF = 0.8935), Richards (IF = 0.8109) and Weibull (IF = 0.7750) models.

The results indicated that biomass estimation by direct and indirect methods can be made amenable for growth

Table 1. Equation parameters for growth kinetics of *A. niger* and *A. oryzae*

Empirical equations	Parameters	*A. niger*		*A. oryzae*	
		Values	Index of fit (IF)	Values	Index of fit (IF)
Linear	K	0.0232	0.7271	0.0087	0.5387
	X_o	0.1036		0.0739	
Exponential	X_o	0.1860	0.6050	0.1032	0.4354
	μ	0.0549		0.042218	
Logistic	K	0.9209	0.8895	2.5554	0.8079
	X_m	0.4908		0.2129	
	μ	-0.4520		-0.5440	
Two phase	k	1	0.8206	0.3172	0.7053
	L	0.0004		0.076957	
	μ	0.1219		0.104101	
Richards	x_α	0.5015	0.8778	0.213949	0.8109
	b	48.314		50.6672	
	k	0.2923		0.4042	
	d	175.348		174.672	
Michaelis- Menten	b_1	0.8208	0.9672	0.2860	0.9596
	b_2	11.5301		5.3780	
Weibull	b_1	0.4826	0.8793	0.2137	0.7750
	b_2	0.4095		0.2105	
	b_3	0.0023		0.0723	
	b_4	3		1.609	
Modified Gompertz	a	0.013932	0.8777	0.004577	0.9035
	b	-3.58367		-3.77012	
	c	0.29079		0.472379	
Morgan-Mercer-Flodin	b_1	0.083302	0.8812	0.004755	0.8935
	b_2	4492.196		24.4837	
	b_3	0.494601		0.202428	
	b_4	4.381786		2.527386	

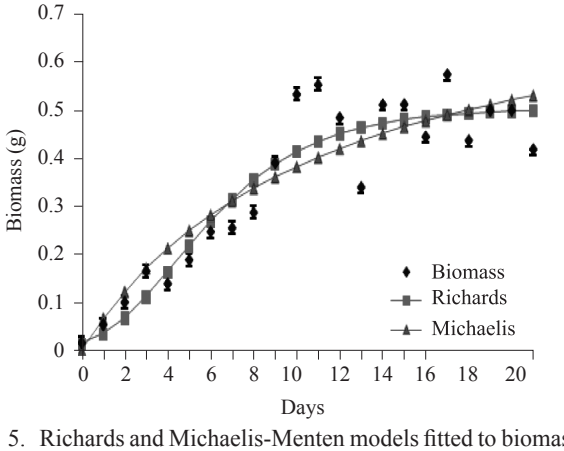

Fig. 5. Richards and Michaelis-Menten models fitted to biomass of *A. niger*

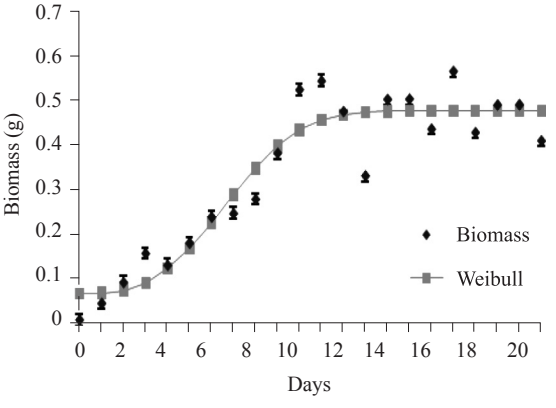

Fig. 6. Weibull model fitted to biomass of *A. niger*

kinetic study of filamentous fungi in SSF. The analysis of growth is an important component of many biological studies and the evolution of mathematical functions such as logistic, Gompertz, Richards and Weibull used to describe population growth have proved useful for a wide range of growth curves (Kingland, 1982). It has been reported that for adequate kinetic analyses, the growth profile should have around 10 data points, with the majority of these located in the regions where the most rapid growth and the acceleration and deceleration phases occur (Viccini *et al.,* 2001). From the results of the present analysis with 21 data points, it is evident that the linear and exponential equations are not appropriate for explaining the growth of *A. niger* and *A. oryzae* strains in SSF. The results

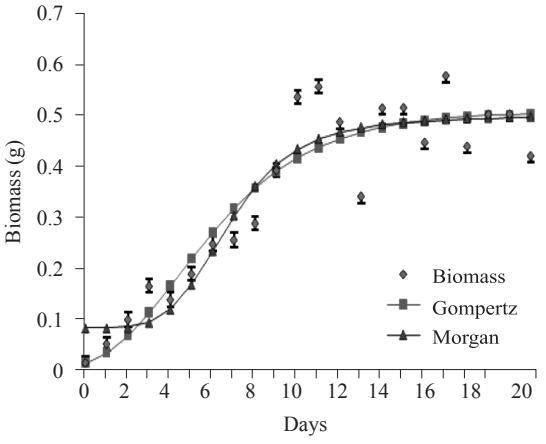

Fig. 7. Modified Gompertz and Morgan-Mercer-Flodin models fitted to biomass of *A. niger*

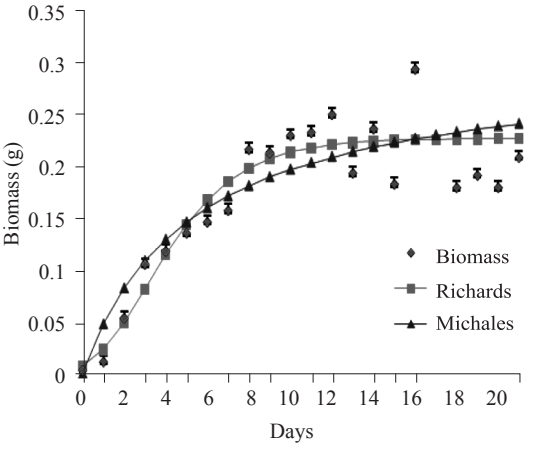

Fig. 8. Richards and Michaelis-Menten models fitted to biomass of *A. oryzae*

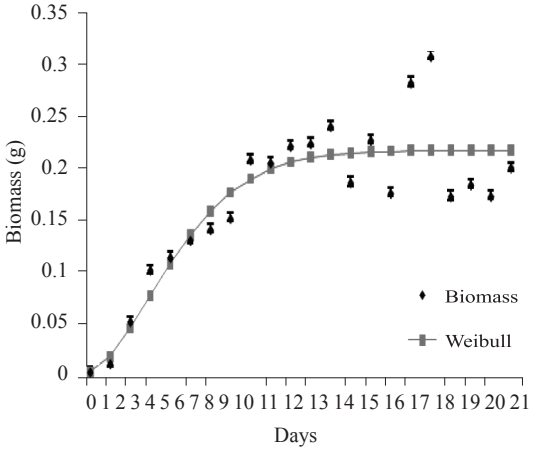

Fig. 9. Weibull model fitted to biomass of *A. oryzae*

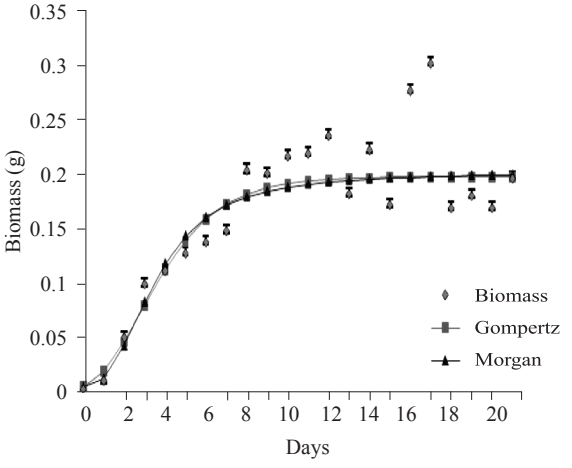

Fig. 10. Modified Gompertz and Morgan-Mercer-Flodin models fitted to biomass of *A. oryzae*

also suggest that for analysis of the growth of fungi, the linear equation for substrate utilisation is not suitable, since it contains no term for the process of differentiation (Metwally *et al*., 1991). In contrast, the Gompertz model does not incorporate the symmetry restriction and has a shorter period of fast growth. Both the Logistic and Gompertz have points of inflection that are always at a fixed proportion of their asymptotic population values. The Richards function is more flexible and can fit asymmetric growth patterns. However; it has more parameters than the Logistic function. The Gompertz function has the same number of parameters as the Logistic function and the Weibull function has the same number of parameters as the Richards function and both can fit asymmetric growth, but they are not very flexible (Yin *et al.*, 2003). In the present analysis, *A. niger* gave similar index of fit for all these models (>0.88) and the most fitting model was that of Michaelis- Menten (IF=0.9672). Similarly, Michaelis-Menten model (IF=0.9596) was the best fitting one for *A. oryzae* followed by modified Gompertz (IF=0.90). The modified Gompertz model had been used mainly to describe growth of bacteria and yeasts as a function of time in liquid media.

The reason for the poor characterisation of growth profiles in SSF is due to the fact that in majority of such works the growth profile is presented without any kinetic analyses, and therefore it is obvious that a good kinetic analysis was not part of the initial motivation of those works. In the present investigation protein and glucosamine levels did not follow the predicted growth models whereas direct biomass value followed it. This shows that protein and glucosamine production follows different kinetics than total biomass possibly due to differences in rate of production of these components at different stages of growth.

In conclusion, the results of the present work show that the growth of *A. niger* and *A. oryzae* can be expressed using logistic and Michelis-Menten growth models. At the initial phase, growth follows logistic equation, whereas, at

later stage it follows the modified logistic model when the nutrient resources become depleted and the fungal growth becomes limited by the availability of the substrate. In order to make growth models particularly beneficial in SSF processes, further research is needed in various domains like understanding of the interaction of varying environmental conditions during SSF on the growth kinetics of the fungi. Studies on fungal biodiversity, ecology and growth kinetics are important in understanding the mangrove ecosystem for its preservation and judicial industrial utilisation. In future, developing structured growth models that explicitly describe SSF might be possible, although this necessitates extensive knowledge about the various factors that control the process.

Acknowledgements

The authors are grateful to the Director, ICAR-CMFRI, Kochi, India for the facilities provided to carry out this research. The first author acknowledges ICAR-Central Institute of fisheries Education (ICAR-CIFE), Mumbai for the financial assistance.

References

Aikat, K. and Bhattacharya, B. C. 2000. Protease extraction in solid state fermentation of wheat bran by a local strain of *Rhizopus oryzae* and growth studies by the soft gel technique. *Proc. Biochem.,* 35: 907-914.

Archer, D. B. and Peberdy, J. F. 1997. The molecular biology of secreted enzyme production by fungi. *Crit. Rev. Biotechnol.,* 17: 273-306.

Asha-Augustine, Imelda-Joseph, and Paulraj, R. 2006. Biomass estimation of *Aspergillus niger* S14 a mangrove fungal isolate and *A. orzyae* NCIM 1212 in solid state fermentation. *J. Mar. Biol. Assoc. India*, 48:139-146.

Bhatnagar, D., Imelda-Joseph and Paulraj, R. 2010. Amylase and acid protease production by solid state fermentation using *Aspergillus niger* from mangrove swamp. *Indian J. Fish.*, 57(1): 45-51.

Draper, N. R. and Smith, H. 1981. *Applied regression analysis, 2nd edn.* John Wiley and Sons, New York, 709 pp.

Imelda-Joseph, Paulraj, R.and Bhatnagar, D. 2008. Effect of solid state fermentation on nutrient composition of selected feed ingredients. *Indian J. Fish.*, 55(4): 327-332.

Kingland, S. 1982. The refractory model: The logistic curve and history of population ecology. *Quart. Rev. Biol.,* 57: 29-51.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurement with the Folin-phenol reagent. *J. Biol. Chem.,* 193:265-271.

Metwally, M., El-Sayed, M., Osman, M., Hanegraaf, P. P. F., Stouthamer, A. H. and Van-Verseveld, H. W. 1991. Bioenergetic consequences of glucoamylase production in carbon-limited chemostat cultures of *Aspergillus niger, Antonie Van Leeuwenhoek*, 59(1): 35-43.

Muralikrishna, P. S., Venkateswarlu, G., Pandey, A. and Rao, L.V. 2003. Biosynthesis of rifamycin SV by *Amycolatopsis mediterranei* MTCC17 in solid cultures. *Biotechnol. Appl. Biochem.*, 37: 311-315.

Nagel, F. J., Tramper, J., Bakker, M. S. and Rinzema, A. 2001. Model for on-line moisture-content control during solid state fermentation. *Biotechnol. Bioeng.,* 72(2): 231-243.

Oxenboll, K., 1994. *Aspergillus* enzymes and industrial uses. In: Powell, K. A., Renwick, A. and Peberdy, J. F. (Eds.), *The genus Aspergillus, from taxonomy and genetics to industrial application*, FEMS Symposium 69, Plenum Press, New York, p147-154.

Pandey, A., Selvakumar, P., Soccol, C. R. and Nigam, P. 1999. Solid state fermentation for the production of industrial enzymes. *Curr. Sci.,* 77(1): 149-162.

Raimbault, M. and Alazard, D. 1980. Culture method to study fungal growth in solid fermentation. *Eur. J. Appl. Microbiol. Biotechnol.,* 9: 199-209.

Ramachandran, S., Roopesh, K., Nampoothiri, K. M., Szakacs, G. and Pandey, A. 2005. Mixed substrate fermentation for the production of phytase by *Rhizopus* spp. using oilcakes as substrates. *Process Biochem.,* 40(5): 1749-1754.

Rosa, M. C. G., De-Silva, J. A. A. and Silva, A. L. N. 1997. Modelling growth in cultures of *Oreochromis niloticus* (L.) and *Cyprinus carpio* L. in Pernambuco, Brazil. *Aquacult. Res.,* 28: 199-204.

Smits, J. P., Rinzema, A., Tramper, J., Schlosser, E. E.and Knol, W. 1996. Accurate determination of process variables in a solid state fermentation system. *Process Biochem.,* 31(7): 669-678.

Viccini, G., Mitchell, D. A., Boit, S. D., Gern, J. C., Rosa, A. S. and Costa, R. M., Dalsenter, F. D. H., Meien, O. F. and Kreiger, N. 2001. Analysis of growth kinetic profiles in solidstate fermentation. *Food Technol. Biotechnol.,* 39(40): 1-23.

Viccini, G., Mitchell, D. A. and Krieger, N. 2003. A model for converting solid state fermentation growth profiles between absolute and relative measurement bases. *Food Technol. Biotechnol., 41* (3): 191-201.

Yin, X., Goudriaan, J. Lantinga, E. A.,Vos, J. and Spiertz, H. J. 2003. A flexible sigmoid function of determinate growth. *Ann. Bot.,* 91: 361-371.

Date of Receipt : 18.03.2014
Date of Acceptance : 07.03.2015