Evaluation of model parameters for growth, tannic acid utilization and tannase production in *Bacillus gottheilii* M2S2 using polyurethane foam blocks as support

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Abstract Production of tannase from *B. gottheilii* M2S2 was studied under solid-state fermentation with an optimized medium consisting of polyurethane foam matrix of dimension $40 \times 40 \times 5$ mm, impregnated with a liquid medium comprising (w/v): 4% tannic acid; 2% NH_4NO_3 ; 0.1% KH_2PO_4 ; 0.2% MgSO_4 ; 0.1% NaCl and 0.05% $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ in distilled water, having a pH of 4.7. Maximum tannase production of 56.87 U/L was obtained after 32 h of fermentation at 32 °C in static condition. This study deals with the evaluation of unstructured kinetic models to understand the behavior of biomass, tannase production and tannic acid degradation, with the fermentation time. The growth rate of *B. gottheilii* M2S2 was 0.0703 h^{-1} at 32 h of fermentation. Product ($Y_{x/s}$) and biomass yield ($Y_{p/s}$) coefficients were estimated as 1.77 U/g of tannic acid and 0.276 g of biomass/g of tannic acid. All the kinetic constants μ , α , β , m and n were evaluated using MATLAB 2015Rb program. The experimental and model-generated data showed a good correlation, which indicated that these models will describe tannase production and fermentation process.

Keywords *Bacillus gottheilii* M2S2 · Polyurethane foam · Tannase · Unstructured kinetic models

Introduction

Tannin acyl hydrolase (E.C. 3.1.1.20), commonly referred as “tannase”, breaks hydrolysable tannic acid into gallic acid and glucose by hydrolysis of ester and depside bonds (Aguilar et al. 2007). It finds potential applications in lots of industrial sectors such as pharmaceutical (for gallic acid and propyl gallate production, used as an antibacterial drug called trimethoprim and antioxidants, respectively), food (clarifying agent in vegetable and fruit juices, preparation of instant tea, revitalizing cold drinks with coffee flavor and degradation of plant phenolic complexes), chemical (treatment of textile and tannery effluents) and beverages (preparation of wine and beer to remove chill haze formation) industries (Aithal and Belur 2013; Aguilar et al. 2007; Rout and Banerjee 2006).

Microbial tannases are gaining prominence because of the economical production and use of renewable resources, in comparison with the traditional chemical methods. Among all tannase producing microorganisms, bacteria have been significant because of their ease in upstream and downstream processing (Vikas et al. 2013; Belur and Mugeraya 2011). The solid-state fermentation have been used in numerous fermentation industries for the production of organic acids, enzymes and other bioactive compounds. Tannase production is predominately carried out in submerged fermentation (SmF) and solid-state fermentation (SSF). Sabu et al. (2006) were the first to report on production of bacterial tannase from *Lactobacillus* sp. ASR-S1 through SSF. Tannin-rich agro-residues such as teri pod (*Caesalpinia digyna*) (Kar et al. 1999; Purohit et al. 2006; Mahapatra et al. 2005; Mukerjee and Banerjee 2006), tamarind seed (*Tamarindus indica*), coffee husk, wheat bran, palm kernel cake (Sabu et al. 2006), Chebulic myrobalan, (Mahapatra et al. 2005; Purohit et al. 2006; Mukerjee and

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Banerjee 2006), leaves of amla (*Phyllanthus emblica*), Keekar (*Acacia nilotica*), jamun (*Syzygium cumini*), jamoa (*Eugenia cuspidate*) and ber (*Ziziphus mauritiana*) (Selwal et al. 2010) have also used for production of tannase under SSF. The moisture content, surface area, pore volume and humidity are the critical parameters in SSF process. The accessibility of nutrients and controlling the critical parameters are the limitations when agro-residues are used as only substrate. Hence, in this study the polyurethane foam (PUF) matrix was used as an inert support to overcome the limitations. The PUF impregnated with a liquid medium was used as a tannin-rich model substrate. The main advantages of impregnated PUF are the uniform porosity, ease in nutrient uptake, shorter lag phase, rapid metabolism and also reduces the difficulty in downstream processes in comparison with fermentation of crude tannin substrates (Subbalaxmi and Vytla 2016).

Statistics on kinetics of fermentation is very much essential for the development of the performance of batch fermentation process (Divyashree et al. 2009). Any fermentation process is generally demonstrated by kinetic equations providing the time progressions for biomass, substrate, and product concentrations (Bouville 2007). Mathematical models of such kinetics are helpful for understanding and design of processes utilizing desired organisms and also will be important tools in the development of balanced approaches for the design and optimization of large-scale bioreactor systems (Viccini et al. 2001).

Recent investigation on tannase is largely focused on selection of strains, media design, downstream processing studies (Mukesh et al. 2015; Mata-Gomez et al. 2015) and very limited work has been done on kinetic modeling for tannase production.

In this study, estimation of kinetic parameters were carried out in various kinetic models in batch mode, to satisfy the experimentally measured biomass, tannic acid content, and tannase activity. The effect of tannase production with biomass and the tannic acid utilization in *Bacillus gottheilii* M2S2 were studied by applying mathematical models, with an inert support PUF impregnated with optimized liquid medium through SSF. To best of our knowledge, we are the first to report on the modeling of uptake and growth kinetics of bacterial strain *Bacillus gottheilii* M2S2 under SSF for tannase production in a tannic acid containing liquid medium impregnated with PUF.

Materials and methods

Chemical and media components

Tannic acid, methyl gallate, bovine serum albumin, rhodanine, glucose and other chemical reagents were of

analytical grade and purchased from Himedia (India) and local suppliers.

Microorganism and culture maintenance

Bacillus gottheilii M2S2 was used in the current study. The strain was isolated from the soil samples of tannery effluent as described in our earlier report (Subbalaxmi and Vytla 2016) and identified based on 16S rRNA sequence analysis and the nucleotide sequence was submitted in Gene Bank (Accession number no. KU866380). The isolate *Bacillus gottheilii* M2S2 was deposited in the Microbial Type Culture Collection and Gene Bank (MTCC), institute of Microbial Technology (IMTECH Chandigarh, India), bearing the number MTCC 12554.

It was grown and maintained on a nutrient agar medium, and preserved at 4 °C. To ensure the viability of the cultures, periodic sub culturing was done every 30 days.

Preparation of inoculum

A bacterial isolate *Bacillus gottheilii* M2S2 was grown in nutrient broth of 50 ml inoculated with 18-h-old culture of 2 ml. Then incubated at 32 °C for 20 h and later the liquid medium was inoculated with the inoculum obtained.

The total number of viable cells of the strain was determined with the colony count technique. It was found that there was a cell density of 4×10^{12} CFU/ml (Subbalaxmi and Vytla 2016).

Solid-state fermentation

Polyurethane foam (PUF) blocks impregnated with a liquid medium were used as an inert support. PUF was obtained from local market (Manipal, India) in the color white. The foam was cut into 4-cm square blocks of thickness of 0.5 cm, washed thrice with warm water, dried for 3 days at 65 °C and autoclaved (20 min at 121 °C) (Mata-Gomez et al. 2015; Rodriguez et al. 2011; Van de and Pyle 2001, 2004). The composition of optimized liquid medium is (w/v): 4% tannic acid; 2% NH_4NO_3 ; 0.1% KH_2PO_4 ; 0.2% MgSO_4 ; 0.1% NaCl and 0.05% $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ in distilled water (Subbalaxmi and Vytla 2016). The liquid medium of pH 4.7 was sterilized at 121 °C for 15 min. Then the 7% of inoculum with 4×10^{12} cells/ml was inoculated into the liquid medium. Afterwards, the inert support material PUF was impregnated by adding the inoculated liquid medium and mixed the whole thoroughly in a sterile 250-ml Erlenmeyer flask and kept for incubation at 32 °C for 60 h. A high moisture content was employed (0.25 g PUF: 10 ml liquid medium) to maintain a high water activity (Mata-Gomez et al. 2015; Rodriguez et al. 2011; Van de and Pyle 2001, 2004). Experiments were

carried out in triplicates and for every 4 h tannic acid content, tannase activity and biomass concentration were determined.

Cell-free extract

The fermented material was retrieved from the Erlenmeyer flask by squeezing and washing with 10 ml citrate buffer (50 mM; pH 5). The fermented material was then cold centrifuged for 15 min at 10,000 rpm to obtain cell-free supernatant. This cell-free supernatant was collected in ampoules and preserved for additional analysis (Mata-Gomez et al. 2015; Rodriguez et al. 2011).

Estimation of biomass

After fermentation period, PUF was squeezed and washed with citrate buffer to extract fermented broth. Then the squeezed PUF and the pellet obtained after centrifuge of broth were dried at 80 °C to constant weight. Total biomass was determined as described below:

$$\begin{aligned} \text{Biomass} = & \{[\text{dry weight of squeezed PUF} \\ & - \text{weight of PUF before impregnation}] \\ & + [\text{dry weight of centrifuge tube with pellet} \\ & - \text{weight of empty centrifuge tube}]\} \end{aligned}$$

Estimation of tannase activity

The activity of tannase was quantified by the spectrophotometric method using substrate such as methyl gallate (Sharma et al. 2000). One unit of tannase is defined as the one micromole of gallic acid formed per minute, under experimental conditions.

Estimation of tannic acid

The tannic acid content in the cell-free extract was quantified using the protein precipitation method of Ann-Hagerman and Larry-Butler (1978).

Estimation of glucose and protein contents

The glucose and protein concentrations in the cell-free extract was determined by dinitrosalicylic acid method (Miller 1959) and Lowry's method (Lowry et al. 1951), respectively.

Mathematical modeling

Logistic growth model

The growth of *Bacillus gottheilii* M2S2 was described by the Monod's:

$$\frac{dX_T}{dt} = \mu X_T, \quad (1)$$

where μ is the specific biomass growth rate (h^{-1}), dX_T/dt is the biomass growth rate (g/l h) and X_T is the concentration of biomass (g/l). Mathematical models of SSF processes most commonly use the logistic equation. The logistic model is free from the concentration of substrate, can be effortlessly accomplished and suitable for the sigmoid profiles of microbial growth, also enables in the determination of kinetic parameters of biological importance (Bailey and Ollis 1986). The growth of microorganism with time is described by the following differential form of logistic equation:

$$\frac{dX}{dt} = \mu X_T \left(1 - \frac{X_T}{X_m} \right), \quad (2)$$

where X_m is the maximum biomass concentration (g/l). The integration of Eq. (2) gives Eq. (3) with the initial conditions of $X_T = X_0$ at $t = 0$, gives following equation:

$$X_T = \frac{X_0 e^{\mu t}}{\left\{ 1 - \left(\frac{X_0}{X_m} \right) (1 - e^{\mu t}) \right\}}. \quad (3)$$

The kinetic parameter, μ is determined by rearranging Eq. (3) as follows:

$$\mu t = \ln \left(\frac{X_m}{X_0} - 1 \right) + \ln \left(\frac{X'}{1 - X'} \right), \quad (4)$$

where $X' = \frac{X_T}{X_m}$, if the experimental data rightly describes the logistic equation, then a plot of $\ln \left(\frac{X'}{1 - X'} \right)$ vs t should give a straight line of slope ' μ ' and intercept $-\ln \left(\frac{X_m}{X_0} - 1 \right)$. The value of X_m is determined from the experimental data (Shuler and Kargi 2002; Kannan et al. 2011).

Rate of tannase production

The growth and non-growth-associated product formation of tannase in *Bacillus gottheilii* M2S2 can be represented by Luedeking-Piret model as shown below (Kannan et al. 2011).

$$\frac{dP_T}{dt} = \alpha \frac{dX_T}{dt} + \beta X_T \quad (5)$$

The first and second terms in Eq. (5) refer to growth and non-growth-associated product formation, respectively. The α and β are the kinetic constants (Hosseini et al. 2015; Biswanath et al. 2012; Kannan et al. 2011; Divyashree et al. 2009).

The equation for tannase production rate (Eq. 6) is attained by integrating Eq. (5) by means of Eq. (3),

$$P_T = P_0 + \alpha A(t) + \beta B(t), \quad (6)$$

where P_0 and P_T are the product concentrations at $t = 0$ and at any instant time ' t ', respectively, and,

$$A(t) = X_0 \left(\frac{e^{\mu t}}{\left(1 - \left(\frac{X_0}{X_m}\right)(1 - e^{\mu t})\right)} - 1 \right) \quad (7)$$

$$B(t) = \frac{X_m}{\mu} \ln \left(1 - \frac{X_0}{X_m} (1 - e^{\mu t}) \right). \quad (8)$$

The kinetics constants α and β in Eq. (6) can be determined by plotting $(P_T - P_0)/B(t)$ vs $A(t)/B(t)$ which is a straight line with slope ' α ' and intercept ' β '.

Rate of tannic acid utilization

Tannic acid is used as an inducer and energy source in the production of tannase. The substrate tannic acid utilization rate can be generally represented by the following equation (Doran 1995).

$$-\frac{dS_T}{dt} = m \frac{dX_T}{dt} + n \frac{dP_T}{dt} + m_s X_T, \quad (9)$$

where m and n are the reciprocals of the yields of biomass and product based on substrate, respectively. In Eq. (9) last term refers maintenance coefficient, which can be determined at the stationary phase ($dX/dt = 0$; $X = X_m$) by the following equation:

$$m_s = \frac{\left(\frac{dS_T}{dt}\right)_{\text{stationary phase}}}{X_m}. \quad (10)$$

Substituting Eq. (5) in Eq. (9) and on rearranging, following equation was obtained:

$$-\frac{dS_T}{dt} = m \frac{dX_T}{dt} + n \left[\alpha \frac{dX_T}{dt} + \beta X_T \right]. \quad (11)$$

Integrating Eq. (11) by means of Eq. (2), through initial conditions ($S_T = S_0$; $t = 0$) and on rearranging, gives the following equation:

$$(S_0 - S_T)/\delta(t) = n \left[\alpha + \frac{\gamma(t)}{\delta(t)} \beta \right] + m, \quad (12)$$

where S_0 and S_T are the initial and final concentrations of substrate at time $t = 0$ and at any instant time ' t ', respectively, and,

$$\delta(t) = X_0 \left(\frac{e^{\mu t}}{\left(1 - \left(\frac{X_0}{X_m}\right)(1 - e^{\mu t})\right)} - 1 \right) \quad (13)$$

$$\gamma(t) = \frac{X_m}{\mu} \ln \left(1 - \frac{X_0}{X_m} (1 - e^{\mu t}) \right). \quad (14)$$

The parameters m and n in Eq. (9) were determined by plotting $[S_0 - S_T]/\delta(t)$ vs $\left[\alpha + \frac{\gamma(t)}{\delta(t)} \beta \right]$, which gives a straight line with slope ' n ' and intercept ' m '.

Determination of other kinetic parameters

Other various kinetic parameters apart from model parameters, such as product yield coefficient based on substrate consumption ($Y_{p/s}$), product yield coefficient based on biomass ($Y_{p/x}$), biomass yield coefficient ($Y_{x/s}$), specific rate of product formation (q_p), specific rate of substrate uptake (q_s) and productivity (P) were estimated according to the methods defined elsewhere (Doran 1995).

Data analysis and modeling

The kinetics of microbial growth, tannic acid utilization and tannase production are consistently expressed in terms of mathematical models. The kinetic parameters of the model Eqs. (2), (5) and (9) were estimated by linearizing these equations as shown in Eqs. (3), (6) and (12), respectively, using MS Excel worksheet. A linear relationship between the specific growth rate, tannic acid utilization and tannase production may possibly be considered as a specific instance and it may not be effective for all bacteria. The MATLAB command 'ode45' which is based on the Runge–Kutta algorithm, was used to solve the differential equations of the proposed mathematical models [Eqs. (2), (5), (9)] and as well to evaluate the significance of kinetic parameters. To estimate precision of the kinetic models, statistical parameter correlation regression coefficient (R^2) was used.

$$R^2 = 1 - \frac{\sum_{i=1}^N (\text{Pr} - \text{Ex})^2}{\sum_{i=1}^N (\text{Ex} - \text{Ex}_{\text{av}})^2}, \quad (15)$$

where Ex , Pr and Ex_{av} are experimental, predicted and average values of experimental, respectively.

Results and discussion

The kinetic results of SSF of tannase production using *Bacillus gottheilii* M2S2 with PUF are shown in Fig. 1. Biomass, tannic acid content, tannase production, glucose and protein concentrations were analyzed over the fermentation time. The production of tannase and biomass was found to reach their maximum at 32 h in the late exponential phase (56.89 U/L of tannase activity and 7.38 g/l of biomass). This maximum tannase activity represented 3.85-fold increase in comparison through without PUF support (results are not shown). Hence, the production of tannase with PUF is feasible and this approach (inert support) can be used for large-scale applications. The decrease in tannase activity was observed after 32 h could be due to the binding of intermediate byproducts competitively or non-competitively to the enzyme active sites. This can be described by the tannase building further

Fig. 1 Kinetic profiles of tannase activity (filled diamond), biomass concentration (asterisk), tannic acid utilization (filled circle), glucose concentration (open circle) and protein concentration (open square) in batch process of tannase production by *B. gottheilii* M2S2 with PUF impregnated liquid medium

glucose and gallic acid available to the microorganism (Mondal et al. 2000). Gallic acid released over time can also inhibit the tannase action competitively, since tannic acid is composed of gallic acid units (Kar et al. 1999). In comparison to this report, *L. plantarum* MTCC 1407 (Kannan et al. 2011), *Bacillus licheniformis* KBR6 (Mondal and Pati 2000), *Lactobacillus* sp. ASR-S1 (Sabu et al. 2006), and *Enterobacter cloacae* MTCC 9125 (Beniwal et al. 2010) produced tannase of 5.22 U/ml, 0.41 U/ml, 0.85

U/gds and 0.69 U/ml after 24, 21, 72 and 48 h, respectively. The strain *B. gottheilii* M2S2 was able to produce tannase in the medium containing sole carbon source as tannic acid. Figure 1 shows similar plots for kinetic profile of biomass growth and tannase activity, which indicates that tannase production is associated with microbial growth.

The rate of tannic acid utilization by *Bacillus gottheilii* M2S2 increased rapidly after 4 h of the fermentation and 56% of the tannic acid was utilized at 60 h of fermentation. Similarly, Kumar et al. (1999) reported that 65% of tannic acid was degraded by *C. freundii* in 24 h under SmF; whereas, Mondal et al. (2000) stated the complete degradation of tannic acid by *B. licheniformis* within 18–21 h of microbial growth under SmF. In this study, the tannic acid solution used throughout is available tannic acid (i.e. non-chemisorbed). The decrease in tannic acid degradation by *B. gottheilii* M2S2 after 32 h of fermentation might be due to the fact that the structure of product gallic acid mimics the tannic acid structure and hence it could bind the enzyme active site competitively and block further enzyme action as reported elsewhere (Kar et al. 1999; Kumar et al. 1999).

The yield coefficients $Y_{x/s}$, $Y_{p/s}$ and $Y_{p/x}$ estimated during the solid-state fermentation process are shown in Table 1. Maximum $Y_{x/s}$, $Y_{p/x}$ and $Y_{p/s}$ were 0.276 g/g

Table 1 Kinetic parameters of growth and tannase production by *B. gottheilii* M2S2 in optimized medium at static condition, temperature 32 °C in shake-flask cultures

Fermentation period, h	Kinetic analysis parameters							
	$Y_{p/x}$, U/g	$Y_{p/s}$, U/g	$Y_{x/s}$, g/g	q_p , U/g h	q_s , g/g h	Tannase activity, U/l	Sp. activity, U/g	P , U/h
0	0	0	0.121	0	0.583	0	0	0
4	2.228	0.319	0.143	0.157	0.491	10.425	2.127	2.606
8	3.751	0.606	0.161	0.264	0.436	18.805	3.917	2.351
12	5.013	0.909	0.181	0.352	0.388	26.838	5.710	2.237
16	6.192	1.259	0.203	0.435	0.346	35.297	7.353	2.206
20	6.643	1.490	0.224	0.467	0.313	40.743	7.835	2.037
24	7.290	1.797	0.247	0.512	0.285	46.743	8.819	1.948
28	7.628	2.071	0.271	0.536	0.259	51.059	9.543	1.824
32	7.700	2.37	0.308	0.541	0.228	56.893	10.344	1.778
36	7.084	2.325	0.328	0.498	0.214	52.336	9.515	1.454
40	6.797	2.344	0.345	0.478	0.204	50.198	9.126	1.255
44	6.849	2.468	0.360	0.481	0.195	50.585	9.197	1.150
48	6.854	2.532	0.369	0.482	0.190	50.604	9.200	1.054
52	6.855	2.580	0.376	0.482	0.187	50.604	9.200	0.973
56	6.856	2.622	0.382	0.482	0.184	50.604	9.200	0.904
60	6.856	2.661	0.388	0.482	0.181	50.604	9.200	0.843

The maximum values of all dependent variables are the mean of triplicates

$Y_{p/x}$ units of tannase produced/g of biomass formed, $Y_{p/s}$ units of tannase produced/g of tannic acid utilized, $Y_{x/s}$ g of biomass produced/g of tannic acid utilized, q_p units of tannase produced/g of biomass formed/h, q_s g of tannic acid utilized/g of biomass produced per h, $Sp. activity$ tannase activity/protein content, P units of tannase produced/total time of fermentation

(average value) of tannic acid, 7.7 U/g of biomass and 1.77 U/g (average value) of tannic acid, respectively, after 32 h of fermentation time. These results specify that tannase production followed the growth of *B. gottheilii* M2S2, with the maximum specific growth rate of 0.0703 h^{-1} . The yield of biomass (0.23 g biomass/g of tannic acid) and product (21.2 U/g of tannic acid) for *A. foetidus* with μ as 0.055 h^{-1} was reported (Mohan et al. 2014). Aguilar et al. (2001) reported that estimation of q_p , together with X_m , helps to differentiate whether productivity is based on a very productive strain (high q_p) or simply because biomass is produced in large quantities (high X_m). In this study, production of tannase in the SSF with PUF was more productive with q_p of 0.541 U/g/h , X_m of 7.38 g/l at 32 h of fermentation and as well due to the strain *B. gottheilii* M2S2 (Table 1). Our results are in good agreement with the results of Aguilar et al. (2001).

The rate of glucose synthesis and tannase production by *B. gottheilii* M2S2 was consistently similar until 32 h (6.4 g/l), and this synthesized glucose could be used by the microorganism for their growth and metabolism. At 32 h of exponential phase glucose synthesis was reduced, the reason could be that their higher concentrations may possibly be repressive on tannase production as reported elsewhere (Sabu et al. 2006; Mondal et al. 2000); and as well this glucose would have been used to produce other byproducts, hence the protein concentration in stationary phase was increased to 14 g/l at 48 h and thereby decreased the tannase production.

Table 2 Values of experimental and predicted kinetic model parameters for tannase production by *B. gottheilii* M2S2 on an inert support PUF impregnated with optimized liquid medium

Model parameters	Experimental values	Predicted values
Biomass (Eq. 2)		
X_m (g/l)	7.39	7.39
X_0 (g/l)	4.133	4.056
μ_m (h^{-1})	0.0703	0.072
Product (Eq. 5)		
α (g/l)	1483	1501
β (h^{-1})	-3.342	0
Substrate (Eq. 9)		
m (g/g)	783.5	769
n (g/U)	0.146	0.252

Estimation of kinetic model parameters μ , α , β , m and n

Tannase production was carried out by *B. gottheilii* M2S2 in batch fermentation with the optimized medium. Microsoft Word Excel-13 was used to determine the kinetic parameters μ (logistic model), α and β (Luedeking–Piret model), m and n (substrate utilization model) as shown in Fig. 2. Logistic model (Eq. 4) was used to fit the growth kinetics data, to determine the specific biomass growth rate (μ) and initial biomass concentration (X_0). μ and X_0 were found to be 0.0703 h^{-1} and 4.06 g/l , respectively, from slope and intercept of Fig. 2a. The calculated X_0 value was found to be similar with an experimental value (4.13 g/l) in

Fig. 2 Estimation of unstructured kinetic model parameters: **a** specific growth rate constant μ , using Eq. (4); **b** growth-associated rate constant ' α ' and non-growth-associated rate constant ' β ' using Eq. (6); and **c** tannic acid utilization kinetic parameters ' m ' and ' n ' using Eq. (12)

Fig. 3 Comparison of kinetic profiles of experimental values (closed symbols) and model fitting (solid line) for tannase activity (filled square), biomass concentration (filled circle) and tannic acid utilization (filled triangle) by *B. gottheilii* M2S2 in shake-flask culture at 32 °C, static condition for 60 h with an inert support PUF under SSF

this study. The correlation coefficient (R^2) was found to be 0.9901. From this experimentation, we established a growth curve of sigmoidal trend, which is comparable to earlier studies on microbial growth (Hosseini et al. 2015; Biswanath et al. 2012; Divyashree et al. 2009).

Luedeking–Piret model of Eq. (6) was considered to determine the mode of tannase production. The kinetic parameters, growth-associated rate constant α and non-growth-associated rate constant β were estimated using Eq. (6), such as 1483 and -3.342 , respectively (Fig. 2b). The correlation coefficient (R^2) was found to be 0.9898.

Fig. 4 Evaluation of experimental vs predicted values for biomass, tannase activity and tannic acid using Eqs. (2), (5) and (9), respectively, for tannase production from *B. gottheilii* M2S2 on inert support PUF

These values signified that the degree of growth-associated rate constant α is much greater than that of non-growth-associated rate constant β . Therefore, the tannase production by *B. gottheilii* M2S2 was found to be growth-associated product formation and it had good agreement with tannase produced from, *L. plantarum* MTCC 1407 (Kannan et al. 2011), *B. licheniformis* (Mondal et al. 2000) and *C. freundii* (Kumar et al. 1999).

In this study, it was observed that the biomass concentration and tannase activity increased with the decrease in the tannic acid concentration present in the optimized medium. An initial tannic acid concentration was found to be 34.26 g/l. Tannic acid degradation was progressively increasing from the start of the fermentation and high degradation was observed at 60 h. Substrate utilization kinetics of Eq. (12) was plotted and the kinetic constants m and n were found to be 783.47 and 0.416 from intercept and slope, respectively (Fig. 2c). The correlation coefficient (R^2) was found to be 0.9494, hence Eq. (12) described 94.94% the experimental data of tannic acid degradation with the predicted data for the same.

Model analysis

The production of tannase by *B. gottheilii* M2S2 under SSF with an inert support PUF impregnated with optimized liquid medium was studied. Modeling was attempted using different kinetic models. The estimated kinetic parameters such as μ , α , β , m and n were used to analyze the model. MATLAB R2015b was used to evaluate the above-

mentioned kinetic parameters. The values of experimentally calculated and simulated kinetic parameters are shown in Table 2. The comparison of experimental and model values of the biomass concentration, tannic acid concentration (substrate) and tannase activity (product) for the system during tannase production from *B. gottheilii* M2S2 is shown in Fig. 3. The correlation coefficient (R^2) value is largely used to describe the goodness of fit for the experimental and model-predicted data (Rajasekar et al. 2015). In this study, R^2 values for biomass concentration, tannase activity and tannic acid concentration were found to be 0.96, 0.98 and 0.87, respectively (Fig. 4). The values of these R^2 signifies that the unstructured kinetic models such as logistic equation (biomass), Luedeking–Piret model (tannase activity) and substrate utilization rate (tannic acid), provide better predictions.

Conclusion

The study on kinetic analysis of *Bacillus gottheilii* M2S2 for tannase production exhibited the maximum tannase activity of 56.89 U/l at 32 h of fermentation, which represents a 3.85-fold increase in production on comparison through without PUF matrix. The logistic model for biomass ($\mu = 0.0703$), Luedeking–Piret equation for tannase activity ($\alpha = 1483$ and $\beta = -3.342$) and substrate utilization rate equation for tannic acid degradation ($m = 783.5$ and $n = 0.146$) were found prominent in predicting the kinetic profiles of tannase production with PUF matrix. The greater value of correlation coefficient R^2 for biomass concentration, tannase activity and tannic acid concentration were found to be 0.96, 0.98 and 0.87, respectively. Hence, these unstructured models could be used to understand the behavior of a system, which is very much needed to study the fermentation process.

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Compliance with ethical standards

Conflicts of interest The authors certify that no actual or potential conflicts of interest in relation to this article exist.

References

Aguilar CN, Augur C, Favela-Torres E, Viniegra-Gonzalez G (2001) Production of tannase by *Aspergillus niger* Aa-20 in submerged

- fermentation and solid state fermentation: influence of glucose and tannic acid. *J Ind Microbiol Biotechnol* 26:296–302
- Aguilar CN, Rodriguez R, Gutierrez-Sanchez G, Augur C, Favela-Torres E (2007) Microbial tannases: advances and perspectives. *Appl Microbiol Biotechnol* 76:47–59
- Aithal M, Belur PD (2013) Enhancement of propyl gallate yield in nonaqueous medium using novel cell-associated tannase of *Bacillus massiliensis*. *Prep Biochem Biotechnol* 43:445–455
- Ann-Hagerman E, Larry-Butler G (1978) Protein precipitation method for the quantitative determination of tannins. *J Agri Food Chem* 26(4):809–812
- Bailey JE, Ollis DF (1986) *Biochemical engineering fundamentals*, 2nd edn. McGraw-Hill Inc, Singapore, pp 373–454
- Belur PD, Mugeraya G (2011) Microbial production of tannase: state of the art. *Res J Microbiol* 6(1):25–40
- Beniwal V, Vinod C, Narender S, Jitendra S (2010) Optimization of process parameters for the production of tannase and gallic acid by *Enterobacter Cloacae* MTCC 9125. *J Am Sci* 6(8):389–397
- Biswanath B, Basak B, Bhattacharya P, Dey A (2012) Kinetic studies of alkaline protease from *Bacillus licheniformis* NCIM-2042. *J Microbiol Biotechnol* 22(12):1758–1766
- Bouville M (2007) Fermentation kinetics including product and substrate inhibitions plus biomass death: a mathematical analysis. *Biotechnol Lett* 29(5):737–741
- Divyashree MS, Navin KR, Shamala TR (2009) A Simple kinetic model for growth and biosynthesis of polyhydroxyalkanoate in *Bacillus flexus*. *New Biotechnol* 26:92–98
- Doran PM (1995) *Bioprocess engineering principles*. Elsevier, California, pp 257–295
- Hosseini VS, Saffari Z, Farhanghi A, Atyabi MS, Norouzian D (2015) Kinetics of alkaline protease production by *Streptomyces griseoflavus* PTCC1130. *Iran J Microbiol* 8(1):8–13
- Kannan N, Aranindan R, Viruthagiri T (2011) Effect of cultural conditions and kinetic studies on extracellular tannase production by *Lactobacillus plantarum* MTCC 1407. *Indian J Biotechnol* 10:321–328
- Kar B, Banerjee R, Bhattacharyya BC (1999) Microbial production of gallic acid by modified solid state fermentation. *J Ind Microbiol Biotechnol* 23:173–177
- Kumar AR, Gunasekaran P, Lakshmanan N (1999) Biodegradation of tannic acid by *Citrobacter freundii* isolated from a tannery effluent. *J Basic Microbiol* 39(3):161–168
- Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. *J Biol Chem* 193:265–275
- Mahapatra K, Ranjan KN, Subhendu SB, Rintu B, Ashok P et al (2005) Purification, characterization and some studies on secondary structure of tannase from *Aspergillus awamori nakazawa*. *Process Biochem* 40:3251–3254
- Mata-Gomez M, Mussatto SI, Rodriguez R, Teixeira JA, Martinez JL et al (2015) Gallic acid production with mouldy polyurethane particles obtained from solid state culture of *Aspergillus niger* GH1. *Appl Biochem Biotechnol* 176:1131–1140
- Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chem* 31:426
- Mohan K, Viruthagiri T, Arun K, Chockalingam (2014) Kinetics and modelling of tannase production using *Aspergillus foetidus* in batch fermentation. *Int J Pharm Pharm Sci* 7(2):64–67
- Mondal KC, Pati BR (2000) Studies on extracellular tannase from newly isolated *Bacillus licheniformis* KBR 6. *J Basic Microbiol* 40(4):223–232
- Mondal KC, Banerjee R, Bikas RP (2000) Tannase production by *Bacillus licheniformis*. *Biotechnol Lett* 22:767–769
- Mukerjee G, Banerjee R (2006) Effects of temperature, pH and additives on the activity of tannase produced by a co-culture of

- Rhizopus oryzae* and *Aspergillus foetidus*. World J Microbiol Biotechnol 22:207–212
- Mukesh K, Shiny R, Vikas B, Raj KS (2015) Optimization of tannase production by a novel *Klebsiella pneumonia* KP715242 using central composite design. Biotechnol Rep 7:128–134
- Purohit JS, Jayati RD, Ranjan KN, Rintu B (2006) Strain improvement for tannase production from co-culture of *Aspergillus foetidus* and *Rhizopus oryzae*. Bioresour Technol 97:795–801
- Rajasekar V, Ramachandra MV, Muthukumaran C (2015) Development of a simple kinetic model and parameter estimation for biomass and nattokinase production by *Bacillus subtilis* 1A752. Austin J Biotechnol Bioeng 2(1):1–5
- Rodriguez D, Luis V, Contreras-Esquivel JC, Raul R, Prado-Barragan AL, Aguilar CN (2011) Optimization of tannase production by *Aspergillus niger* in solid-state packed-bed bioreactor. J Microbiol Biotechnol 21(9):960–967
- Rout S, Banerjee R (2006) Production of tannase under mSSF and its application in fruit juice Debittering. Indian J Biotechnol 5:346–350
- Sabu A, Augur C, Swati C, Ashok P (2006) Tannase production by *Lactobacillus* sp. ASR-S1 under solid-state fermentation. Process Biochem 41:575–580
- Selwal MK, Yadav A, Selwal KK, Aggarwal NK, Gupta R et al (2010) Optimization of cultural conditions for tannase production by *Pseudomonas aeruginosa* IIIB 8914 under submerged fermentation. World J Microbiol Biotechnol 26:599–605
- Sharma S, Bhat TK, Dawra RK (2000) A Spectrophotometric method for assay of tannase using rhodanine. Anal Biochem 279:85–89
- Shuler ML, Kargi F (2002) Bioprocess engineering: basic concepts, 2nd edn. Pearson Education Inc, London, pp 175–209
- Subbalaxmi S, Vytla RM (2016) Process optimization for tannase production by *Bacillus gottheilii* M2S2 on inert polyurethane foam support. Biocatal Agri Biotechnol 7:48–55
- Van de LJ, Pyle DL (2001) Solid-state fermentation and bioremediation: development of a continuous process for the production of fungal tannase. Chem Eng J 84:15–123
- Van de LJ, Pyle DL (2004) Solid-state fermentation: a continuous process for fungal tannase production. Biotechnol Bioeng 87(7):924–929
- Vicini G, Mitchell AD, Boit DS, Gern CJ, da Rosa SA et al (2001) Analysis of growth kinetic profiles in SSF Fermentation. Food Technol Biotechnol 39(4):271–294
- Vikas B, Anil K, Gunjan G, Vinod C (2013) A novel low molecular weight acido-thermophilic tannase from *Enterobacter cloacae* MTCC 9125. Biocatal Agri Biotechnol 2:132–137