Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 2, 2015

Original Article

KINETICS AND MODELING OF TANNASE PRODUCTION USING *ASPERGILLUS FOETIDUS* IN BATCH FERMENTATION

MOHAN KUPPUSAMY[1*], VIRUTHAGIRI THANGAVELU[2], ARUN KUMAR, CHOCKALINGAM[3]

[1]Associate Professor of Petrochemical Technology, Sri Ramanathan Engineering College, Tirupur, India, [2]Professor of Chemical Engineering, Annamalai University, Annamalai nagar 608002, India, [3]Assistant Professor of Petrochemical Technology, Sri Ramanathan Engineering College, Tirupur, India.
Email: skmohan75@gmail.com

Received: 23 Sep 2014 Revised and Accepted: 25 Oct 2014

ABSTRACT

Objective: To produce tannase enzyme using *Aspergillus foetidus* with red gram husk as substrate in batch fermentor and investigate the suitable un structured kinetic model for the system.

Methods: The present study was done via two steps. At first to study the maximum production of tannase enzyme by *Aspergillus foetidus* (MTCC 3557) using red gram husk as a substrate in a modular ferment or followed by to develop the kinetic model of tannase production.

Results: The maximum tannase activity and biomass concentration were found to be 157.26 U/ml and 7.12 g/l respectively at the end of 96 hours of fermentation. The biomass yield coefficient ($Y_{X/S}$) and the product yield coefficient ($Y_{P/S}$) were found to be 0.23 g of biomass/g of substrate and 21.2 U/g of substrate respectively. Logistic model, Luedeking-Piret model and substrate utilization kinetic model were found to represent closely the experimental data of growth kinetics, product formation kinetics and substrate utilization kinetics respectively.

Conclusion: Tannase enzyme production was studied using *A. foetidus* with redgram husk as substrate by modular fermenter and suitable models were predicted. The kinetic parameters were also estimated by fitting the data to the model using the Lineweaver-Burk method.

Keywords: *Aspergillus foetidus*, Tannase, Kinetics and Modeling, Redgram husk.

INTRODUCTION

Tannase (tannin acyl hydrolase, E. C.3.1.1.20) is an inducible, extracellular enzyme that catalyzes the breakdown of ester (galloyl ester of an alcohol moiety) and depside bonds (galloyl ester of gallic acid) present in hydrolysable tannins [1]. Tannase have wide distribution in plants, animals, and microbes [2]. Bacteria [3], yeast [4] and filamentous fungi [5] [6] are known tannase producers. Species of *Aspergilli* were reported as the best tannase producers in submerged and solid state fermentations [7]. At present, tannase is used in different industrial sectors, mainly, for preparation of gallic acid, instant tea, acron wine, coffee flavoured soft drinks, high-grade leather tannin, clarification of beer, fruit juices [8] [9] [10]. Gallic acid, a hydrolytic product of tannin, has different uses like chemical synthesis of propyl gallate and trimethoprim which have application in the food and pharmaceutical industries [11]. The utilization of agro-industrial wastes, on one hand, provides alternative substrates and, on the other hand helps to solve pollution problems by eliminating the need for disposal of the wastes. The nature of the substrate employed is the most important factor affecting fermentation processes, and its selection depends upon several factors mainly related to cost and availability and, thus, may necessitate the screening of several agro industrial residues [12]. In addition, Kinetic model describing the behavior of microbiological systems can be a highly appreciated tool and can be reduce tests to eliminate extreme possibilities [13]. Here for the first time, we are reporting the use of red gram husk (agrowaste) for tannase production, which are very cheap and readily available substrate in this part of the world. In the present research work, the tannase enzyme production was studied by submerged fermentation technique using *Aspergillus foetidus* with red gram husk as substrate in a modular ferment or and various unstructured kinetic models were used to characterize the fermentation process.

MATERIALS AND METHODS

Microorganism maintenance and inoculum preparation

The fungal organism *Aspergillus foetidus* (MTCC 3557) was obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. *Aspergillus foetidus* (MTCC 3557) was maintained in Modified Czapek Dox agar media with a composition of sucrose 30 g/l, sodium nitrate 2 g/l, magnesium glycero phosphate 0.50 g/l, potassium chloride 0.50 g/l, dipotassium sulphate 0.35 g/l, ferrous sulphate 0.01 g/l and agar 12 g/l at 30ºC for 7 days.

The inoculum required for submerged fermentation was prepared by growing the organism using Modified Czapek Dox medium (excluding agar) harvesting the spores from 96 hours old culture (grown at 35ºC). The spore suspension was collected in sterile falcon tube and stored at 4ºC until the actual study. The medium components and chemicals used in this study were procured from Himedia Ltd, Mumbai, India.

Batch fermentation

A modular fermenter (New Brunswick, USA) of 3L capacity was used to study the kinetics of tannase enzyme production. The Modified Czapek Dox Medium of 1L and 3% (w/v) of substrate redgram husk was sterilized for 15 minutes at 121°C and was cooled after the sterilization. 50 ml of seed culture of *Aspergillus foetidus* was used to inoculate the sterile medium. The production of tannase was carried out under optimum operating conditions at 35°C, an initial pH of 5.5 and with an inducer tannic acid concentration of 3% (w/v) for a fermentation period of 120 hours. The samples were drawn at regular time intervals and analyzed for tannase activity, biomass concentration and residual substrate concentration.

Tannase assay

0.05 ml of enzyme solution was incubated with 0.3 ml of 1.0 % (w/v) tannic acid and 0.2 M acetate buffer (pH 5.0) at 40°C for 10 min and then the enzyme production was stopped by cooling to 0°C by the addition of 3 ml Bovine Serum Albumin (1 mg/ml), which precipitates the remaining tannic acid. Simultaneously, a control without the enzyme was incubated and the samples were analyzed. The tubes were then centrifuged (5000 x g, 10 min) and the precipitate was dissolved in 3 ml of Sodium Dodecyl Sulphate-triethanolamine (1% (w/v) SDS in 5 % (v/v) triethanolamine)

solution and the absorbency was measured at 530 nm after addition of 1 ml of $FeCl_3$ (0.01 M $FeCl_3$ in 0.01 N HCl).

One Unit of Tannase activity is defined as the amount of enzyme required to hydrolyze one micro mole of ester linkage of tannic acid in 1 min at specific condition [14].

Biomass estimation

A known volume of fermented broth was taken and centrifuged. Then the supernatant separated out from the fermented broth for analyzing the activity and the settled cell mass was dried and weighed. The difference between initial and final weight of the centrifuge tube will be the weight of the cell mass [15].

Estimation of tannin

The estimation of tannin content was done the protein precipitation method of Haggerman and Butler [16]. Dried substrates were ground finely in methanol and kept overnight at 4°C. One ml of extract was taken in a tube and 3 ml of Bovine Serum Albumin solution was added and kept for 15 min at room temperature. The tubes were centrifuged at 5000 x g for 10 min, supernatant was discarded and pellet was dissolved in 3 ml of Sodium Dodecyl Sulphate-triethanolamine solution. One ml of $FeCl_3$ solution was added and tubes were kept for 15 min at room temperature for color stabilization. The color was read at 510 nm against the blank using UV Spectrophotometer.

Kinetic models

Logistic growth model

The logistic growth model is a substrate independent model. The logistic model states that the rate of growth of the cell is proportional to the cell mass concentration present at any time [17]. Logistic growth model is presented in below form:

$$X = \frac{X_o e^{kt}}{\left[1 - \frac{X_o}{X_s}\left(1 - e^{kt}\right)\right]} \quad \ldots\ldots\ldots (1)$$

Where X is the biomass concentration (g L^{-1}), X_0 is the initial biomass concentration (g L^{-1}) k is the rate constant (h^{-1}) and t is time (hr).

Product formation kinetic model

Luedeking-Piret Model

According to this model, the product formation rate depends upon both the instantaneous cell mass concentration (X) and growth rate (dX/dt) in a linear manner [18].

$$\frac{dp}{dt} = \alpha \frac{dX}{dt} + \beta X \quad (2)$$

Where p is the product concentration (g L^{-1}), α is the growth associated product formation constant and β is the non-growth associated product formation constant, (h^{-1})

Substrate utilization kinetics model

The substrate utilization kinetics is given by the equation (3), which considers substrate conversion to cell mass, to product formation and substrate consumption for cell maintenance [19]

$$\frac{dS}{dt} = \frac{1}{Y'_{X/S}} \frac{dX}{dt} - \frac{1}{Y'_{P/S}} \frac{dp}{dt} - kX \quad (3)$$

Where $Y'_{X/S}$ is the biomass yield coefficient and $Y'_{P/S}$ is the product yield coefficient.

RESULTS AND DISCUSSION

The tannase enzyme production was carried out under optimum operating conditions at 35°C, an initial pH of 5.5 and with an inducer tannic acid concentration of 3% (w/v) for a fermentation period of 120 hours. The samples were drawn at regular time intervals and analyzed for tannase activity, biomass concentration and residual substrate concentration and the results are shown in Fig.1. The tannase activity was found to increase with respect to fermentation time and reaches a maximum of 157.26 U/ml at the end of 96 hours of fermentation and then decreases gradually till the end of fermentation. The biomass concentration reaches a maximum of 7.12 g/l at 96 hours and further there was no increase in biomass concentration till the end of the fermentation.

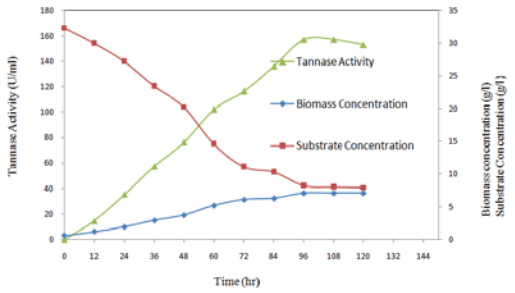

Fig. 1: Tannase enzyme Production using *A. foetidus* with RGH as substrate under optimum conditions

The lag phase of 12 hours and an exponential phase of 84 hours were observed for the growth of fungi *A. foetidus* and tannase enzyme production was very significant during the exponential phase of the microorganism. The rate of substrate utilization was found to increase rapidly till 96 hours in an exponential manner and it was found to be very low in the stationary phase upto 120 hours. The biomass yield coefficient ($Y_{X/S}$) was found to be 0.23 g of biomass/g of substrate and the product yield coefficient ($Y_{P/S}$) was found to be 21.2 U/g of substrate. table 1 also showed that the kinetics of tannase enzyme production using *A. foetidus* with redgram husk as substrate.

The unstructured models provide a good approximation of the fermentation profile even though the complete mechanism of microbial growth is not considered in the models. Logistic model (R^2 = 0.98) used to estimate specific growth rate (0.061 h^{-1}). Substrate utilization kinetic model (R^2 = 0.983) was used to find the yield coefficient (0.377) Luedeking-Piret model (R^2 = 0.942) was used to find growth associated parameter 'α' (18.02) and non growth associated parameter 'β' (0.041).

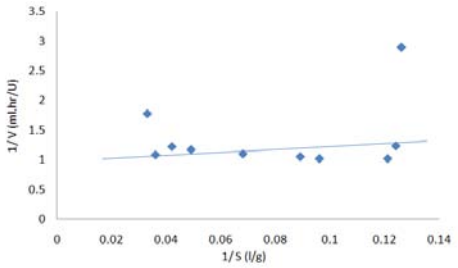

Fig. 2: Line weaver-Burk Plot for Tannase Production using *A. foetidus* with RGH

From the Lineweaver-Burk plot K_m and V_{max} values were found to be 4.126 g/l and 1.25 U/ml. hr respectively. The Michaelis Menten kinetic model for maximum tannase production was found to be

$$V = 1.25\,(S)/4.126 + (S) \quad \ldots\ldots (4)$$

Table 1 shows model parameters for tannase enzyme production with RGH as substrate. The rate of tannase production experimental values were compared with model predicted values given by the Eqn. (4) and is given in table 2 and table 3.

Table 1: Model Parameters for Tannase enzyme Produc tion with RGH as substrate

Microorganism-*A. foetidus* and Substrate-Redgram husk						
Logistic Growth Model		**Substrate utilization Model**		**Luedeking-Piret Model**		
k (h^{-1})	**R^2**	**$Y_{X/S}$**	**R^2**	**k**	**β**	**R^2**
0.055	0.966	0.304	0.941	21.2	0.035	0.981

Table 2: Kinetics of Tannase enzyme Production using *A. foetidus* with RGH

Time (hr)	Tannic acid concentration (S), g/l	Biomass concentration (X), g/l	Tannase Activity (P), U/ml
0	32.33	0.61	0.00
12	30.02	1.20	14.62
24	27.22	1.92	35.32
36	23.44	2.92	57.52
48	20.21	3.75	76.23
60	14.62	5.20	102.12
72	11.14	6.12	116.45
84	10.34	6.32	136.12
96	8.24	7.12	157.26
108	8.04	7.06	152.26
120	7.89	7.00	147.16

Table 3: Line weaver Burk plot data for production of tannase

Time (hr)	Rate of product formation (Expt) V= dP/dt U/ml. hr	Rate of Product formation (Predict) V= dP/dt U/ml. hr	Line weaver Burk Plot	
			1 / S (l / g)	1 / V (ml. hr/U)
0	0.00	1.104	0.030	0.00
12	0.345	1.094	0.033	1.775
24	0.562	1.080	0.036	1.280
36	0.781	1.057	0.042	1.221
48	0.819	1.032	0.049	1.170
60	0.854	0.967	0.068	1.096
72	0.912	0.904	0.089	1.049
84	0.953	0.885	0.096	1.017
96	0.983	0.824	0.121	1.011
108	0.979	0.817	0.124	1.231
120	0.812	0.811	0.126	2.898

CONCLUSION

Batch fermentation of tannase enzyme production was studied using *Aspergillus foetidus* (MTCC 3557) with red gram husk as substrate by modular fermentor. The logistic model for microbial growth, Luedeking-Piret model for tannase production and substrate utilization kinetic model for tannic acid utilization kinetics were found with high coefficient of determination (R^2) values of 0.966, 0.941 and 0.981 respectively. The kinetic parameters were also estimated by fitting the data to the model using the Line weaver-Burk method.

ACKNOWLEDGMENT

The authors gratefully acknowledge the Bioprocess Research Laboratory, Department of Chemical Engineering, Annamalai University for providing the necessary facilities for the successful completion of this research work.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Lekha PK, Lonsane BK. Production and application of tannin acyl hydrolase; state of the art. Adv Appl Microbiol 1997;44:215–60.
2. Aguilar CN, Rodríguez R, Gutiérrez-Sánchez, Augur C, Favela-Torres E, Prado-Barragan LA, *et al*. Microbial tannases: advances and perspectives. Appl Microbiol Biotechnol 2007;76:47–59.
3. Deschamps AM, Otuk G, Lebeault JM. Production of tannase and degradation of chestnut tannins by bacteria. J Ferment Technol 1983;61:55-9.
4. Aoki K, Shinke R, Nishira H. Purification and some properties of yeast tannase. Agr Biol Chem 1976;40(1):79-85.
5. Bajpai B, Patil S. Tannin acyl hydrolase (EC 3.1.1.20) activity of *Aspergillus*, *Penicilium*, *Fusarium* and *Trichoderma*. World J Microbiol Biotechnol 1996;12:217-20.
6. Bradoo S, Gupta R, Saxena RK. Screening of extracellular tannase producing fungi: development of a rapid and simple plate assay. J Gen Appl Microbiol 1996;42:325-9.
7. Bhat TK, Singh B, Sharma OP. Microbial degradation of tannins–a current perspective. Biodegradation 1998;9:343-57.
8. Chae SK, Yu TJ. Experimental manufacture of acron wine by fungal tannase. Hanguk Sipkum Kwahakhoechi 1983;15:326–32.
9. Mohan SK, Viruthagiri T, Arunkumar C. Statistical Optimization of Process parameters for the production of tannase by *Aspergillus flavus* under submerged fermentation. 3 Biotech 2014;4(2):159–66.
10. Coggon P, Sanderson GW. Manufacture of instant tea. Patent Ger Offen 1973;110:54-8.
11. Mohan Kuppusamy, Viruthagiri Thangavelu, Arunkumar Chockalingam. Optimization of submerged fermentative production of Tannase by *Aspergillus flavus.* Int J Chem Tech Res 2012;4(4):1461-7.

12. Couto SR, Sanroman MA. Application of solid-state fermentation to food industry-a review. J Food Engg 2006;76(3):291–302.
13. Lin Y, Tanaka S. Ethanol fermentation from bio mass resources: current state and prospectus. Appl Microbiol Biotechnol 2006;69:627-42.
14. Mondal KC, Banerjee D, Jana M, Pati BR. Calorimetric assay method for determination of the tannase activity. Anal Biochem 2001;295:168-71.
15. Narasimha G, Sridevi A, Viswanath B, Subhosh Chandra M, Rajasekar reddy B. Nutrient effects on production of cellulytic enzymes by *A. niger*. Afr J Biotechnol 2005;5(5):472-6.
16. Haggerman AE, Butler LG. Protein precipitation method for determination of tannins. J Agric Food Chem 1978;26:809–12.
17. James Bailey, David Ollis. Biochemical Engineering Fundamentals, 2nd ed; 2010.
18. Luedeking R, Piret EL. A Kinetic study of the lactic acid fermentation. J Biochem Microbiol Technol Eng 1959;67(6):1393-412.
19. Weiss RM, Ollis DF. Extracellular microbial polysaccharides: Substrate, biomass and Product kinetic equations for batch xanthan gum fermentation. Biotechnol Bioengg 1966;22:859-73.