International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol. 3, No.4, pp 1845-1850, Oct-Dec 2011

Production of Endoglucanase in Mixed Culture of *Trichderma viride* and *Aspergillus niger*-Kinetics and Modeling

Kavitha.S.[1]*, Nagarajan.P.[2]

[1]Research scholar, Department of Chemical Engineering, Annamalai University Chidambaram, Tamil Nadu (INDIA)

[2]Associate professor, Department of Chemical Engineering, Annamalai University (INDIA).

Corres.author: kavitha2124@yahoo.com

Abstract: The fermentation of endoglucanase was studied by mixed culture of *Trichoderma Viride* and *Aspergillus Niger* using agro waste as substrate. The effects of substrate concentration and inoculum size, cell mass and endoglucanase activity were studied in the batch experiment. Substrate utilization kinetics was also studied out. Differential equations for cell mass rate, product formation rate and substrate utilization rate as functions of initial cell concentration, cell concentration at any time and stationary cell concentrations were used to predict the kinetic parameters and the kinetic pattern obtained from the batch experiments were used to calculate the models. A Mathematical model was presented for the batch fermentation of endoglucanase from mixed culture using different concentration of rice bran and wheat bran. The objective of this research was to reduce the cost of endoglucanase production by optimization of fermentation parameters and modeling of the fermentation process.
Keywords; *Aspergillus Niger, Trichoderma Viride*, Kinetics, Endoglucanase, Fermentation.

INTRODUCTION

Lignocelluloses materials are an alternative feedstock as they are available in large quantities at a relatively low cost. Cellulosic material is the most abundant renewable carbon source in the world[1,2] . Microbial conversion of cellulose / ligno cellulosic biomass into useful products is a complex process involving combined action of three enzymes namely endoglucanase, exoglucanase and to β-glucosidase.[3]

Cellulases have a wide range of applications in various industries, such as textile, laundry, food and feed, pulp and paper, baking, alcohol from biomass, waste treatment, pharmaceutical, protoplast production, genetic engg and pollution treatment[4-7].

Cellulose may be hydrolyzed using enzymes to produce glucose for the production of ethanol.[8] Cellulose production is the most expensive step during ethanol production from cellulosic biomass accounting for approximately 40% of the total cost.

The optimization of fermentation conditions is an important problem in the development of economically feasible bioprocesses .Generally, economic restriction force industrial process to work in a very small range of operating conditions. For some kinetic model describing, the behavior of microbiological system can be a highly appreciated

tool and can be reduce tests to eliminate extreme possibilities .[9,10]

In the present studies, optimization of process conditions such as substrate concentration, temperature, P^H and fermentation time for the production of endoglucanase from agro residues using mixed culture and various kinetic models evaluated and the constants were predicted.

MATERIALS AND METHODS

MICRO ORGANISMS AND CULTURE MAINTENANCE

Aspergillus Niger MTCC 281 and *Trichoderma Viride* MTCC 167 strains obtained from the stock culture of Microbial Type Culture Collection, Chandigarh and maintained on 4% potato –dextrose- agar medium. The cultures maintained by regular transfer onto new slants and were stored at 4˚C.

SUBSTRATE

RB and WB dried at 65^{O}C overnight and used in the fermentation medium.[1] both those substrates collected from the respective industries.

INOCULUM PREPARATION

Fungal cultures grown on two different PDA slants and the spores harvested aseptically from 6 days old PDA slants. Sterile distilled water (2ml) was added to each fungal agar slants and shaken vigorously for preparing uniform suspension which was used as inoculums.

PRODUCTION MEDIUM

The production medium contained (g/l) KH_2PO_4 – 1.0, $CaCl_2$-0.1, $MgSO_4.7H_2O$–0.2, $(N H_4)_2 SO_4$ – 0.5, KCL-0.5,L-asperginie,yeast extract and 5g of RB and WB (different proportions) were mixed with the liquid medium in 1:3 ratio in 250 ml Erlenmeyer flasks and autoclaved at 121 ^{O}C for 20 min. The mixed substrate inoculated with 1 ml spores containing 10^7 spores /ml.[11]

ANALYTICAL TECHNIQUES

Fermentation was carried out on mixture of substrate hydrolyzed with trace elements. The endoglucanase activity, substrate concentration, pH, temperature and biomass were determined every 24 hours for 6 days. Culture supernatant was determined according to Mandels et al.[12].

A reactive mixture of 0.5 ml of 1% (W/V) carboxymethyl cellulose solution in 0.05 M citrate buffer (pH 4.8) and 0.5 ml of culture supernatant was incubated at 50 ^{O}C after incubation for 30 min the reducing sugars released were assayed by adding 3 ml of DNS reagent. Controls were prepared with 10 min boiled enzyme .[13]

One unit of endoglucanase activity expressed as the amount of enzyme required to release 1μ mol reducing sugars per ml under the above assay conditions by using glucose as standard curve.[14]

For cellulose evaluation, anthrone method was implemented 1ml of sample, 8ml of 0.1% anthrone solution and 4 ml of distilled water, being this solution incubated in boiling water for 15 min. The spectrophotometer measuring made against crude cellulose to 620 nm.[3,9]

For biomass estimation, 5ml portion of culture broth centrifuged for 20 minutes and the supernatant discharged. The resulting pellets were dried and dry weight was estimated.[8,15,16]

KINETIC MODEL

GROWTH KINETICS

The Monod equation relates the specific growth rate μ and substrate concentration described by equation.[17]

$$\mu = \frac{\mu_m s}{K_s + s} \qquad (1)$$

The Monod constant Ks and maximum growth rate μ_m were calculated from equation.[8,18,19] Under optimal growth conditions when the inhibitory effect of substrates and product play no role, the rate of cell growth kinetics is given by

$$\frac{dx}{dt} = \mu_o x \qquad (2)$$

Where μ_0 is a constant defined as the initial specific growth rate equation implies that X increases with time regardless of substrate availability. In reality the growth of cell governed by a hyperbolic relationship and logistic equation given by

$$\frac{dx}{dt} = \mu_o \left[1 - \frac{x}{X_m}\right] x \qquad (3)$$

The logistic equation utilized to describe the kinetics of several polysaccharides fermentation systems.[19,20] The integrated form of eqn (1) using $X=X_0$ at t = 0 gives a value of X as a function of time t.

$$X = \frac{X_o e^{\mu_n t}}{1 - \left(\frac{X_o}{X_m}\right)(1 - e^{\mu_n t})} \qquad (4)$$

Rearrangement of eqn (4) gives eqn (5)

$$\ln \frac{x}{x_m - x} = \mu_m t - \ln\left(\frac{x_m}{x_o} - 1\right) \quad (5)$$

The graph was plotted between ln [X/X_m-X] Vs time t. From the graph, the intercept ln (X_m/X_0–1) and slope found out.[18]

Product formation:

Product formation kinetics described by Leudeking – Piret model.[21] in this work, model used to predict the enzyme concentration during the time course of fermentation. The Leudeking Piret model combines both growths associated and non-growth associated contributions. According to this model, the product formation rate depends upon both the instantaneous biomass concentration, X and growth rate, dx/dt in a linear manner.

Where α and β were growth and non-growth associated constants that may vary with fermentation conditions.

$$\beta = (dp/dt)\, st / X_m \quad (6)$$

The non-growth associated constant β calculated from eqn (7) the experimentally obtained cell growth curve extended to the stationary phase and the concentration of the cell mass at the stationary phase obtained. This was the asymptotic value of the cell mass concentration, the slope of the product concentration curve at stationary phase found out. This value divided by the cell mass concentration at the stationary phase.

In order to express P as a function of time eqn (6) rearranged.

$$dp = \alpha\ dx + \beta\, x dt \quad (7)$$

However, X is a function of t. This is given by eqn (11)

$$dp = \alpha\ dx + \beta \int x(t)\, dt \quad (8)$$

$$x(t) = \frac{x_o e^{\mu_m t}}{1 - \left(\frac{x_o}{x_m}\right)(1 - e^{\mu_m t})} \quad (9)$$

Insert the above expression.

$$dp = \alpha dx + \frac{\beta\, x_o e^{\mu_m t}}{1 - \left(\frac{x_o}{x_m}\right)(1 - e^{\mu_m t})}\, dt \quad (10)$$

Integrating eqn (11), using logistic eqn for x (t) with P=0 at t=0 condition for form a product formation rate.

$$p = \alpha x_o \left[\frac{e^{\mu_m t}}{1 - \left(\frac{x_o}{x_m}\right)(1 - e^{\mu_m t})} - 1\right] + \beta \frac{x_o}{\mu_m} \ln 1 - \left(\frac{x_o}{x_m}\right)(1 - e^{\mu_m t}) \quad (11)$$

Equation (12) written in the following form,

$$P = A(t) + \beta B(t) \quad (12)$$

Where

$$A(t) = X_o \left[\frac{e^{\mu_m t}}{1 - \left(\frac{x_o}{x_m}\right)(1 - e^{\mu_m t})} - 1\right] \quad (13)$$

$$B(t) = \frac{x_o}{\mu_m} \ln \left(1 - \frac{x_o}{x_m}\right)(1 - e^{\mu_m t}) \quad (14)$$

The graph was plotted between [P] Vs A (t) gives the constant α.[22]

Substrate utilization kinetics:

A part of the substrate used for conversion of cell mass, a part used for product formation and another part for maintenance therefore,

$$\frac{ds}{dt} = \frac{1}{y_{x/s}} \frac{dx}{dt} + \frac{1}{y_{p/s}} dp/dt + k_e x \quad (15)$$

Sub eqn (6) in eqn (14)

$$\frac{-ds}{dt} = \frac{1}{y_{x/s}} \frac{dx}{dt} + \frac{1}{y_{p/s}} \left(x \frac{-dx}{dt} + \beta x\right) k_e x \quad (16)$$

Simplifying,

$$\frac{-ds}{dt} = \gamma \frac{dx}{dt} + \eta x \quad (17)$$

Where,

$$\gamma = \frac{\alpha}{y_{p/s}} + \frac{1}{y_{x/s}} \quad (18)$$

$$\eta = \frac{\beta}{y_{p/s}} + k_e \quad (19)$$

Here,

$$y_{x/s} = \frac{x - x_0}{s - s_0} \quad (20)$$

$$y_{p/s} = \frac{P - P_0}{s_0 - s} \quad (21)$$

Substituting Eqn (4) into eqn (15) and integrating yields the following equation,

$$S = S_o - \gamma X_o \left[\frac{e^{\mu_m t}}{1 - \left(\frac{X_o}{X_m}\right)(1 - e^{\mu_m t})} - 1 \right] - \eta \frac{X_m}{\mu_m} \left[\ln 1 - \left(\frac{X_o}{X_m}\right)(1 - e^{\mu_m t}) \right] \quad (22)$$

Where the last term on the right hand side signifies the S may continue to diminish due to product formation and maintenance even after X reaches X_m.[18]

RESULT AND DISCUSSION

Examination of experimental data obtained from batch culture showed that the logistic model was applicable for this particular system. Fermentation of endoglucanase by mixed culture in batch fermentation showed a classical growth trend.

The logistic model makes possible generalized batch kinetics of mixed culture growth during the fermentation of endoglucanase. For product formation and substrate utilization kinetics, used to stimulate a wide range of substrate concentrations. The model parameters were first evaluated by solving the eqn (5), (7) and (13).These values were then, used to calculate the kinetic parameters.

Product formation parameters calculated from eqn (8).The graph was plotted between [P] vs. A (t). The growth associated product formation constant α was found out from slope and find out the kinetics parameters β, X_m and μ_m from eqn (7) and (5). The values used to calculate product formation rate.

At any instant, from the experimental values of cell mass concentration X, product concentration P and substrate concentration S, the yield coefficient η and γ estimated from equation (12) and (13). From eqn (9) μ_m replaced by k, the graph plotted between $\ln (x/x_m) / (1-x/x_m)$ vs. time t, the constant k found out from the slope.

The models then used to predict the cell mass concentration, endoglucanase production and substrate concentration respectively. The kinetic data showed also that substrate consumption was due to both growth and non-growth metabolic activities. The predicted values were compared to experimental values .Variations in the parameter values were determined in each case and relationship were developed for the prediction of those parameter values. The estimated parameters values in Table (1).[15,21-23]

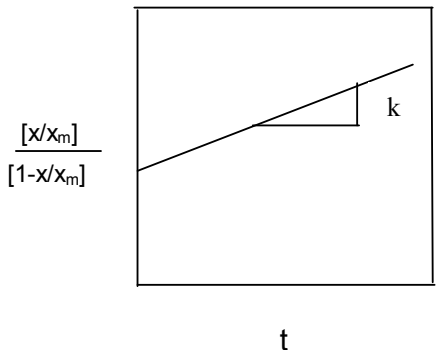

Fig. 1 Evaluation of k using eqn (9)

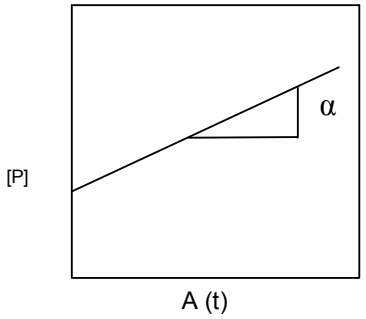

Fig. 2 Evaluation of α using eqn (8):

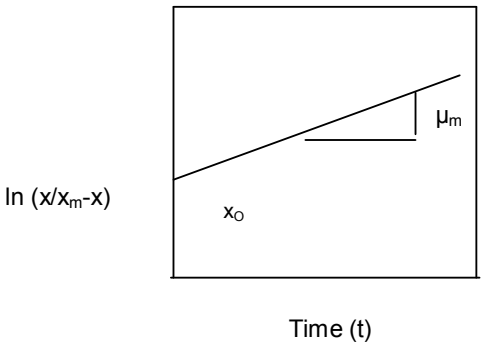

Fig. 3 Determination of Xo and μ_m using eqn (5)

Table 1.Values of the Kinetic Parameters

Parameters	Value of Kinetic Parameters
X_m	6.7
X_0	3.52
μ_m	0.02
α	0.13
β	0.08
η	0.04
γ	3.07
k	0.03

CONCLUSION

Fermentative production of endoglucanase using mixed culture of *Trichoderma Viride* and *Aspergillus Niger* was investigated. The logistic model used for describing biomass formation, the Leudeking Piret model used for describing endoglucanase production and the modified Leudeking Piret model used for describing substrate consumption. The logistic model was found to be a suitable model in representing the batch kinetics of endoglucanase using mixed culture. The fitting of the model proposed to the experimental data shows a high accuracy of the model for predicting the endoglucanase production.

ACKNOWLEDGMENT

The authors express their sincere thanks to University Grants Commission (UGC) New Delhi for the financial support and Department of Chemical engineering, Annamalai University for providing the necessary facilities for this research work.

REFERENCES

1. Benkun Qi, Risheng Yaoa, Ying Yua, Yuan Chena. (2007): Influence of Different Rations of rice straw to wheat bran on production of cellulolytic enzymes by Trichoderma Viride in solid-state fermentation. EJEAF Che. 6(9) [2341 – 2349]
2. Chellapandi P. Jani. H.M (2008): Production Endoglucanase by the native strains of Strptomyces isolates in submerged fermentation. Bra. J. Microbial 39:122-127
3. Kavitha.S, Nagarajan.P (2011).Production of endoglucanase by Aspergillus Niger using agro residue, Int.J.Che.Eng.Research Vol (3), pp.89-96.
4. Howard, R.L., Abotsi, E., Jancen, E.L. and Howard. (2003): Lignocellulosic Biotechnology: Issue of Bioconversion and Enzyme production. Afr .J Biotechnol.2 (12):602-619.
5. Acharya, P.B., D.K. Acharya, and H.A. Modi, (2008): Optimization for cellulase production by Aspergillus Niger using saw dust as substrate. Afr. J. Biotechnol., 7: 4147-4152
6. Benkun Qi, Risheng Yaoa, Ying Yua, Yuan Chena. (2007): Influence of Different Rations of rice straw to wheat bran on production of cellulolytic enzymes by Trichoderma Viride in solid-state fermentation. EJEAF Che. 6(9) [2341 – 2349
7. Bhat.M.K.,Bhat.S,1997.cellulose degrading enzymes ad their potential industrial applications.biotechnol.Adv.,15:583-620
8. Muthuvelayudham. R and Viruthagiri, (2006): Fermentative production and kinetics of cellulose protein on Trichoderma reesei using sugarcane bagasse and rice straw. African Journal of Biotechnology Vol. 5 (20), pp. 1873-1881.
9. Muthuvelayudham R, Viruthagiri T (2007). Optimization and modeling of cellulose protein from Trichoderma reesei Rut C30 using mixed substrate. Afr. J. Biotechnol. Vol. 6(1): 041-046.
10. Wong.K.K.Y.and J.N. Saddler, (1993): Applications of hemicellulase in the food feed, pulp and paper insustries.In: Coughlan MP, Hazelwood GP, editors.Hemicellulose and hemicellulase. London: Portland press, 127-143
11. Muhammad Mushin Javed, Ikram – Ul – Haq, Tehmin Saleem Khan and Zafar Siddiq (2005). Cotton Saccharifying activity of cellulases produced by co-culture of *Aspergillus Niger* and *Trichoderma Viride*. Research. J. of Agri. and Bio.Sci. 1(3): 241-245.
12. Mandels. M (1985): Applications of cellulose.Bio chem... soc Trans B;414-415
13. Miller GL (1959): Use of dinitrosalicyclic acid reagent for determination of reducing sugar. Analytical hemistry 31: 426-428
14. Ghose. T. K. (1987): Measurement of cellulase activities. Pure & Applied Chemistry 59: 257–268
15. Kavitha.S, Nagarajan.P (2011). Fermentative production of endoglucanase- Kinetics and Modeling, Int.J.Eng.Sci.Technol. Vol (3), pp.1889 - 1893.

16. Kavitha.S, Nagarajan.P (2010). Fermentative production of Endoglucanase CHEMCON 2010, Ind.Chem. Engg. Congress. 310-311.

17. Monod .J (1949). The growth of bacterial cultures. Annu Rev Microbial. 3:371-394.

18. Ramanathan. M, Rathinasabhabathy.C.S, Dhanasegar. R, Viruthagiri.T (2007).Batch kinetics and modeling of glutathione synthesis from Candida Utilis utilizing glucose. Indian chemical Engineer. vol(49). pp.21-28

19. Dhanasekar R, Viruthagiri T, Sabarathinam P.L. (2002). Poly (3-hydroxybutyrate) synthesis from mutant strain Azobacter Vinelandi utilizing glucose in a batch reactor. Biochem. Engg. J. 10: 1-8.

20. Mohamad.F, Tayeb.O.E, Aboulwafa.M (2007). Optimization of the industrial production of bacterial α amylase in Egypt Analysis of kinetic data for enzyme production by two strains of bacillus amyloliquefaciens. Afr.J.Biotechnol. Vol.7(24);4537- 4543

21. Leudeking R, Piret EL (1959). A kinetic study of the lactic acid fermentation. J Biochem Microbial Technol Eng. 1: 393-412

22. Shanmugaprakash.M. (2010) A Simple unstructured mathematical model for ethanol fermentation by mutant neurospora crassa. Int. J.che.eng. research.vol. (2):pp.13-21.

23. Bharathiraja.B and Jayamuthu nagai. J, (2008): Production and kinetics of cellulose enzyme from sawdust hydrolyzed using Trichoderma reesei 9926a.Aadvanced biotech, 32.

*****