Biochemical Engineering Journal 77 (2013) 49–57

Contents lists available at SciVerse ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Kinetic modeling and implementation of superior process strategies for β-galactosidase production during submerged fermentation in a stirred tank bioreactor

Huzaifa S. Choonia*, S.S. Lele

Food Engineering and Technology Department, Institute of Chemical Technology (Deemed University), Matunga, Mumbai 400 019, India

ARTICLE INFO

Article history:
Received 22 October 2012
Received in revised form 24 April 2013
Accepted 26 April 2013
Available online 3 May 2013

Keywords:
Lactobacillus acidophilus
β-galactosidase
Stirred tank bioreactor
Submerged fermentation
Fed-batch fermentation
Semi-continuous fermentation

ABSTRACT

This paper reports development and implementation of superior fermentation strategies for β-galactosidase production by *Lactobacillus acidophilus* in a stirred-tank bioreactor. Process parameters (aeration and agitation) were optimized for the process by application of Central Composite Design. Aeration rate of 0.5 vvm and agitation speed of 250 rpm were most suitable for β-galactosidase production (2001.2 U/L). Further improvement of the operation in pH controlled environment resulted in 2135 U/L of β-galactosidase with productivity of 142.39 U/L h. Kinetic modeling for biomass and enzyme production and substrate utilization were carried out at the aforementioned pH controlled conditions. The logistic regression model (X_0 = 0.01 g/L; X_{max} = 2.948 g/L; μ_{max} = 0.59/h; R^2 = 0.97) was used for mathematical interpretation of biomass production. Mercier's model proved to be better than Luedeking–Piret model in describing β-galactosidase production (P_0 = 0.7942 U/L; P_{max} = 2169.3 U/L; P_r = 0.696/h; R^2 = 0.99) whereas the latter was more efficient in mathematical illustration of lactose utilization (m = 0.187 g/g h; $Y_{x/s}$ = 0.301 g/L; R^2 = 0.98) among the two used in this study. Strategies like fed-batch fermentation (3694.6 U/L) and semi-continuous fermentation (5551.9 U/L) further enhanced β-galactosidase production by 1.8 and 2.8 fold respectively.

1. Introduction

Digestion of lactose requires its hydrolysis into its component saccharides (glucose and galactose) by the action of intestinal β-galactosidase (β-D-galactoside galactohydrolyse; E.C. 3.2.1.23) [1]. However, absence of this enzyme gives rise to a condition called 'lactose-intolerance' [2]. Since this problem is prevalent in more than half of world's population, enzymatic hydrolysis of lactose using β-galactosidase is one of the most important biotechnological processes in the food industry [3].

Although β-galactosidase can be produced from a variety of sources such as bacteria, yeast and mold [4], they are commercially produced by GRAS (Generally Recognized as Safe by the FDA) microorganisms (e.g., yeast of the genus *Kluyveromyces* and fungus of the genus *Aspergillus*). *Lactobacillus acidophilus*, a GRAS organism, isolated by our group from fermented *Eleusine coracana* was found to be a potential source of this enzyme [5,6].

Production requires development of efficient fermentative medium that ensures high enzyme production. Once a medium is established, it is necessary to carry the process from laboratory set-up to a pilot plant and eventually for industrial or commercial processing. However, microbial processes are inherently complex, and it is critical to understand, control, and optimize them [7]. Kinetic modeling of a fermentation process is regarded as an indispensable step on account of their ability to provide useful information for the analysis, design and optimal operation conditions for the production of a target biomolecule in bioreactor [8–10].

Different types of models, classified as structured and unstructured models, have been used for kinetic modeling. Although structured models can explain complex microbial systems at the molecular levels, relatively simpler unstructured kinetic models have been used more widely for practical applications [11,12]. Several unstructured kinetic models, like, Monod model for biomass

Abbreviations: K_La, volumetric mass transfer coefficient; C_L, concentration of dissolved oxygen in fermentation broth (mmol/L); C^*, saturated oxygen concentration (mmol/L); K_L, oxygen mass transfer coefficient (cm/h); a, gas/liquid interface area per liquid volume (cm^2/mL); X_0, biomass at time t = 0 (g/L); X, biomass at time t (g/L); X_{max}, maximum biomass (g/L); μ_{max}, maximum specific growth rate (/h); t, time (h); P_0, initial product concentration (U/L); P, β-galactosidase activity (U/L); P_{max}, maximum concentration of β-galactosidase (U/L); P_r, ratio between the initial volumetric rate of product formation (r_p) and P_0; α, growth associated product formation coefficient (g cells/h); β, non-growth associated product formation coefficient [(units/(g h)]; S_0, initial concentration of substrate (g/L); S, final concentration of substrate (g/L); $Y_{P/S}$, product yield for enzyme per substrate consumption (U/g); $Y_{X/S}$, product yield for biomass per substrate consumption (g/g).

* Corresponding author. Tel.: +91 022 33611111; fax: +91 022 24145614.
E-mail address: huzichoonia@gmail.com (H.S. Choonia).

1369-703X/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bej.2013.04.021

production and Luedeking–Piret (LP) model for product formation and substrate utilization, have been proposed to describe the fermentation of glucose to organic and amino acids such as citric, lactic, and glutamic acids [13,14]. However, very few reports are available on kinetic modeling and scale-up studies of β-galactosidase production by *Lactobacillus* species.

Thus, the main objective of the present work was to investigate the effect of different factors such as aeration rate, agitation speed, pH control and DO control on β-galactosidase production and productivity during scale up of the fermentation process in a 5 L bioreactor using our indigenously isolated *L. acidophilus*. Kinetic modeling for biomass production, β-galactosidase production and lactose consumption for the optimized experimental run was developed using unstructured models. Strategies like fed-batch and semi-continuous modes of fermentation were also implemented to achieve our goal of maximizing β-galactosidase production.

2. Materials and methods

2.1. Production medium for β-galactosidase

Production of β-galactosidase by indigenously isolated *L. acidophilus* was carried out by growing them in the following optimized medium (not reported): lactose (10 g/L), yeast extract (20 g/L), mycological peptone (10 g/L), magnesium sulphate (50 mg/L) and manganese sulphate (25 mg/L) purchased from Himedia Labs, Mumbai, India and tri-ammonium citrate (4 g/L), potassium acetate (2.5 g/L) and dipotassium hydrogen phosphate (4 g/L) purchased from S. D. Fine Chemicals, Mumbai, India. pH of the medium was adjusted to 6.5 before sterilization and inoculated (3% v/v) by using the cell pellet suspension in saline having an absorbance of 1.0 at 660 nm from 18 h seed grown in the same medium.

2.2. Bioreactor studies

Bioreactor studies for β-galactosidase production were carried out in a 5 L Sartorius BioStat B5 bioreactor. Sterile fermentation medium (2 L) was inoculated through the inoculation port with the help of one of the four peristaltic pumps of the bioreactor. Bioreactor temperature was controlled by a thermocouple that maintained the temperature throughout the runs. The medium pH was recorded automatically using a sterile pH electrode (Mettler-Toledo). Foam formation, if any, was controlled by a level electrode and using sterile silicon oil as the anti-foam agent. Dissolved oxygen (DO) was continuously monitored and controlled using the DO probe (Hamilton). The pH and DO probes were calibrated in each run, once the medium temperature was maintained at 25 °C, but before inoculating the medium.

Three different strategies that were implemented for maximizing enzyme production include Batch fermentation that included effect of aeration and agitation, Fed-Batch (variable volume) mode of operation and Semi-continuous mode of operation.

Table 1a
CCD design of experiments for β-galactosidase production, its productivity and K_La.

Run	Agitation (rpm)	Aeration (vvm)	Total activity (U/L)	Productivity (U/L h)	K_La (/h)
A	250 (−1)[a]	0 (−1)	1208.3 ± 2.9	50.34	–
B	450 (+1)	0 (−1)	1760.2 ± 1.8	117.35	–
C	250 (−1)	0.5 (+1)	2001.2 ± 3.7	133.41	12.48
D	450 (+1)	0.5 (+1)	1820.6 ± 4.2	121.38	48.12
E	350 (0)	0.2 (0)	1642.0 ± 1.9	102.63	12.36

[a] Number in bracket depicts the coded value of the independent variables.

2.2.1. Batch fermentation for β-galactosidase

Effect of aeration and agitation on production and productivity of β-galactosidase was investigated by applying a Centered Composite Design (CCD) using Design Expert Software Version 6.0.10 trial version (State Ease, Minneapolis, MN). The experimental design for the two independent variables, aeration rate and agitation speed, comprised of 5 experimental runs at three levels with 4 factorial points (2^2) and one center point. This design of experiments is depicted in Table 1a. The optimized process with respect to aeration and agitation was used for pH controlled and DO controlled operations, the details of which are shown in Table 1b.

Sampling to estimate enzyme production, biomass production [optical density (O.D) at 660 nm wave-length and dry cell weight (DCW)] and substrate (lactose) utilization was done through the sampling port every 3 h during the course of fermentation up to the 15th hour after which samples were drawn at 2 h intervals. The fermentation broth from the bioreactor was harvested once microorganism entered the stationary growth phase in terms of the optical density.

Volumetric mass transfer coefficient (K_La) was calculated for all the runs with varying aeration and agitation specifications. K_La was calculated using the static gassing out technique in presence of the same concentration of cells that were present during that fermentation batch, but by using dead cells in place of viable ones [15].

The bioreactor condition that resulted in maximum enzyme production, in this case the pH controlled run, was modeled for biomass production using logistic equation and β-galactosidase production and lactose utilization using two models, namely, Luedeking–Piret model and Mercier's model. Experimental data were fitted to proposed models using Solver of Microsoft Excel 2007 by nonlinear regression using the least-squares method.

2.2.2. Fed-batch fermentation for β-galactosidase production

Optimized fermentation conditions with respect to aeration and agitation obtained during batch studies were used in variable volume fed-batch fermentation for enhancing β-galactosidase production. Fed-batch fermentation is nothing but batch cultures that are fed continuously or sequentially with medium without the removal of fermentation broth. Depending on the volume of fresh-feed added, it is categorized as variable-volume and fixed-volume fed batch fermentation [16]. Fermentation was initiated with 2 L media. Once the organism entered the stationary growth phase (measured in terms of optical density), corresponding to 15 h from the time of inoculation, first feed of sterile concentrated media

Table 1b
Effect of pH stat and DO stat fermentation on β-galactosidase production and its productivity.

Run	Agitation (rpm)	Aeration (vvm)	% DO	pH	Total activity (U/L)	Productivity (U/L h)
C	250	0.5	Uncontrolled	Uncontrolled	2001.2 ± 3.7	133.41
F	250	0.5	Uncontrolled	Controlled	2135.8 ± 1.6	142.39
G	250	NA	5%	Controlled	2076.1 ± 4.2	115.34