Biochemical Engineering Journal 53 (2010) 131–136

Contents lists available at ScienceDirect

Biochemical Engineering Journal

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

Batch and fed-batch modeling of carotenoids production by *Xanthophyllomyces dendrorhous* using *Yucca fillifera* date juice as substrate

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ARTICLE INFO

Article history:
Received 4 March 2010
Received in revised form
14 September 2010
Accepted 4 October 2010

Key words:
Astaxanthin
Xanthophyllomyces dendrorhous
Mathematical model
Batch
Fed-batch
Carotenoids

ABSTRACT

Two reliable mathematical models for batch and fed-batch cultures were developed to describe the microbial growth and the carotenoids production of the yeast strain 25-2 of *Xanthophyllomyces dendrorhous* (formerly *Phaffia rhodozyma*). The carbon source was date juice obtained from the *Yucca fillifera*. In order to determine the best batch model two different specific growth rate combinations were evaluated, one for substrate saturation with product inhibition and other for substrate and product inhibition. The best specific growth resulted in the combination for terms related to both substrate and product inhibition. The batch culture model predicts biomass and carotenoids production, as well as substrate consumption at sugar concentrations ranging from 5, 10, 20 and 40 g l^{-1}, with mean values for the coefficient of determination of 0.9836, 0.9775, 0.9828 and 0.9651, respectively. The substrate concentration for 40 g l^{-1} was the weakest prediction for all the experimental data set; therefore a correction term was added to improve prediction from the mathematical model. The batch model was then extended to fed-batch culture with an inlet substrate concentration of 65 g l^{-1} where the mean value of 0.9868 for the coefficient of determination was obtained.

1. Introduction

The astaxanthin (3,3′-dihydroxi-β,β-carotene-4,4′-dione) is widely distributed in nature and is the main pigment in crustaceans and salmonids, as well as various birds including certain flamingoes, the scarlet ibis and many other microorganisms [1]. Thus, there is a growing interest in the use of astaxanthin as a pigment for the aquaculture and poultry industries because of these animals cannot synthesize astaxanthin and consequently it is desirable to be included in the feed to obtain a color appealing to consumers [2]. Moreover, astaxanthin is a vitamin A precursor and has antioxidant activity preventing some forms of cancer [3,4]. In economical terms astaxanthin is important because its market value is greater than US$ 100 million per year [5]. Although the pigment astaxanthin can be produced by synthetic chemical technology, some of the by-products resulting from such chemical processes may have undesirable side effects when consumed. It is for this reason the production of carotenoids from microbial sources has been a focus of extensive research [6].

The yeast *Xanthophyllomyces dendrorhous* (formerly *Phaffia rhodozyma*) synthesizes astaxanthin as its main carotenoid (85% of the total carotenoids content) [7]. Several substrate carbon sources have been used to produce carotenoids by *X. dendrorhous* cultures including glycerol [8], sugar cane juice [9], cellobiose, glucose and sorbitol [10,11], among others [12,13]. Another source for producing carotenoids is juice date from the *Yucca fillifera* [14]. The production of carotenoid by the yeast *P. rhodozyma* in batch cultures is affected by high substrate concentrations [15]. Therefore, diverse studies have reported on the production of carotenoids in fed-batch cultures [16–18].

Better methods for measurement, monitoring and modeling have become necessary to accomplish the scientific and industrial development of modern biotechnology. A more detailed knowledge about the biosystems will allow us a better phenomena understanding and operational control [19]. Therefore the optimization and control of bioprocesses can be performed from mathematical models describing the kinetics of microbial growth, substrate uptake and product formation [20]. In this context, few kinetics models have been reported for *X. dendrorhous*. A model based

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doi:10.1016/j.bej.2010.10.004

Nomenclature

D	dilution rate (h^{-1})
F	flow rate ($l\,h^{-1}$)
K_i	glucose inhibition constant ($g\,l^{-1}$)
K_p	carotenoids inhibition constant ($\mu g\,l^{-1}$)
K_s	glucose saturation constant ($g\,l^{-1}$)
m	number of variables
m_s	maintenance cellular coefficient ($g\,g^{-1}\,h^{-1}$)
n	total number of experimental data points
P	carotenoid concentration ($\mu g\,l^{-1}$)
R^2	coefficient of determination
S	substrate concentration ($g\,l^{-1}$)
$S(0)$	initial substrate concentration ($g\,l^{-1}$)
S_f	inlet substrate concentration ($g\,l^{-1}$)
V	volume (l)
X	cell concentration ($g\,l^{-1}$)
W_{ij}	weight of each variable
y_i	experimental data points
$\bar{y}$	mean value for each variable
$Y_{x/s}$	biomass yield coefficient on glucose ($g\,g^{-1}$)
$Y40_{x/s}$	correction parameter for the biomass yield coefficient on glucose ($g\,g^{-1}$)

Greek letters

α	growth-associated product formation coefficient ($\mu g\,g^{-1}$)
β	non-growth-associated product formation coefficient ($\mu g\,g^{-1}\,h^{-1}$)
γ	exponent term for the product inhibition model
Δ_{ij}	difference between the model and the experimental value
μ	specific growth rate (h^{-1})
μ_{max}	maximum specific growth rate (h^{-1})

on mass balance approach and Monod growth kinetics was used to describe a fed-batch culture enabling the calculation of a feed regime to obtain the maximum yield of cells and pigment [21]. Other mathematical models were used to calculate kinetic parameters for the growth of the yeast and the biomass productivity [22]. The growth on glucose and overflow metabolism in batch and fed-batch cultures for astaxanthin production was reported in [23]. Reliability on this biotechnological system is relevant and open issue; particularly for high substrate concentration.

The aim of this manuscript is to report a suitable and reliable model to describe the kinetics of carotenoids production by the strain 25-2 of *X. dendrorhous* in batch culture. Initially, the study is done about batch culture and the results were extended to a fed-batch culture. The carbon source was date juice obtained from the *Y. fillifera*. Thus, the main contribution of this manuscript is to provide detailed information on model kinetics that can be used in developing future control and operation schemes for the mutant strain 25-2 of *X. dendrorhous* when growth on date juice.

2. Materials and methods

2.1. Microorganism

The 25-2 astaxanthin-overproducing mutant was derived from the wild type *X. dendrorhous* ATCC 24202 [24]. The strain was stored at 4 °C and subcultured every month for maintenance. It was conserved in our laboratory collection.

2.2. Culture media

Dates of the *Y. fillifera* were collected from the state of Zacatecas, México. The juice was extracted by dissolution for 1 h at 60 °C in a stirred tank. The juice was separated from the pulp by successive filtrations and the resulting solution was centrifuged at $5000 \times g$ to remove suspended solids. Finally, the juice was clarified using H_3PO_4 and CaO as described in [25].

2.3. Date juice sugar composition

The date juice was analyzed by HPLC to determine its sugar composition, finding fructose 60%, glucose 39.5% and other sugars 0.5%.

2.4. Inoculums conditions

Loopfuls of the mutant strain of *X. dendrorhous* 25-2 were used to inoculate 10 ml of YM broth Difco (1% glucose, 0.5% Bacto-peptone, 0.3% malt extract and 0.3% yeast extract). The pre-culture broth was inoculated into a 500 ml Erlenmeyer flask containing 100 ml of YM broth and incubated for 24 h on a rotary shaker at 250 rpm.

2.5. Bioreactor fermentations

The fermentation experiments were carried out in a 3 l Applikon bioreactor. The bioreactor was sterilized at 121 °C for 15 min. The following physical variables were controlled: pH, 6; temperature, 20 °C; agitation speed, 900 rpm; and the dissolved oxygen concentration, 75%. Samples were taken every 6 h. The Bioexpert™ software was used for data acquisition from an Applikon biocontroller ADI 1035. Batch fermentations were carried out in duplicate with a working volume of 2 l. Four different initial substrate concentrations were used, 5, 10, 20, and 40 $g\,l^{-1}$, of date juice media. Fed-batch fermentation was carried out in a 3 l Applikon bioreactor with an initial volume of 1.2 l and a substrate concentration of 15 $g\,l^{-1}$ of date juice media. A liquid reservoir containing 65 $g\,l^{-1}$ of date juice media was used to feed the fermentor at a flow rate of 0.00021 $l\,h^{-1}$.

2.6. Analytical methods

Cell growth was measured in terms of turbidity at 600 nm. Biomass concentration was measured as dry cell mass. The reducing sugar concentration in the medium was determined using 3,5-dinitrosalicylic acid reagent [26]. The carotenoids concentrations were determined by the DMSO method [27].

3. Kinetic model

The following assumptions were made to build the mathematical model:

1. Although the date juice is composed mainly by fructose and glucose the sugar concentrations in the media were treated as a single substrate.
2. There is no oxygen limitation in the culture.
3. There is no limitation by nitrogen.
4. Ethanol and glycerol production are negligible.

3.1. Batch model

A differential mass balance to describe the biomass is given by:

$$\frac{dX}{dt} = \mu X \quad (1)$$