Biochemical Engineering Journal 79 (2013) 57–70

Contents lists available at ScienceDirect

Biochemical Engineering Journal

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

Five-step continuous production of PHB analyzed by elementary flux, modes, yield space analysis and high structured metabolic model

Markan Lopar[a], Ivna Vrana Špoljarić[a], Aid Atlić[b], Martin Koller[b], Gerhart Braunegg[b], Predrag Horvat[a,*]

[a] Department of Biochemical Engineering, Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6/IV, HR-10000 Zagreb, Croatia
[b] Institute of Biotechnology and Biochemical Engineering, Graz University of Technology, Petersgasse 12, A-8010 Graz, Austria

ARTICLE INFO

Article history:
Received 25 February 2013
Received in revised form 5 July 2013
Accepted 8 July 2013
Available online 17 July 2013

Keywords:
Cupriavidus necator
Elementary modes
High-structured mathematical model
Metabolic fluxes
poly(3-Hydroxybutyrate)
Yield space analysis

ABSTRACT

A high structured metabolic model for PHB synthesis by *Cupriavidus necator* DSM 545 consisting of 43 mass balance equations related to the same number of intracellular compounds was established. The metabolic state of cells cultivated in a continuously operated five stage bioreactor cascade was analyzed by help of elementary flux modes and two-dimensional yield space. Two different C-source feeding strategies were performed. Concerning PHB and biomass yields, values of the more efficient strategy were used as the data source for elementary modes and metabolic flux calculations, respectively. Metabolic fluxes were calculated from experimental yield data using a combination of elementary modes by applying the quadratic programming approach, in which the sum of squared weighting factors was minimized. Two different metabolic situations concerning activity of glucose-6-phosphate isomerase were tested. The high structured metabolic model was validated by comparison of experimental data from 24 h batch cultivation and simulated results.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Poly(hydroxyalkanoates) (PHAs) are biodegradable intracellular polyesters synthesized by various eubacterial genera and several archaea [1–3] with a variety of important functions in various ecosystems [4], mainly serving as an intracellular energy- and C source reservoir [5]. The homopolyester poly([*R*]-3-hydroxybutyrate) (PHB) is the best characterized and most intensively investigated type among a great number of known different PHAs. By methods of industrial biotechnology (recently known as "white biotechnology") they can be produced from renewable resources of first and second generation (lignocelluloses wastes, grains, beet and cane sugar, whey, biodiesel and biodiesel waste, i.e. glycerol and waste lipids) [6–8]. These polyesters can be separated from the surrounding microbial cells and, after appropriate processing, they can serve as biodegradable substitutes for petrochemical plastics such as poly(ethylene) (PE) and poly(propylene) (PP) [9–12].

Considering their production costs and some material properties, PHAs are still inferior to petro-chemically originated plastics [13–15], but recent oil price fluctuation lead to increased attention for these biopolymers. Continuous production based on renewable resources is a promising tool for the reduction of production costs and for improving sustainability aspects in PHAs life cycle.

The first experiments dealing with continuous production of PHAs performed in a one stage chemostat system [16,17] were followed by experiments of Koyama and Doi [18] as well as by Yu et al. [19]. In these experiments, the applied dilution rate was found as the most important factor influencing number-average molecular mass (M_n) of PHAs (decisive for the processing properties). Three different types of microbial producers were reported in the past concerning PHA production kinetics:

(i) Strains that express strict separation between growth phase and PHA production phase under N or P limitation (i.e. *Pseudomonas* sp. 2F, *Methylomonas extorquens* [20]).
(ii) Strains that accumulate PHA to a certain extent under balanced nutritional conditions and amplified PHA accumulation in non-growth phase caused by N or P limitation (i.e. *Cupriavidus necator*).
(iii) Strains that feature high formation rates for PHA even without any limitation of an essential growth component (i.e. *Azahydromonas lata* DSM 1122 [2,21], *Pseudomonas putida* GPo1 ATTC 29347 [2,22,23].

For the microorganisms mentioned under (i) and (ii) it was reasonable to apply two stage continuous cultivation where the first

* Corresponding author. Tel.: +385 1 46 05 166; fax: +385 1 48 36 424.
E-mail addresses: mlopar@pbf.hr (M. Lopar), ivrana@pbf.hr (I. Vrana Špoljarić), aid.atlic1@gmail.com (A. Atlić), martin.koller@tugraz.at (M. Koller), g.braunegg@tugraz.at (G. Braunegg), phorvat@pbf.hr (P. Horvat).

1369-703X/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bej.2013.07.003

stage was intended for extensive biomass growth and the second stage for non growth-associated PHA synthesis as well as for achieving of appropriate molecular mass [24–28].

Chemostats have been used in the past also for special purposes i.e. investigation of C/N ratio as regulating factor of PHA synthesis [29–33].

Recently Atlić et al. [34] have tested a five stage continuous process constructed to achieve different but tailored process conditions in each step of the cascade, regarding types and concentrations of substrates and co-substrates as well as parameter values like temperature, pH-value and dissolved oxygen. This "cascade" strategy was chosen in order to act as a suitable tool for designing novel biopolymers for special applications, e.g. block-polymers [35] with alternating "soft" and "hard" segments or polymers with desired molecular mass and controlled polydispersity index.

The five-stage system was intended to provide a complete nutrient supply in the first reactor (balanced biomass growth), furthermore, the subsequent, second vessel was intended to complete the consumption of nitrogen source (growth-associated PHA synthesis), the aim of the last three reactors was to force the cells to non-growth associated synthesis of PHA under nitrogen-free, but C-source reach conditions. In addition, it was expected from the mentioned five stage system to provide information about cells tolerance to long term N-limitation (concerning cell damages and loss of the PHA synthesis). This way, from the bioprocess engineering point of view, each of the three different metabolic states of the production strain *C. necator* was reflected by different stage of the technological reactor system.

Furthermore, the just mentioned five stage bioreactor system was optimized by Horvat et al. using low structured, formal-kinetic mathematical model [36]. However, the applied low structured kinetic model was not able to predict the transient states between three different metabolic states, and, therefore, its achievements were limited. It is possible to solve such problems by applying high structured mathematical models of metabolic networks. In this work the reconstruction of metabolic network for *C. necator* DSM 545 was based on genome structure and data originated from databases as GeneDB, Kyotto Encyclopedia of Genes and Genomes (KEGG), BioSilico, University of Minnesota Biocatalyst/Biodegradation Database (UMBBD), Transport DB, ExPASy, BRENDA.

The five-step continuous process was analyzed by metabolic engineering methods as useful tools for getting a deeper insight into the metabolic and physiological situations of the cells in each step of the reactor system. Metabolic engineering methods described by Stephanopoulos et al. [37] as well as by Gombert and Nielsen [38] were additionally improved and new approaches have been developed for analysis of complex metabolic networks: flux balance analysis [39–41], elementary (flux) mode analysis [41,42], extreme pathways approach [43–45], flux variability analysis [46,47], minimal metabolic behaviors [48] and dynamic simulation and parameter estimation [49].

Elementary modes (EMs) are the smallest sub-networks that allow a metabolic reconstruction network to operate under steady state conditions [42]. EMs defined as a set of vectors that originate from the stoichiometric matrix of a metabolic network are characterized by three main properties:

(a) In a given network a unique set of elementary modes exists.
(b) 'Non-decomposability' means that any removed reaction in any elementary mode consequently excludes the whole elementary mode, so it cannot operate as a functional unit.
(c) All routes through a metabolic network consistent with property (b) are parts of elementary modes set.

In complex metabolic networks the combinatorial overproduction of number of EMs is possible. In such cases, by using "yield analysis" (YA) the "overproduced" set of EMs can be reduced to a minimal subset of EMs that is able to describe the related metabolic situation [50]. The solution space in yield analysis of metabolic pathways is a bounded convex hull in the yield space. Relative metabolic fluxes for the whole network of pathways can be calculated by appropriate software. In this work the elementary modes concept and metabolic yield analysis were applied in order to analyze metabolic situations in PHB producing cells in all steps of the continuous five stage process. According to our knowledge, this is the first time that a high structured metabolic model, the elementary modes concept and metabolic yield analysis were applied together for analyzing of continuous multistage production of PHB.

2. Materials and methods

2.1. Five-stage bioreactor cascade

The continuous cascade system consisting of five bioreactors is shown in Fig. 1. The cultivation procedures, as well as the information regarding fermentation experiments and obtained results were described earlier by Atlić et al. [34]. Briefly, in the first 23 h the

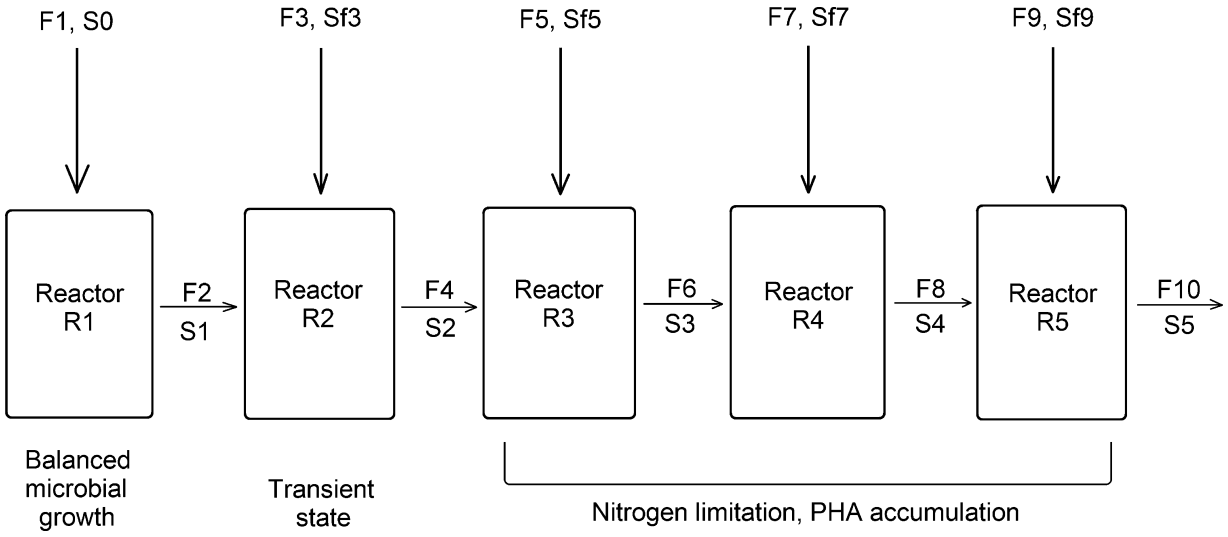

Fig. 1. Five-step bioreactor cascade for continuous PHB production.

Download English Version:

https://daneshyari.com/en/article/3268

Download Persian Version:

https://daneshyari.com/article/3268

Daneshyari.com