Biochemical Engineering Journal 105 (2016) 159–167

Contents lists available at ScienceDirect

Biochemical Engineering Journal

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

Regular article

Microbial production of 2,3-butanediol through a two-stage pH and agitation strategy in 150 l bioreactor

Anchal Priya[a,b], Prem Dureja[b], Pooja Talukdar[b], Rohit Rathi[b], Banwari Lal[a,b,*], Priyangshu M Sarma[a,b,*]

[a] TERI University, 10 Institutional Area, Vasant Kunj, New Delhi-110070, India
[b] TERI, Darbari Seth Block, India Habitat Centre, New Delhi-110003, India

ARTICLE INFO

Article history:
Received 21 May 2015
Received in revised form
18 September 2015
Accepted 20 September 2015
Available online 25 September 2015

Keywords:
Glucose
Microbial growth
Fermentation
Fed-batch culture
150 l bioreactor
2,3-Butanediol (2,3-BD)

ABSTRACT

This study was focused on scaling up of 2,3-butanediol (2,3-BD) production, via fermentation in a 150 l bioreactor using the newly isolated strain *Enterobacter cloacae* TERI BD 18. Operational parameters were customized for the strain *E. cloacae* using a fed- batch strategy, which enhanced the glucose to 2,3-BD conversion yield (0.44 g/g). Furthermore, dual stage pH and agitation control regime was considered where in the initial 10 h the pH value was 7.5 and the agitation rate used was 200 rpm favoring bacterial growth, while these values were subsequently changed to a pH value of 6.5 and an agitation rate of 150 rpm leading to enhanced accumulation of 2,3-BD. Production of 85 g/l of 2,3-BD was achieved with ethanol and acetoin as the only by-products. Total productivity of 1.73 g/l/h with a yield of 0.48 g of 2,3-BD per g of glucose was achieved in the 150 l bioreactor. Additionally, the total fermentation time was reduced to 50 h with this strategy. The integrated approach of fed-batch strategy together with dual regime in pH and agitation control favors 2,3-BD production in the pilot scale bioreactor system employed in this study.

1. Introduction

2,3 Butanediol (2,3-BD), a precursor of butadiene is widely used in the rubber industry. It is a versatile chemical and can be used as a platform compound for industrial purposes [1,2]. Additionally, 2,3-BD has potential applications in the manufacture of printing inks, perfumes, fumigants, moistening, softening agents, explosives, plasticizers, food and pharmaceuticals [2,3,4]. Also, it has a low freezing point of −60 °C which makes it a promising candidate for commercial use as an antifreeze agent [4]. As crude oil reserves have become increasingly scarce, bio-refineries that integrate biomass conversion processes to produce fuels, power, and chemicals from renewable resources are currently at various stages of development [3,5].

The use of chemical processes to produce platform chemicals requires high-energy input and expensive catalysts [6]. The development of the biotechnological methods for commercial scale production of 2,3-BD was set in the middle of the last century and then discontinued because of less expensive routes of chemical synthesis. During the last decades, the rising prices of petroleum and the new wave of white technology have revived significant interest in the production of 2,3-BD [7].

Thus, microbial production of 2,3-BD will alleviate the dependence on oil supply for the production of platform chemicals. 2,3-BD can be produced from carbohydrates by various bacterial species such as enteric bacteria of the genera *Enterobacter* and *Klebsiella* and also by bacilli such as *Paenibacillus polymyxa* via the mixed acid–butanediol fermentation pathway during anaerobic or microaerobic condition [3,5,8]. Recently, aerobic production of 2,3 BD has also been reported [9].

Several strategies have been used to enhance 2,3-BD production, such as introducing super productive recombinant strains, optimizing fermentation conditions, and building mathematical models [10–13]. Although 2,3-BD production has improved by adopting these methods, it is not commercially viable for economical industrial production. Low cost feed stocks, agricultural waste products as carbon source [6,14] has also been proposed but their pretreatment makes the bioprocess tedious and expensive to implement at larger scale. The cost for transportation (in bulk) of the raw materials required in large-scale production is a matter of concern [4]. Further, these complex carbon sources may also cause hindrance

* Corresponding authors at: TERI University, 10 Institutional Area, Vasant Kunj, New Delhi-110070, India. Fax: +91 11 24682144.
E-mail addresses: banwaril@teri.res.in (B. Lal), priyangshumsarma@yahoo.co.in (P.M. Sarma).

http://dx.doi.org/10.1016/j.bej.2015.09.016
1369-703X/

in downstream processing hence increasing the cost of 2,3-BD production. Although some 2,3-BD fermentations have been established in laboratory studies, it has not been produced on a large scale. The primary economic barriers to the commercialization of microbial 2,3-BD production might be the unavailability of substrate in bulk, low efficiency of substrate utilization, or byproduct formation [15].

Based on the above highlighted points, the main aim of the present work is to develop a cost-effective and feasible strategy for an economical production of 2,3-butanediol up to 150 l fermentation system. For industrial application large-scale fermentation system is an essential requirement with methods, which can be easily adopted. Therefore, it is necessary to improve 2,3-BD production in an economical way by selecting high-yield strains and systematically optimizing the fermentation conditions by adopting simpler and cost effective methods.

Culture condition such as carbon source, pH, and temperature varies significantly from species to species [16]. Moreover, the efficiency of any bio-system is strictly temperature and pH dependent owing to strong dependence on enzymatic activity and cellular metabolism. Controlling the culture temperature and pH can enhance metabolic pathway for the bioconversion of glucose to 2,3-BD [12]. Thus, for an efficient industrial process to be developed in large-scale fermentation system, simple media and customized physiological parameters is a necessity.

In this study, *Enterobacter cloacae* TERI BD18 was isolated which was easy to cultivate in a simple media with commercial grade glucose as carbon source. Fed-batch fermentation strategy was developed by controlling the two-stage pH and dual agitation control. Further, residual feed rate methodology in 150 l bioreactor was adopted giving enhanced production of 2,3-BD. As per the author's knowledge, this is the first report of fed-batch developed together with two-stage pH and agitation control strategy in 150 l fermentation system for enhanced microbial 2,3-BD production for industrial application.

2. Materials and methods

2.1. Media and culture condition

The culture was maintained on Luria–Bertani (LB) agar slant at 4 °C. The seed medium for inoculum was composed of (g/l): 10 g peptone, 3 g beef extract, 5 g NaCl at pH 6.5. For seed preparation a full loop of the culture from a fresh slant tube was inoculated in 250 ml Erlenmeyer flasks containing 100 ml of fresh seed medium and cultivated for 12 h at 37 °C with shaking at 200 rpm.

For the production of 2,3-BD, fermentation medium consisting of (g/l): 30 g glucose, 3 g K_2HPO_4, 7 g KH_2PO_4, 4 g $(NH_4)_2$ SO_4, 0.2 g $MgSO_4{\cdot}7H_2O$, 1 g $Na_3C_6H_5O_7{\cdot}2H_2O$ was used during fermentation studies. The media components used in the 150 l fermentation study were of commercial grade.

2.2. Analytical methods

The bacterial growth in each experiment was monitored by measuring the increase in optical density (OD) at 600 nm using UV-visible spectrophotometer (UV-2450, Shimadzu, Japan). The dry cell weight (DCW) concentrations were determined by standard method [17].

The concentrations of 2,3-BD, acetoin, ethanol and acetic acid in liquid phase were analysed with GC 7890A (Agilent Ltd., USA) equipped with flame ionization detector and DB-WAXetr column (30 m × 530 μm × 1 μm). The oven temperature was kept at 60 °C for 1 min and raised to 200 °C at the rate of 15 °C/min hold for 1 min and increase to 220 °C at the rate of 10 °C/min and hold for 15 min. The injector and detector temperatures were 250 °C and 300 °C respectively. Helium was used as the carrier gas at a flow rate of 3 ml/min. Authenticated standards (2,3-BD, acetoin, ethanol) procured from Sigma–Aldrich (Germany) were used for the identification of retention time. The calibration curve obtained for all the standards showed R^2 value more than 0.998.

Low molecular weight organic acids and residual glucose were analysed by High Performance Liquid Chromatography (HPLC, Agilent 1100 series, USA) equipped with AminexR HPX-87H, (300 mm × 7.8 mm) column (BIORAD, CA, USA). Sulphuric acid (0.005 M H_2SO_4) was used as mobile phase with the flow rate of 0.6–0.9 ml for 21 min in RID detector (optimum temperature 80 °C) as described by Singh et al. [18].

All the mentioned methods were calibrated by injecting standards with the range of concentration. The calibration curve was obtained for all the standards with R^2 approximately 0.998. The methods were found accurate and precise.

The 2,3-BD yield was calculated as the total amount of 2,3-BD (g) and acetoin (g) produced by amount (g) of substrate (glucose) consumed (2,3-BD g/g glucose). Both 2,3-BD and its precursor acetoin were the target products and their combination was expressed as the total production yield [4,8].

2.3. Selection of microorganisms

Initially, ten different types of bacterial strains were isolated from soil samples contaminated with petroleum hydrocarbons from an oil field in Mehsana (23.6000°N, 72.4000°E) located in the western state of Gujarat, India. The bacterial strains were routinely sub cultured and stock cultures were maintained in 25% glycerol at −70 °C for subsequent studies.

The bacterial strain designated as TERI BD 18 showing maximum production of 2,3 BD was identified by sequencing of the 16S rDNA gene as described by Sarma et al. [19]. Sequences were analyzed and identified using microseq microbial identification and analysis software and nucleotide–nucleotide Blast (Blast N) of NCBI database.

2.4. Operational parameters

The operational parameters of initial concentration of glucose, initial pH, temperature, agitation and inoculum size were optimized in 3 l bioreactor with 2 l of fermentation medium. Five percent of seed inoculum was used in the optimization studies. Batch fermentation was carried out with a range of initial glucose concentration of 10, 20, 30, 40, 50, 60 and 70 g/l. Thereafter, the effect of initial medium pH (pH 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5) on 2,3-BD production by TERI BD 18 was studied. The pH was adjusted with 2 N HCl and 2 N KOH solutions. Subsequently, to investigate the effect of temperature on growth and 2,3-BD production by TERI BD 18, the experimental set was incubated at temperatures 25, 30, 37 and 40 °C. The study of temperature optimization was performed at pH 7.5 and agitation of 200 rpm.

Similarly the effect of different rate of agitation 100, 150, 200, and 300 rpm were studied for analyzing the effect on 2,3-BD production at pH 7.5, temperature 37 °C.

To reduce the overall cost of 2,3-BD production in large fermentation system different inoculum size in the range of 1–5% was evaluated at pH 7.5, temperature 37 °C and 200 rpm. All the experiments were performed in duplicate and the data points are average of the duplicate ± standard deviation (less than 5% of average).

2.5. Batch and fed-batch fermentations

Batch and Fed-batch fermentations were conducted initially in 3 l and then in a 150 l fermentation system. Bioreactor (150 l,