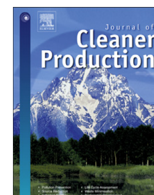
Journal of Cleaner Production 168 (2017) 420–427

Contents lists available at ScienceDirect

Journal of Cleaner Production

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



Two-stage continuous culture – Technology boosting erythritol production

Magdalena Rakicka*, Anita Rywińska, Zbigniew Lazar, Waldemar Rymowicz

Department of Biotechnology and Food Microbiology, Wroclaw University of Environmental and Life Sciences, 37 Chełmońskiego St., 51-630 Wroclaw, Poland

A R T I C L E I N F O

Article history:
Received 7 January 2017
Received in revised form
25 August 2017
Accepted 5 September 2017
Available online 6 September 2017

Keywords:
Yarrowia lipolytica
Erythritol
Chemostat
Two-stage culture
Glycerol

A B S T R A C T

Yield and product titer are two important parameters for economic evaluation of any biotechnological process. The published studies on erythritol biosynthesis are based mainly on batch and fed-batch cultures, which do not fully utilize the production potential of the cells. In the current work, a two-stage continuous process of erythritol production from glycerol by an industrially relevant strain of *Yarrowia lipolytica* is proposed. The proposed process significantly enhanced titer of secreted erythritol and reduced process costs compared to the conventional batch cultures on glycerol. The two-stage chemostat process with glycerol resulted in 199.4 g dm^{-3} of erythritol with overall yield of 0.66 g g^{-1} and productivity of 0.8 g dm^{-3} h^{-1}. These results represent almost 2.5-fold higher titer of erythritol compared to commonly used batch cultures of *Y. lipolytica* and almost 2 times higher titer and 1.3-fold increase in the product yield compared to a previously published continuous process with glycerol as a substrate. This study showed the potential of a two-stage continuous process using a genetically unmodified strain of *Y. lipolytica* for efficient erythritol production from raw glycerol originating from different industries.

© 2017 Elsevier Ltd. All rights reserved.

1. Introduction

Increasing the yield of production of important chemicals may be achieved by improvement of culture parameters combined with efficient refining processes. Increased concentration of the product may be obtained by applying fed-batch, repeated batch, continuous or multistage cultures instead of simple batch culture. The fed-batch processes are so far the most popular methods for increasing the product titer (McLarty et al., 2016; Shuler and Kargi, 2002). It provides controlled culture conditions and easy substrate concentration monitoring, and allows to obtain high cell density in the culture medium. The biosynthesis of byproducts as well as the catabolite repression effects are strictly controlled to maximize the conversion of the substrate into the desired product. However, more attention should be devoted to monitoring the accumulation of toxins, so that they do not exceed the inhibitory concentration in the medium (Lennox et al., 2001). Simultaneously, the concentration of the available nutrients should not become the limiting parameter. Over the last years, these aspects have been addressed in several reports focused on the design and application of a series of bioreactors (Chang et al., 2011). Usually, these bioreactors act as continuous stirred-tank reactors (CSTR). This type of culture has many advantages: it is easy to operate, of simple construction, and replacement of biocatalysts and maintenance are not troublesome (De Gooijer et al., 1996). All processes conducted in a series of bioreactors can be divided into two main groups: processes with a constant overall stoichiometry, which can be performed in one bioreactor; and processes with segregation in two or more bioreactors, which may lead to a higher metabolite concentration, higher productivity and efficiency of the process, a higher conversion degree, or all of the above (De Gooijer et al., 1996). The second group consists of processes, such as biogas production or nitrification, where the stoichiometry is variable and the descriptive kinetic equation changes during the process (De Gooijer et al., 1996). Nowadays, the yield, specific productivity and product titer are being improved mainly through metabolic engineering approaches (Stephanopoulos, 2007). However, the processes with genetically modified organisms still raise many doubts in industrial applications, especially for food production. Due to these

* Corresponding author.
E-mail addresses: magdalena.rakicka@upwr.edu.pl (M. Rakicka), anita.rywinska@upwr.edu.pl (A. Rywińska), zbigniew.lazar@up.wroc.pl (Z. Lazar), waldemar.rymowicz@up.wroc.pl (W. Rymowicz).

http://dx.doi.org/10.1016/j.jclepro.2017.09.060
0959-6526/© 2017 Elsevier Ltd. All rights reserved.

restrictions, it is desirable to develop a process with industrially relevant strains that are not genetically modified, with improved production parameters. Furthermore, to increase the profitability of the whole technology, application of cheap substrates combined with the utilization of the remaining biomass for animal feeding is of even more interest.

In response to these expectations, the aim of the present study was to enhance erythritol (ERY) titer, yield and productivity from glycerol (GLY) by the *Y. lipolytica* Wratislavia K1 strain in a continuous two-stage chemostat culture limited by nitrogen and phosphorus. In the previous study on ERY production from GLY in chemostat culture it was found that 103.4 g dm^{-3} of ERY is a limit value which may be obtained in chemostat culture (Rakicka et al., 2016). By operating the two chemostat cultures in tandem, the concentration of the carbon source and the ERY titer increased. The aim of the second chemostat is complete utilization of the carbon from the medium, reduce the negative impact of high substrate and product concentration on the cells, provide the optimal aeration of the culture as well as maintaining the biomass concentration on high, reasonable level. Moreover, the ability of this strain to produce well-dispersed, unicellular populations offers the possibility to improve ERY biosynthesis. The proposed two-stage technology of sweetener production results in a high concentration of ERY in the culture broth with a low level of undesired byproducts. It is a waste-free process, as the yeast biomass has a high protein level as recommended by the FAO/WHO for animal feed application. To our knowledge, this is the first report on two-stage chemostat technology for high ERY biosynthesis using a strain of *Y. lipolytica* from different raw GLY.

2. Materials and methods

2.1. Microorganisms

Y. lipolytica Wratislavia K1 used in this study originated from the yeast culture collection of the Department of Biotechnology and Food Microbiology, Wrocław University of Environmental and Life Sciences in Poland. The Wratislavia K1 strain was isolated from Wratislavia 1.31 in the course of continuous citric acid production from glucose in a nitrogen-limited chemostat (Rywińska et al., 2011).

2.2. Substrate

Technical glycerol (GLY), crude glycerol (GLY-1) derived from biodiesel production and crude glycerol (GLY-2) derived from soap production were used as a source of carbon and energy. The composition of all substrates is listed in Table 1.

2.3. Media and culture conditions

The inoculum medium consisted of: technical GLY − 50 g, yeast extract − 3 g, malt extract − 3 g, and peptone − 5 g in 1 dm^3 of distilled water. Growth of cells was carried out in 0.3 dm^3 flasks containing 0.05 dm^3 of growth medium on a rotary shaker (CERTOMAT IS, Sartorius, Germany) at 30 °C and 2.3 Hz for 72 h. The inoculum (OD_{600} = 10, 0.2 dm^3), representing 10% of the working volume, was introduced aseptically into the bioreactor containing the appropriate medium.

The pilot two-stage CSTR with two different dilution rates was conducted in bioreactors in chemostat mode with technical GLY as the substrate in nitrogen and phosphorus limited conditions. A schematic representation of the laboratory-scale two-stage continuous production process is presented in Fig. 1. The chemostat cultures began in two independent bioreactors: Chemostat reactor 1 (first stage) and Chemostat reactor 2 (second stage) (BIOSTAT B-PLUS, Sartorius, Germany). Both bioreactors contained the same medium consisting of (g dm^{-3}): technical GLY − 100; $(NH_4)_2SO_4$ − 2.3; KH_2PO_4 − 0.22; $MgSO_4 \times 7 H_2O$ − 1; yeast extract − 1; NaCl − 26.5 (Rywińska et al., 2015). The volume of the two bioreactors was different: 1.5 dm^3 in the 1st and 2.0 dm^3 in the 2nd reactor. After 24 h of batch culture the continuous biosynthesis began at different dilution rates: 0.01 h^{-1} in the 1st and 0.008 h^{-1} in the 2nd reactor. The overall dilution rate (D_{ov}) of the two-stage process was 0.004 h^{-1}. The feeding medium introduced to the 1st bioreactor consisted of (g dm^{-3}): technical GLY − 300; $(NH_4)_2SO_4$ − 4.6, KH_2PO_4 − 0.22, $MgSO_4 \times 7 H_2O$ − 1.0, yeast extract − 1.0 in tap water. The feeding medium introduced to the 2nd bioreactor consisted of (g dm^{-3}): technical GLY − 200; $(NH_4)_2SO_4$ − 4.6, KH_2PO_4 − 0.22, $MgSO_4 \times 7 H_2O$ − 1.0, yeast extract − 1.0, NaCl − 26.5 in tap water. After reaching a steady state, both bioreactors were operated in tandem with feeding medium pumped into the 1st bioreactor (GLY_{inI} 300 g dm^{-3}). The effluent from the 1st fermenter was fed (5.0 cm^3 h^{-1}) directly to the 2nd reactor. The volume of the 2nd stage was kept constant at 2 dm^3.

The two-stage CSTR on raw substrates. Based on the results obtained from the previous experiments, the two-stage continuous cultures on raw GLY were conducted using the conditions described above. In these experiments crude GLY from the biodiesel industry and from soap production were used as a carbon and energy source (GLY-1 and GLY-2, respectively).

All CSTR were cultured at 30 °C, with 13.3 Hz and 0.6 m^{-1} for the stirring rate and aeration, respectively. The pH was maintained automatically at 3 by the addition of 5 mol dm^{-3} NaOH solution. The bioreactor with appropriate medium was autoclaved for 20 min at 121 °C. All cultures were conducted in two biological replicates. All pump tubing and bioreactors were autoclaved separately and joined aseptically after sterilization. Sterile medium was pumped from the feed reservoir to the bioreactor.

2.4. Analytical methods

Ten milliliters of culture broth was centrifuged (5 min, 2700 RCF). The biomass was washed with distilled water and filtered on 0.45 μm pore-size membranes. Biomass concentration was determined gravimetrically after drying at 105 °C and expressed in grams of cell dry weight per liter (g dm^{-3}). Protein concentration in the biomass was analyzed using the Kjeldahl method (1883). Concentrations of GLY, ERY, arabitol (ARA), mannitol (MAN), citric acid (CA) and α-ketoglutaric acid (KGA) were measured in culture supernatants by high-performance liquid chromatography (Dionex-Thermo Fisher Scientific, UK) using a Carbohydrate

Table 1
Composition of glycerol used in present study.

Symbol	Substrate	Waste product derived from	Glycerol content (%)	Nitrogen content (%)	NaCl (%)	Ash (%)	Water (%)
GLY	technical glycerol (control)	biodiesel industry	96	0	0	0	4,00
GLY-1	crude glycerol	biodiesel industry	83	0.014	4.30	6.34	6.35
GLY-2	crude glycerol	soap production	76	0.041	7.59	8.76	3.60

Download English Version:

https://daneshyari.com/en/article/5479813

Download Persian Version:

https://daneshyari.com/article/5479813

Daneshyari.com