Electronic Journal of Biotechnology 17 (2014) 72–78

Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

An increasing of the efficiency of microbiological synthesis of 1,3-propanediol from crude glycerol by the concentration of biomass

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ARTICLE INFO

Article history:
Received 9 September 2013
Accepted 23 December 2013
Available online 30 January 2014

Keywords:
C. butyricum
HSP protein
Microfiltration

ABSTRACT

Background: 1,3-Propanodiol (1,3-PD), is used in the production of polytrimethylene terephthalate (PTT), an aromatic polyester that exhibits high elastic recoveries. It is also employed as a supplement with low solidification properties, a solvent and a lubricant in the formof propylene glycol. 1,3-PD is effectively synthesized by a microbiological way from crude glycerol. The main problem of this technology is using a high concentration of glycerol, which is a limiting factor for bacteria cells growth (especially in batch fermentation).
Results: In this work, the influence of different glycerol concentration in batch fermentation on *Clostridium butyricum* DSP1 metabolism was investigated. The biomass was concentrated for two times with the use of membrane module (in case of increasing kinetic parameters). Increased optical density of bacteria cells six times increased the productivity of 1,3-PD in cultivation with 20 g/L of glycerol at the beginning of the process, and more than two times in cultivation with 60–80 g/L. Also the possibility of complete attenuation of 140 g/L of crude glycerol in the batch fermentation was investigated. During the cultivation, changes of protein profiles were analyzed. The most significant changes were observed in the cultivation in the medium supplemented with 80 g/L of glycerol. They related mainly to the DNA protein reconstructive systems, protective proteins (HSP), and also the enzymatic catalysts connected with glycerol metabolic pathway.
Conclusions: The application of filtration module in batch fermentation of crude glycerol by *C. butyricum* DSP1 significantly increased the productivity of the process.

1. Introduction

The production of biofuels from renewable energy is one of the most important issues of the industrial biotechnology of the 21st century. One example of this process is the production of biodiesel from rapeseed oil. During this process, crude glycerol, as a by-product, is synthesized. There are a number of well-known methods of the application of crude glycerol, *e.g.* microbial utilization to 1,3-Propylene glycol (1,3-PD) using chemical synthesis of polyesters and polyurethanes [1,2,3,4]. Biotechnological production of 1,3-PD (with microorganisms) is a good alternative to a chemical way which generated huge cost and toxic by-products [5]. A very important issue is also the industrial application of crude glycerol — a by-product from biodiesel production. Microbiological synthesis of 1,3-PD is mainly carried out by bacteria from the genera *Clostridium*, *Klebsiella*, *Citrobacter* and *Lactobacillus* [3,6,7,8]. However, microbiological synthesis of 1,3-PD has some limitations, *e.g.* in batch and fed-batch fermentations' high concentration of glycerol increases the osmotic pressure which is a factor limiting the growth of bacterial biomass [9,10,11]. The maximum density of *Clostridium butyricum* cells in propanediol fermentation is 0.61–3.4 g/L (in batch process) and 4.2 g/L (in fed-batch process) and depends mostly on the concentration and purity of raw material used [7,12], while the productivity is 0.3–2.3 g/L/h in batch fermentation, 0.7–2.9 g/L/h in fed-batch fermentation, and 16.2 g/L/h in continuous process [13,14,15,16,17]. Among favorable solutions in order to improve some kinetic properties of a biotechnological way of 1,3-PD production there is biomass concentration. The advantage of this method is that it applies the process of microfiltration (MF). During MF small molecules, bacteria cells, viruses, particles of plant raw materials, and particles of fat are removed. Thus, the color of permeate can change, and its turbidity can decrease. MF results from different hydrostatic pressure between both sides of the membrane. It is commonly used in food industry, among other processes in cold sterilization of beer, wine, milk and in clarification of fruit juice. In biotechnology, it is a convenient sterilization method applied to media containing thermolabile compounds. Furthermore, filtration is commonly utilized in concentration of bacterial

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Peer review under responsibility of Pontificia Universidad Católica de Valparaíso.

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http://dx.doi.org/10.1016/j.ejbt.2013.12.010

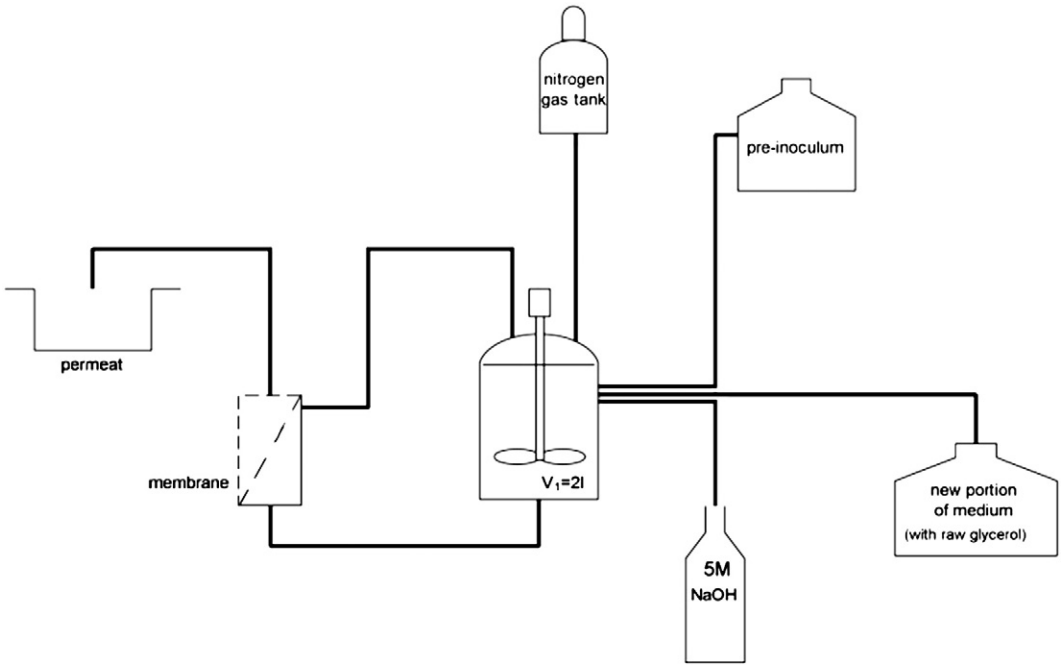

Fig. 1. The block diagram of the fermentation process with filtration module.

biomass in the production of industrially useful enzymes, therapeutic proteins, *etc.* MF is also used to concentrate algae biomass during bioethanol production [18,19].

The main aim of using filtration module in 1,3-PD from crude glycerol by microbiological way is to increase the kinetic parameters of that process and recirculation of biomass. The application of MF process in 1,3-PD production by *C. butyricum* makes it possible to concentrate biomass in closed systems which are a very important quality with respect to anaerobic microorganisms. In this work, the possibility of using MF process for biomass concentration of *C. butyricum* cells and in the resulting process of improving kinetic parameters of 1,3-PD production was investigated.

2. Materials and methods

2.1. Microorganism

In the conversion process of crude glycerol to 1,3-PD a bacterial strain, *C. butyricum* DSP 1, was used. *C. butyricum* DSP1 was previously isolated from ruminal fluid and collected at the Department of Biotechnology and

Table 1
Experimental results of *C. butyricum* DSP 1 during batch cultivation in 2-L bioreactor, at various initial crude glycerol concentrations without biomass recycling.

Parameter/concentration of raw glycerol	20	40	60	80	100	120	140
Time of fermentation (h)	17.5	22.5	25.5	33.5	76	108	120
Max biomass, X_{max} (g/L)	0.9	1.3	1.4	1.2	0.8	0.5	0.5
Max 1,3-PD concentration, $1,3PD_{max}$ (g/L)	9.33 ± 0.12	18.83 ± 0.18	32.54 ± 0.98	37.59 ± 0.75	48.12 ± 0.22	11.22 ± 0.43	1.43 ± 0.09
1,3-PD productivity $P_{1,3\text{-PD}}$ (g/L/h)	0.53	0.83	1.28	1.13	0.63	0.1	0.01
1,3-PD yield, $Y_{1,3\text{-PD}}$ ($g_{1,3\ PD}/g_{Gly}$)	0.47	0.47	0.54	0.47	0.48	0.48	0.47
Max butyric acid concentration, $\But_{max}$ (g/L)	1.14 ± 0.08	2.23 ± 0.08	3.82 ± 0.07	4.81 ± 0.05	5.52 ± 0.06	0.02 ± 0.00	0.04 ± 0.00
Butyric acid productivity P_{But} (g/L/h)	0.34	0.27	0.29	0.32	0.21	<0.00	<0.00
Butyric acid yield, Y_{But} (g_{But}/g_{Gly})	0.06	0.05	0.07	0.06	0.05	<0.00	<0.00
Max acetic acid concentration, Ace_{max} (g/L)	0.71 ± 0.01	1.12 ± 0.03	2.2 ± 0.02	2.12 ± 0.03	2.8 ± 0.02	0.01 ± 0.00	0.02 ± 0.00
Acetic acid productivity P_{Ace} (g/L/h)	0.04	0.05	0.07	0.07	0.04	<0.00	<0.00
Acetic acid yield, Y_{Ace} (g_{Lac}/g_{Gly})	0.03	0.03	0.04	0.03	0.00	<0.00	<0.00
Max lactic acid concentration Lac_{max} (g/L)	1.04 ± 0.02	1.24 ± 0.03	2.66 ± 0.04	3.12 ± 0.04	3.36 ± 0.04	0.01 ± 0.04	0.02 ± 0.04
Lactic acid productivity P_{Lac} (g/L/h)	0.06	0.05	0.10	0.01	0.04	<0.00	<0.00
Lactic acid yield Y_{Lac} (g_{Lac}/g_{Gly})	0.05	0.03	0.04	0.04	0.03	<0.00	<0.00