Bioresource Technology 152 (2014) 499–504

Contents lists available at ScienceDirect

Bioresource Technology

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

Heading for an economic industrial upgrading of crude glycerol from biodiesel production to 1,3-propanediol by *Lactobacillus diolivorans*

Stefan Pflügl [a,b], Hans Marx [b,*], Diethard Mattanovich [b,c], Michael Sauer [b,c]

[a] School of Bioengineering, FH Campus Wien – University of Applied Sciences, Muthgasse 62, 1190 Vienna, Austria
[b] Department of Biotechnology, BOKU – VIBT University of Natural Resources and Life Sciences, Muthgasse 18, 1190 Vienna, Austria
[c] Austrian Centre of Industrial Biotechnology (ACIB GmbH), Muthgasse 11, 1190 Vienna, Austria

HIGHLIGHTS

- *Lactobacillus diolivorans* efficiently converts crude glycerol to 1,3-propanediol.
- *L. diolivorans* is not inhibited by 0.7 g/l furfural and 0.3 g/l 5-HMF.
- Conversion of biobased resources to up to 85 g/l with a productivity of 0.45 g/l h.

ARTICLE INFO

Article history:
Received 29 July 2013
Received in revised form 11 November 2013
Accepted 14 November 2013
Available online 27 November 2013

Keywords:
1,3-Propanediol
Microbial conversion of crude glycerol
Lignocellulosic hydrolysate
Industrial production
Inhibitory effects by crude substrates

ABSTRACT

Lactobacillus diolivorans was evaluated as a potential organism for production of 1,3-propanediol under industrially relevant conditions. Crude glycerol of different origins has been tested and showed no inhibitory effects on growth or production. Using crude glycerol from biodiesel production from palm oil 85 g/l 1,3-propanediol have been obtained with a productivity of 0.45 g/l h in a fed-batch cultivation. Sugar necessary for the formation of biomass was replaced with a hydrolysate from lignocellulosic material resulting in 75 g/l 1,3-propanediol and a productivity of 0.36 g/l h. Lignocellulosic hydrolysate contained the potential inhibitors furfural and 5-hydroxymethylfurfural at concentrations of 0.7 and 0.3 g/l, respectively. Addition of furfural and 5-hydroxymethylfurfural to batch cultures in said concentrations did not show inhibitory effects on growth or 1,3-propanediol production.

1. Introduction

The production of biodiesel increased dramatically over the past years (Almeida et al., 2012). Biodiesel is produced via transesterification. Plant fat or oil reacts with an alcohol (usually methanol) to fatty acid (FA) esters, thereby liberating glycerol (Ma and Hanna, 1999). Glycerol from biodiesel production, referred to as crude or raw glycerol, amounts to 10% of the total biodiesel production volume (Almeida et al., 2012). The overall economic efficiency of biodiesel production depends on glycerol as additional source of income. However, with increasing production of biodiesel, glycerol prices have seen a sharp decrease with prices as low as $110/t (Kerr et al., 2007). Therefore, glycerol has become a waste product rather than a by-product of biodiesel production (Yang et al., 2012). In order to improve income of a biodiesel biorefinery, glycerol has to undergo a value-adding step to produce high-value chemicals such as 1,3-propanediol.

However, crude glycerol from biodiesel production contains a number of impurities such as methanol (usually used for transesterification), triglycerides, salts (as catalyst), moisture and soap (Yang et al., 2012). Some of the impurities, in particular free fatty acids, have been reported to be inhibiting for microbial fermentations such as the production of 1,3-propanediol production with *Clostridium butyricum* (Petitdemange et al., 1995; Chatzifragkou et al., 2010; Chatzifragkou and Papanikolaou, 2012).

As reported previously, *Lactobacillus diolivorans* is a good natural producer of 1,3-propanediol from glycerol (Pflügl et al., 2012). However, 1,3-propanediol production with *L. diolivorans* requires the addition of a sugar (e.g. glucose), as the organism is not able to grow on glycerol as the main source of carbon. Pure D-glucose is an expensive carbon source for biomass formation, thereby increasing production costs. Hydrolysates from lignocellulosic material are a cheap alternative (Heer and Sauer, 2008). Lignocellulosic hydrolysates often contain toxic compounds which may inhibit microbial fermentations, as reported for example for production of ethanol with *Saccharomyces cerevisiae* (Palmqvist and Hahn-Hägerdal, 2000; Almeida et al., 2007).

* Corresponding author. Tel.: +43 1 3189900401.
E-mail address: hans.marx@boku.ac.at (H. Marx).

0960-8524/$ - see front matter © 2013 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.biortech.2013.11.041

The overarching aim of this study was to evaluate the potential of *L. diolivorans* as a production host for 1,3-propanediol under actual industrial and economic conditions. Therefore, the ability of *L. diolivorans* to produce 1,3-propanediol with crude glycerol from different origins was tested. D-glucose was replaced by lignocellulosic hydrolysate and potential inhibitory effects have been evaluated.

2. Methods

2.1. Microorganism and medium

L. diolivorans DSM 14421 (LMG 19667) was used for all experiments in this study. Cells were maintained at −80 °C in culture broth supplemented with 10% (w/v) glycerol.

MRS medium as developed by De Man et al. (1960) was used in a modified form for all cultivations in this study (Pflügl et al., 2012). For the batch phase, MRS was supplemented with 3% (w/v) D-glucose or other sugars and 1% (w/v) pharma grade or crude glycerol.

During fed-batch cultivations a glucose/glycerol solution with a molar ratio of 0.1 was used as feed medium. The concentration of glycerol in the feed solution was 500 g/l and the concentration of D-glucose was 97.8 g/l. For cultivations with lignocellulosic hydrolysate the total sugar content was adjusted to 97.8 g/l. The actual concentrations of the feed solution were determined for all cultivations and used for the calculations and showed deviations of no more than 20%. 5 mg/l vitamin B_{12} was added to the batch and feed medium in all fed-batch cultivations. 100% (w/v) Struktol® SB 2121 (Schill + Seilacher, Hamburg, Germany) was used as antifoam agent for the fed-batch cultures and was only added as necessary.

2.2. Preparation of crude glycerol

Crude glycerol was obtained as follows: the crude suspension from the biodiesel production process containing glycerol was adjusted to pH 7, when necessary, and autoclaved for 20 min at 121 °C. A two phase system was obtained, with an organic phase containing fatty acids and an aqueous phase containing glycerol. Subsequently, the organic phase was removed. The glycerol concentration from the aqueous phase was determined by HPLC analysis and used as a stock solution for addition to the culture medium.

2.3. Batch and fed-batch cultures

For all cultivations the fedbatch-pro® bioreactor system (DASGIP AG, Jülich, Germany) was used. Technical specifications of the reactor system, preparation of cultivations and culture conditions were as described previously (Pflügl et al., 2012).

For all cultivations, 700 ml culture medium was inoculated to an OD_{600} of 0.1 with 2% (v/v) inoculum from an exponentially growing preculture. For the fed-batch cultivations, the separately sterilized feed solution was added at a rate of 1.5 ml/h after glycerol was consumed from the batch medium.

2.4. Analytical procedures

12 ml samples were taken at regular intervals throughout the whole cultivation duration. Biomass production was determined by measuring optical density at 600 nm. OD_{600} values were converted into cell dry mass (CDM) with a previously established correlation (Pflügl et al., 2012). The lowest CDM concentration detectable is 0.125 g/l derived from the lowest detectable OD_{600} value of 0.1.

The concentrations of D-glucose, D-xylose, D-fructose, L-arabinose, glycerol, 1,3-propanediol, 3-hydroxypropionic acid, lactic acid, acetic acid and ethanol in the culture broth were determined by HPLC analysis (Shimadzu, Korneuburg, Austria) with a Rezex ROA-Organic Acid H + column (300 mm × 7.8 mm, Phenomenex, USA). The column was operated at 60 °C, 1.0 ml/min flow rate and 0.004 M H_2SO_4 as mobile phase. Detectors used were a refraction index detector (RID-10A, Shimadzu, Korneuburg, Austria) and a photodiode array detector (SPD-M20A, Shimadzu, Korneuburg, Austria). Sample preparation and detection limits of HPLC measurements were as described previously (Pflügl et al., 2012).

Lignocellulosic hydrolysate was analyzed with a Rezex RPM Monosaccharide Pb + 2 column (300 mm × 7.8 mm, Phenomenex, USA). The column was operated at 80 °C temperature, 0.6 ml/min flow rate and water as mobile phase (same detectors as above). Samples were filtrated, and 10 µl of sample were injected for analysis of D-glucose, D-xylose, L-arabinose, mannose, galactose, furfural and 5-hydroxymethyl furfural. Technical replicates of the analyses were within a 5% margin.

CO_2 in the fermentation off-gas was quantified. Together with biomass concentrations and the produced metabolites carbon balances were set up as described previously (Pflügl et al., 2012). Within the margin of error, complete carbon recovery was observed for all cultivations.

2.5. Preparation of lignocellulosic hydrolysate

Commercially available wood chips for heating purposes containing 30% (w/w) spruce and 70% (w/w) beech were treated with steam explosion for 15 min at 121 °C and 2 bar pressure. Samples were stored at −20 °C until they were further treated. For enzymatic digestion, dry matter of the sample was determined, which ranged between 20% and 30% (w/w). The amount of dry material was adjusted to 20 g/l with water, and Cellic® enzyme (Novozymes®, USA) according to the manufactures recommendation. Digestions were performed in 2 l shake flasks at 50 °C and 220 rpm. After 72 h, digestion was completed. The final solution of lignocellulosic hydrolysate was obtained by centrifugation and used as a stock for preparation of either batch or feed medium.

Lignocellulosic hydrolysates contained up to 58 and 18 g/l D-glucose and D-xylose, respectively. The final feed medium contained 97.8 g/l total sugars and 500 g/l glycerol. Pure D-glucose and D-xylose were added in the same ratio as contained in the original lignocellulosic hydrolysate when necessary.

3. Results and discussion

3.1. Different crude glycerols in fed-batch with L. diolivorans DSM 14421

As reported previously, *L. diolivorans* is a good producer of 1,3-propanediol from glycerol in a fed-batch process cofermenting D-glucose and glycerol (Pflügl et al., 2012). For an industrial scale production process, glycerol would not be used as pharma grade glycerol, but as crude glycerol produced during biodiesel production. However, this form of glycerol contains a number of impurities, some of which have been reported to have inhibitory effects on microbial fermentations. The source of inhibition has been identified mainly as free fatty acids remaining after incomplete transesterification (Venkataramanan et al., 2012; Nguyen et al., 2013). Subsequently, the production potential of 1,3-propanediol by *L. diolivorans* with crude glycerol was evaluated in fed-batch cultivations. The cultivations were carried out on MRS medium supplemented with 3% (w/v) D-glucose, 1% (w/v) crude glycerol as batch medium and a glucose-glycerol solution as feed. Glucose is