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Process strategies for enhanced production of 1,3-propanediol by *Lactobacillus reuteri* using glycerol as a co-substrate

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ABSTRACT

1,3-Propanediol (PDO) is a bulk chemical used in the synthesis of polymers for terephthalates, cosmetics and lubricants, among other things. PDO can be produced by microorganisms growing anaerobically on glycerol. The objective of this study was to analyze the behavior of *Lactobacillus reuteri* ATCC23272 when cultivated in batch, repeated batch and continuous modes during the conversion of glycerol into 1,3-propanediol, with regards to the main parameters for viable industrial production: productivity (Q_{PDO}), PDO concentration and yield on glycerol ($Y_{PDO/GLY}$). Cultures were performed in an MRS medium with glucose and glycerol as co-substrates, at 37 °C. Besides the bioreactor operation mode, the following features were evaluated: anaerobiosis, limited oxygen respiration, pH 5.5 and 6.2. In batch mode, the best condition was anaerobiosis at pH 5.5, which resulted in $Y_{PDO/GLY}$ and Q_{PDO} of 0.66 $g_{PDO}\,g_{GLY}^{-1}$ and 1.42 $g\,L^{-1}\,h^{-1}$, respectively. In repeated batch mode, the highest level of productivity was 4.12 $g\,L^{-1}\,h^{-1}$, when cells were first decanted and 80% of the liquid phase was replaced with fresh medium. In chemostat mode, $Y_{PDO/GLY}$ of 0.70 $g_{PDO}\,g_{GLY}^{-1}$ was achieved and productivity in the steady state was 20% higher (4.92 $g\,L^{-1}\,h^{-1}$), compared to the best result in the repeated batch mode. The highest PDO productivity, Q_{PDO}, in chemostat mode, was due to the highest rate of glucose consumption, which can be directly related to PDO productivity.

1. Introduction

The current demand for biofuels and biopolymers is driving research to increase production efficiency and economy. 1,3-propanediol (PDO) is an important intermediary in the production of polymers obtained from petrochemical compounds [1]. However, PDO can also be produced by biochemical processes using glycerol, a by-product from the production of biodiesel, as raw material. For every 90 m^3 of biodiesel produced, 10 m^3 of glycerol is released [2,3].

Some bacterial strains such as *Klebsiella*, *Citrobacter*, *Clostridium* and *Lactobacillus* are naturally able to convert glycerol into 1,3-propanediol [4–8]. *Lactobacillus reuteri* is a heterofermentative bacterium, known to inhabit the gastrointestinal tracts of humans, pigs, birds and other animals [9]. The main advantages of using this microorganism are its ability to produce 3-HPA (3-hydroxypropionaldehyde) and PDO, and the fact that it is not pathogenic or genetically modified. The disadvantage is that *L. reuteri* does not grow on glycerol as the sole carbon source, hence the need for cofermentation (a mixture of glycerol and sugar in culture medium) in the production of 1,3-propanediol [10,11].

L. reuteri metabolism has been proposed by Talarico et al. [12], as shown in Fig. 1. It can be observed that sugar fermentation results in lactate, CO_2, acetate and ethanol using glucose as an electron acceptor [13,14]. There is NADH regeneration in lactate and ethanol formation and there is ATP gain in acetate production. Initially, glycerol is converted by the glycerol dehydratase enzyme (coenzyme B12-dependent) in 3-HPA, which is subsequently reduced by the propanediol oxidoreductase enzyme (NAD^+-dependent oxidoreductase), thus producing PDO [12].

PDO can be produced in a reactor using different operational modes, such as batch, repeated batch and continuous (chemostat). Many papers have been published about the batch and repeated batch modes, but few articles on PDO production in chemostat mode are available. In the main studies, *Clostridium* and *Klebsiella* are considered the best candidates for 1,3-propanediol production

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Nomenclature

PDO	1,3-propanediol
3-HPA	3-hydroxypropionaldehyde
$Y_{PDO/GLY}$	1,3-propanediol yield on glycerol, $g_{PDO}\ g_{GLY}^{-1}$
Q_{PDO}	productivity of 1,3-propanediol, $g\ L^{-1}\ h^{-1}$
D	dilution rate, h^{-1}
X	dry weight, $g\ L^{-1}$
CV	coefficient of variation
$\alpha \times V$	a specific portion of fermentation broth, α is a fraction ($0 > \alpha > 1$) and V is a working volume of bioreactor
$Y_{LAC/GLC}$	lactate yield on glucose, $g_{LAC}\ g_{GLC}^{-1}$
$Y_{X/GLC}$	cells yields on glucose, $g_{LAC}\ g_{GLC}^{-1}$
$Y_{ACE/GLC}$	acetate yield on glucose, $g_{ACE}\ g_{GLC}^{-1}$
$Y_{ETH/GLC}$	ethanol yield on glucose, $g_{ETH}\ g_{GLC}^{-1}$
Q_{GLC}	global consumption rates of glucose, $g\ L^{-1}\ h^{-1}$
Q_{GLY}	global consumption rates of glycerol, $g\ L^{-1}\ h^{-1}$
μ_x	specific growth rate, h^{-1}
μ_{PDO}	global specific PDO production rate, $g_{PDO}\ g_{CELL}^{-1}\ h^{-1}$

[15]. Table 1 shows the main results found in literature in terms of $Y_{PDO/GLY}$ and Q_{PDO} for the production of PDO via microbiological route in different process types.

The theoretical mass conversion from glycerol to PDO is $0.83\ g_{PDO}\ g_{GLY}^{-1}$, and as shown in Table 1, the nearest yields to this theoretical value were achieved with the genus *Lactobacillus*, except the one found by El-Ziney et al. [21]. The reason for these highest yield values may be because, contrary to other microorganisms, this species does not use glycerol as its carbon source.

According to Table 1, the highest productivity was found in the continuous mode (chemostat). González-Pajuelo et al. [27] carried out chemostat experiments with *Clostridium butyricum* that resulted in productivity of $10.3\ g\ L^{-1}\ h^{-1}$ and $Y_{PDO/GLY}$ of $0.53\ g_{PDO}\ g_{GLY}^{-1}$, with a dilution rate, D, equal to $0.3\ h^{-1}$. Menzel et al. [28] cultivated *Klebsiella pneumoniae* in chemostat mode and achieved a PDO concentration of 35.2–48.5 $g\ L^{-1}$ and productivity of 4.9–8.8 $g\ L^{-1}\ h^{-1}$.

Papanikolaou et al. [19] carried out experiments in a two-stage continuous process. The first stage was intended to achieve high productivity, whereas the second stage was intended to increase the final PDO concentration. This strategy was used because, according to the authors, it is difficult to obtain a high PDO concentration and an increase in Q_{PDO} simultaneously. In this process, the PDO concentration, $Y_{PDO/GLY}$ and Q_{PDO} achieved were 41–46 $g\ L^{-1}$, $0.53\ g_{PDO}\ g_{GLY}^{-1}$ and $3.4\ g\ L^{-1}\ h^{-1}$, respectively.

Several methods are used for achieving high productivity, for example, cell immobilization and cell recycling. Pflugmacher and Gottschalk [30] carried out continuous tests with *Citrobacter freundii*. They were immobilized on modified polyurethane carrier particles PUR 90/16. The PDO productivity achieved was equal to $8.2\ g\ L^{-1}\ h^{-1}$ with $D = 0.5\ h^{-1}$. Reimann et al. [29] reported continuous cultivation with cell recycling using hollow-fiber membrane modules for PDO production by *C. butyricum*. In this study, the dilution rate was $0.5\ h^{-1}$ and PDO concentration achieved was $26.5\ g\ L^{-1}$ ($56\ g\ L^{-1}$ of glycerol was added) and Q_{PDO} was $13.3\ g\ L^{-1}\ h^{-1}$. However, this process could only be run for short time periods because of the risk of clogging the membrane. In these continuous process studies, microorganisms were grown in a culture medium with glycerol as the sole carbon source.

With regards to *C. butyricum* and *K. pneumoniae*, there are many reports exploring different bioprocesses in order to obtain PDO. On the other hand, there are only a few papers that describe co-fermentation of glucose and glycerol by *Lactobacillus* sp. for PDO production [21,31]. El-Ziney et al. [21] carried out cultivations in batch and continuous (chemostat) modes with *L. reuteri* 12002 in order to evaluate the production of 3-HPA and other metabolites. The batch mode favoured PDO production, compared to the continuous process ($D = 0.17\ h^{-1}$). PDO concentration was $4.4\ g\ L^{-1}$ and $4.4\ g\ L^{-1}$ in batch and chemostat modes, respectively; however, PDO productivity was better ($0.43\ g\ L^{-1}\ h^{-1}$ compared to $0.37\ g\ L^{-1}\ h^{-1}$) in chemostat mode. It was not possible to calculate $Y_{PDO/GLY}$, since glycerol consumption was not found in this paper.

In this study, the objective was to increase PDO productivity (Q_{PDO}) with *L. reuteri*. Accordingly, PDO production was carried out under different modes of reactor operation, namely batch, repeated

Table 1
Main published results for PDO production via biotechnological route.

Process type	Microorganism	Titer PDO ($g\ L^{-1}$)	$Y_{PDO/GLY}$ ($g_{PDO}\ g_{GLY}^{-1}$)	Q_{PDO} ($g\ L^{-1}\ h^{-1}$)	Reference
Batch	*C. butyricum*	56	0.51	1.90	Biebl et al. [16]
	K. pneumoniae	20.4	0.51[a]	1.95	Xiu et al. [17]
	C. butyricum	19.6	0.57	1.96	Zhu and Fang [18]
	C. butyricum	16.5	0.55	1.20	Papanikolaou et al. [19]
	L. reuteri	13	0.70[a]	1.08	Tobajas et al. [11]
	L. reuteri	28.7	0.78[a]	1.05	Baeza-Jiménez et al. [20]
	L. reuteri	4.4	–	0.32	El-Ziney et al. [21]
	L. diolivorans	21.8	0.58	0.85	Pflügl et al. [22]
Fed-batch	*C. butyricum*	93.7	0.52	3.30	Wilkens et al. [23]
	K. pneumoniae	71.6	0.54	1.93	Jin et al. [24]
	L. diolivorans	85.4	0.64[a]	0.46	Pflügl et al. [22]
Repeated batch	*C. diolis*	67.8	0.53	1.04	Kaur et al. [25]
	K. pneumoniae	66	0.50	3.30[b]	Xue et al. [26]
Continuous	*C. butyricum*	30	0.53	10.3	González-Pajuelo et al. [27]
	K. pneumoniae	48.5	–	4.9–8.8	Menzel et al. [28]
	L. reuteri	2.5	–	0.43	El-Ziney et al. [21]
	C. butyricum	46	0.53	3.4[c]	Papanikolaou et al. [19]
	C. butyricum	26.6	–	13.3[d]	Reimann et al. [29]
	C. freundii	16.3	0.53	8.2[e]	Pflugmacher and Gottschalk [30]

[a] Co-fermentation glucose/glycerol.
[b] Repeated fed-batch.
[c] Two-stage continuous process.
[d] Continuous process with cell recycling.
[e] Anaerobic fixed bed reactor with effluent recycle.