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1,3-Propanediol production from glycerol with a novel biocatalyst *Shimwellia blattae* ATCC 33430: Operational conditions and kinetics in batch cultivations

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Abstract

Shimwellia blattae ATCC 33430 as biocatalyst in the conversion of 1,3-propanediol from glycerol is herein evaluated. Several operational conditions in batch cultivations, employing pure and raw glycerol as sole carbon source, were studied. Temperature was studied at shaken bottle scale, while pH control strategy, together with the influence of raw glycerol and its impurities during fermentation were studied employing a 2 L STBR. Thereafter, fluid dynamic conditions were considered by changing the stirring speed and the gas supply (air or nitrogen) in the same scale-up experiments. The best results were obtained at a termperature of 37ºC, an agitation rate of 200 rpm, with free pH evolution from 6.9 and subsequent control at 6.5 and no gas supply during the fermentation, employing an initial concentration of 30 g/L of raw glycerol. Under these conditions, the biocatalyst is competitive, leading to results in line with other previous works in the literature in batch conditions, reaching a final concentration of 1,3-propanediol of 13.84 g/L, with a yield of 0.45 g/g and a productivity of 1.19 g/(L·h) from raw glycerol.

1. Introduction

The current global industrialization model, sustained by progressive depletion of petroleum stocks has raised the worldwide need for alternative energy sources, such as biofuels, being biodiesel considered among the most important (Luque & Melero, 2012). This compound is the main product of the trans-esterification of vegetable or animal fatty acids with short chain alcohols, producing a concentrated water-rich glycerol (the so-called raw or crude glycerol) as by-product. The current global increase of biodiesel demand has caused a high accumulation of raw glycerol, currently regarded as waste. Hence, several glycerol conversion processes have been developed in order to valorize this compound into high added value products, where microbial biotechnology plays a key role (Johnson & Taconi, 2007).

1,3-propanediol (PDO) has long been considered a specialty platform chemical, due to its high number of applications in the new polymer industry, cosmetics, personal care and cleaning products (Kaur et al., 2012). PDO is mainly used as a monomer for poly-tri-methyl-terephthalate (PTT) production (representing approximately 90% of its total market share), as well as other polyesters with important uses in the plastic and fiber industries (Witt et al., 1994; Zeng & Sabra, 2011). The chemical production of PDO requires high pressure and temperature conditions as well as expensive catalysts; therefore, the microbial conversion of glycerol to PDO has recently gained a great deal of attention and has been subsequently studied in a large number of papers, where different bacterial species (e.g. *Klebsiella, Clostridium, Lactobacillus* and *Enterobacter*) have been used as biocatalysts under aerobic or micro-aerobic conditions (Kaur et al., 2012). The best results have been attained by genetically engineered strains of *Klebsiella pneumoniae* and *Clostridium butyricum* (Chatzifragkou et al., 2012; Jin et al., 2011). Glycerol assimilation follows two different coupled pathways. Through the oxidative pathway (a), the substrate is successively transformed into pyruvate through dihydroxyacetone (DHA) and phosphoenolpyruvate (PEP), releasing succinic acid to recover reducing power (NAD) and

produce ATP. Then, pyruvate is converted to formic acid, lactic acid, 2,3-butanediol (via acetoin) and acetyl-CoA, that is oxidized into ethanol and acetic acid to generate NAD and ATP (Gungormusler et al., 2010). Through the reductive pathway (b), glycerol is dehydrated to 3-hydroxypropionaldehyde by glycerol dehydratase (GDHt), and subsequently reduced to PDO by 1,3-propanediol dehydrogenase (PDO DH) (Zeng & Biebl, 2002). The catalytic activity of both enzymes depends on the presence of different co-factors: GDHt is coenzyme-B_{12} dependent, while PDO DH is $NADH+H^+$ dependent (Kaur et al., 2012). The enzymes of the reductive pathway are encoded by *dha* operon, where GDHt is considered as a rate-limiting enzyme of the pathway (Seyfried et al., 1996). PDO synthesis is associated to cell growth, since biomass production is the only pathway branch that provides $NADH+H^+$ (Andres et al., 2004).

Even though the best PDO production results from glycerol were obtained by *K. pneumoniae*, the use of this bacterium in an industrial process is not desirable., due to its pathogenicity (Pflugl et al., 2012). More recent works have reported strains of *L. diolivorans* (Pflugl et al., 2012), *L. reuteri* (Vieira et al., 2015), *C. freundii and H. alvei* (Drozdzynska et al., 2014) to be producers of 1,3-propanediol, as well as a novel *K. oxytoca* strain (Wojtusik et al., 2015) (see Table 1). *S. blattae* ATCC 33430 is capable of producing PDO from glycerol by the enzymatic system described below (Andres et al., 2004) and it's also employed to synthesize poly(3-hydroxypropionate), a biopolymer derived from 3-HPA, by engineered strains that may accumulate almost 10% w/w [CDW] of this product inside the cells (Heinrich et al., 2013).

The aim of this work is to study the capability of a novel strain *S. blattae* ATCC 33430 in 1,3-propanediol production from glycerol, and to determine adequate experimental batch conditions such as temperature, pH control strategy, fluid dynamic conditions (stirrer speed and whether sparging or not with air or nitrogen is favorable) and the use of pure and raw glycerol. Moreover, the scale-up from shaken bottle to stirred tank bioreactor (STBR) has been studied.

2. Methods

2.1 Microorganism and medium

S. blattae ATCC 33430 was the biocatalyst used in all the experiments carried out in this study. Cells were stored at -80ºC in 50% w/w glycerol/saline serum medium prior to inoculation. The medium employed was M92x medium (Rhee et al., 1997) for all cultures, the composition of which being as follows (per liter of deionized water): 2 g NH_4Cl, 6 g KH_2PO_4, 12 g Na_2HPO_4, 1 g NaCl, 0.246 g $MgSO_4 \cdot 7H_2O$, 0.011 g $CaCl_2$ and 1.5 g yeast extract. The initial concentration of glycerol (pure or raw) was approximately 30 g/L in every run, due to the drag of metabolites from inoculation stage. In raw glycerol experiments, the substrate was taken from a concentrate coming directly from biodiesel production with the following composition (w/w basis): 65.8% glycerol, 3% water, 4.49% chlorides and 0.48% phosphates.

In order to ensure the same metabolic state of the cells in every experiment, two previous growth steps were made before each run in anaerobic conditions, bubbling commercial nitrogen before sterilization into the bottles containing the culture medium. These two previous growth steps were carried out at a temperature of 37 ºC in 100 mL shaken bottles, with 90 mL of culture medium, in an orbital shaker at 210 rpm for 4.5 and 12 h, respectively. The temperature study was carried out at shaken-bottle scale, at 210 rpm with a working volume of 10 mL. The rest of variables were studied in a 2 L STBR BIOSTAT B-Plus. The initial biomass concentration was fixed at 0.1 g/L in both bottle and bioreactor scale. Culture samples were taken during each run to determine biomass concentration by optical density at 600 nm and to quantify the concentration of different metabolites by HPLC, previously removing the biomass by centrifugation (14,000 x g during 10 min.).

2.2 Batch STBR cultures

All variables, except temperature (see Table 2), were studied in a 2L STBR BIOSTAT® B-Plus (Sartorius AG, Germany), with a working volume of 2000 mL and 15% (v/v) inoculum from an exponentially growing pre-culture, as indicated before. The bioreactor consisted of a non-baffled cylindrical vessel of inner diameter 13.7 cm and height 20 cm, with agitation by means of a dual six-blade Rushton turbine system (5.3 cm diameter) coupled to an electrical motor (Kollmorgen 3ΦPM, Danaher Motion, Czech Republic). The reactor was previously sterilized by autoclaving at 121ºC for 20 min. Runs were carried out at 37 ºC of temperature and the pH control strategy was monitored with a sensor (EasyFerm Plus K8 200, HAMILTON, Switzerland) coupled to the bioreactor system, pH being controlled by 2 M NaOH and 2 M HCl solutions. Cultures were carried out in micro-aerobic or anaerobic conditions, sparging air (0.04 or 0.08 vvm), nitrogen (1 vvm) or without gas supply (see Table 3). The stirring speed was fixed at 200 rpm in all runs, except in the study of this variable, in which it was changed from 100 to 400 rpm.

2.3 Analytical procedures

Biomass concentration was determined by the measurement of optical density at 600 nm (Shimadzu UV-visible spectrophotometer UV-1603) with prior calibration, where the Cell dry mass (105ºC until constant weight) (CDM) and the optical density (OD_{600}) are related according to:

$$\text{CDM}\ \left(\frac{\text{g}}{\text{L}}\right) = 0.403\ \text{x}\ \text{OD}_{600} \qquad (1)$$

Glycerol and the main metabolic pathway products, such as 1,3-propanediol, lactic acid, succinic acid, acetic acid, formic acid and ethanol were determined by HPLC (Agilent technologies, 1100 Series), with a Rezex ROA-Organic Acid H+ (8%) column (300 x 7.8 mm,

Phenomenex), coupled to a Waters 2414 Refractive Index Detector. The mobile phase employed was H_2SO_4 0.005 M at a flow rate of 0.6 mL/min (Esteban et al., 2015a; Esteban et al., 2015b) . Column temperature was controlled at 65 ºC, while refractive index detector operated at 55ºC. The sample volume was 5 µL.

2.4 Calculations

All bioconversion results were referred to PDO yield *(Y_{PG})* and productivity *(P_{PG})*, given by Eqs. (2) and (3), respectively:

$$Y_{PG} = \frac{C_{PDO}}{C_{G_0} - C_G} \quad (2)$$

$$P_{PG} = \frac{C_{PD0}}{t} \quad (3)$$

where C_{PDO} [g/L] is the PDO concentration at different times, C_{G0} [g/L] is the initial glycerol concentration, C_G [g/L] is the glycerol concentration and t [h] is the fermentation time.

3. Results and discussion

3.1 Selection of the optimum process temperature

The first step of this work was the study of the temperature during the batch fermentation process, at shaken-bottle scale. Pure glycerol was used as the sole carbon source and temperature was modified from 33 to 39ºC. As can be observed in Table 2, the best results were obtained at 37ºC, where the maximum 1,3-propanediol concentration was reached. Though this value was very similar to that achieved at 39ºC, the productivity was lower at the latter temperature. During this study, the fermentation stopped in every run after 11 h, remaining 40% (w/w) of initial substrate. Since final pH values *(pH_F)* were virtually identical

at any temperature, this study shows the possible inhibition of the process by low pH values, due to the synthesis of organic acids according to the metabolic pathway. This effect has also been reported by other authors in the literature (Azbar et al., 2010; Jolly et al., 2014; Vieira et al., 2015), indicating the importance of developing an appropriate pH control strategy in order to enhance the PDO production.

3.2 Influence of initial pH value

The fermentation was scaled-up to a STBR, varying the initial pH from 6.9 (M92x value) to 8.1, whose results are shown in Table 2 and Figure 1. When the starting pH value was more basic, biomass growth and substrate uptake were enhanced. Higher pH_F values allowed for the completion of the fermentation in 16 hours, reaching 11.26 g/L of PDO. However, working at a higher initial pH had a negative effect on the production rate of PDO by way of increasing the batch time necessary to reach production results comparable to those given in the literature (Wojtusik et al., 2015; Zhang et al., 2007). The highest PDO production rate was reached at pH values ranging from 6.7 to 6.0 (see Figure 1, A), which leads to conclude that this variable must be controlled within this interval to increase PDO yield and productivity.

3.3 Development of pH control strategy

The advantage of controlling the pH evolution during the 1,3-propanediol production bioprocess from glycerol has been described by several authors in the literature. (Petrov & Stoyanov, 2012) have developed a specific strategy called *"forced pH fluctuations"*, based on the introduction of consecutive pulses of base buffer with definite Δ pH amplitude during the fermentation process. This technique increases by 10% the maximal amount of PDO attained, as well as additional 22% higher productivity and 35% higher yield values (Petrov & Stoyanov, 2012). A suitable pH control (maintained at a value of 7.0) improves PDO

production by *Klebsiella oxytoca* FMCC-197 from the same initial amount of raw glycerol in batch cultivations doubling the final mass to volume concentration of PDO, also obtaining 29% higher yield and reducing fermentation time from 96 to 32 h (Metsoviti et al., 2012). This strategy was successfully tested in batch experiments with other biocatalysts, such as *Klebsiella pneumoniae* (Vieira et al., 2015), *Citrobacter freundii* (Drozdzynska et al., 2014)and *Lactobacillus diolivorans* (Pflugl et al., 2012) (see Table 1).

Due to the fact that experimental results using higher initial pH values from 6.9 led to a remarkable decrease in the PDO production rate, it was decided to start with this value, allowing for the free evolution of this variable during the first hours of the process. Thereafter, pH was controlled within the optimal range shown in the previous section (6.7 to 6.0), using pH forced fluctuations or maintaining it constant to 6.5 or 6.0 values (see Table 2). The best results were obtained by controlling pH at 6.5 after free evolution from 6.9. Under these conditions, PDO synthesis, substrate uptake rate and biomass growth were improved, compared with experiments without pH control (see Figure 2-A). Runs featuring pH control from 6.5 to 6.9 were very similar to each other, yet the values of yield and productivity were slightly higher when the pH forced fluctuations method was not used: 0.49 vs 0.45 g/g and 1.25 vs 1.14 g/(L·h), respectively. Moreover, residual glycerol was clearly reduced when pH was controlled within 6.9 to 6.5, using the forced fluctuations method or keeping it constant to 6.5. These results resemble those obtained by other authors in the literature (see Table 1).

pH control allowed for better growth conditions, reaching 1.3 g/L of dry biomass by the end of the fermentation process. This improvement leads in turn to more biocatalyst production, increasing substrate uptake rate and product formation and reducing the inhibitory effect of acid conditions, due to the synthesis of by-products.

3.4 Influence of the use of raw glycerol

In order to evaluate the industrial application of this bioprocess, the use of raw glycerol as substrate has been considered under the same experimental conditions as those employed in the previously described assays utilizing pure glycerol in 2 L STBR, namely: 37ºC of temperature, 200 rpm of stirrer speed, 30 g/L of initial substrate concentration and the aforementioned pH control strategy (free evolution to 6.9 to 6.5 and subsequent control at 6.5). The results are shown in Table 3, where it can be seen that the final concentration of 1,3-propanediol was higher than that obtained with pure glycerol, while yield and productivity were slightly lower (see Table 2 and 3). No residual glycerol was observed after 12 hours and the biomass growth was higher with raw glycerol (1.5 g/L, see Figures 2-D and 3-C). This phenomenon could be ascribed to the presence of phosphates and salts in raw glycerol, which can help to buffer pH medium in the early stages of the fermentation, improving growth, uptake substrate and PDO production rates (Chatzifragkou et al., 2012; Jalasutram et al., 2011; Metsoviti et al., 2012). Considering these results, raw glycerol has been employed to determine the optimal stirring speed and the effect of sparging gas (air or nitrogen) during fermentation.

3.5 Study of the stirring speed

The influence of the stirring speed employing raw glycerol has been evaluated in STBR under the previously described batch conditions. Five different stirring conditions were tested from 100 to 400 rpm (see Table 3). The consumption of initial substrate increased as stirring speed escalated, improving C_{PDO} yield and productivity values. From 200 to 400 rpm of stirrer speed, no residual glycerol was detected in HPLC analysis after at 12 hours. Biomass growth was affected from 300 rpm, a fact more clearly observed at 400 rpm (see Figure 3-E), where microbial growth rate was lower and the total dry biomass measured was reduced by 12%. This decline involved a decrease in PDO production, in terms of final concentration, yield and productivity. This stirring speed study reflected that 1,3-propanediol is a product partially associated to growth, which leads to conclude that microbial growth and product synthesis

were closely coupled and interdependent. PDO production results were very similar when the stirring speed employed was 200 and 300 (see Figure 3). Due to the need of reducing operational process costs, 200 rpm was selected as the optimal stirring speed value in bioreactor batch cultures.

3.6 Study of aeration strategy

S. blattae is an anaerobic facultative bacterial strain. Recent works employing microorganisms with similar metabolic pathways highlight the importance of studying the influence of aeration strategies in the bioprocess development (Wang et al., 2011). Small airflow supplied as sparged bubbles from the bottom of the reactor can increase the specific activity of glycerol dehydratase improving PDO production and yield under high glycerol concentrations (Wang et al., 2011; Zheng et al., 2008). Based on airflow values employed in the literature, an experimental plan was designed, testing three aeration conditions of air (0.04 and 0.08 vvm) and nitrogen (1 vvm). The results are presented in Figure 4, comparing the profile concentrations of biomass, glycerol, PDO and organic acids in different runs without gas supply (Figure 4-A). For this system, the air supply failed to bring any improvement in the conversion of the bioprocess, compared to runs in the absence of gas (see Table 3). Total PDO concentration was sharply reduced when airflow was increased and high quantities of residual glycerol (13.4% at 0.04 vvm and 32.6% at 0.08 vvm) were detected. Moreover, biomass growth rate was clearly negatively affected by oxygen supply, especially at longer times, in the latter part of the batch experiment (see Figures 4-B and 4-C).

Supplying high nitrogen gas flow to create a completely anaerobic environment did not provide any better results than batches in the absence of gas. Substrate uptake rate was lower when nitrogen was present during fermentation, remaining 14.5% of residual glycerol at 12 hours. Yield and productivity values were slightly higher than those obtained in the run carried out without gas supply, and the total concentration of PDO obtained were very similar to each

other. The synthesis of organic acids (lactic, succinic, acetic, formic) was not affected by air or nitrogen supply, since these concentration profiles were very similar in the different conditions employed, except in the run with the highest airflow (Figure 4-C), where the final concentration of by-products was lower, because the glycerol conversion was much slower compared to the rest of the runs.

Kinetics of 1,3-propanediol production bioprocess

The evolutions throughout time of the concentrations of biomass, glycerol and PDO in all the runs conducted under different operational conditions are given in Figures 1 to 4. Moreover, in Figures 1 and 2, the evolution of pH is shown, and in Figure 4, the concentrations of the main by-products are added.

Figure 1 depicts the influence of the initial pH values in the different runs in which this variable is not controlled, i.e., it was allowed to vary freely. As can be seen, when the initial pH is increased, all the rates decrease; thus, glycerol uptake, PDO production and growth rates are smaller when the initial pH is increased from 6.9 to 8.1. Nevertheless, the maximum biomass and 1,3-propanediol concentrations are very similar from one run to another, around 0.9-1 g/L and 10 g/L, respectively, although the time needed increases at higher initial pH.

The effect of pH control is shown in Figure 2. As can be observed, when the pH value is not allowed to reach a value under 6.5, the substrate uptake rate and the PDO production rates are much higher than those attained when the pH takes lower values. Also, in the case of control of pH at 6.5, the maximum biomass concentration is higher, although the growth rates are similar in all cases. As previously indicated, the maximum PDO concentration is reached when the pH is controlled from 6.9 to 6.5.

The effect of stirring speed is given in Figure 3. In this figure it is evident that the rates of glycerol uptake, growth and PDO production, increase as the stirring speed intensifies from

100 to 200 rpm. For stirrer speed higher than 200 rpm, the results are similar, although this may not be evident for growth rate. The latter effect must be due to the fact that the concentration of the carbon source, glycerol, decreases to zero. The maximum PDO production rate is observed at 200 and 300 rpm of stirring speed, then falling when 400 rpm is used.

Finally, Figure 4 shows the influence of gas supply (air, nitrogen or no gas supply). As can be seen, when air is bubbled, all the rates decline the rates of glycerol uptake, growth, PDO production and even that of the by-product production. This means that the bacterial metabolism is negatively affected by the presence of oxygen. The best results, as previously indicated, are reached in the run conducted without any gas supply under strict anaerobic conditions. PDO production by *S. blattae* ATCC 33430 using raw glycerol as sole carbon substrate was very similar to other previous works that employed risk class 2 strains (see Table 1). Recently published studies carried out by other class 1 strains have reached promising amount of PDO (yielding 0.66 g/g and productivities of 1.42 g/(L·h)), although its viability has not been tested using raw glycerol yet (Vieira et al., 2015).

4. Conclusions

S. blattae ATCC 33430 is presented as a class 1 suitable biocatalyst for the conversion of glycerol into 1,3-propanediol, studying several process variables at shaken bottle and STBR batch cultivations. The optimal culture was carried out employing 30 g/L of substrate, a temperature of 37ºC, controlling pH at 6.5 after free evolution from 6.9, with a stirring speed of 200 rpm and without any gas supply. Further conversion was also tested using raw glycerol, reaching similar results to those obtained with pure glycerol: 13.84 g/L of 1,3-PDO, with a yield of 0.44 g/g and a productivity of 1.15 g/(L·h).

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Legend of Tables and Figures

Table 1. 1,3-propanediol production by different bacterial strains cultivated on substrates containing glycerol as sole or co-substrate in batch fermentations. **Table 2.** Overview of C_{PDO}, Y_{PG}, residual substrate and P_{PG} for the batch cultivations of *S. blattae* using pure glycerol.

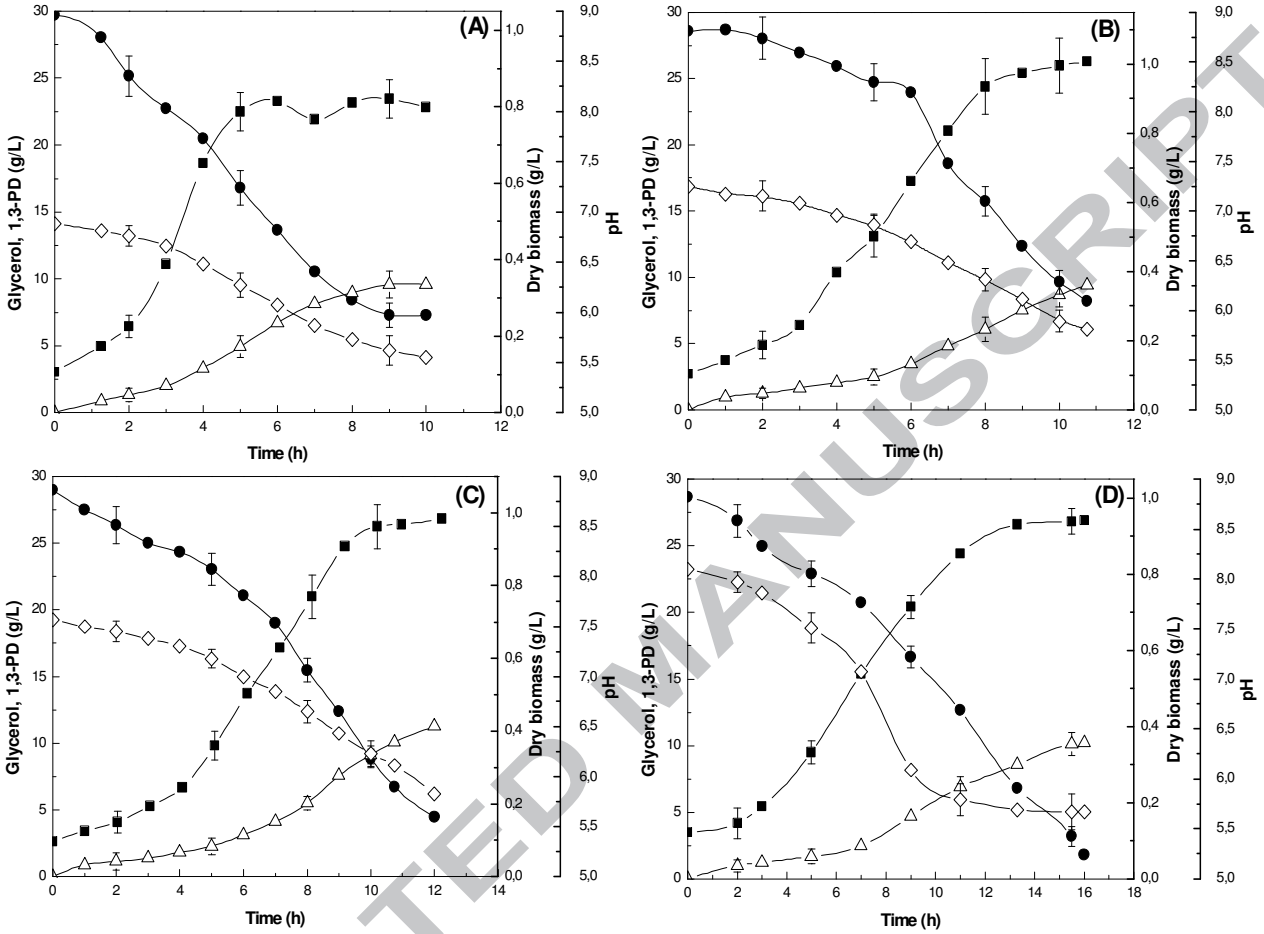
Table 3. Overview of residual substrate, C_{PDO}, Y_{PG} and P_{PG} for the batch cultivations of *S. blattae* using crude glycerol.

Figure 1. Influence of initial pH in STBR batch cultivations: time of course of the concentrations of glycerol (closed circles), 1,3-propanediol (open triangles), dry biomass (closed squares) and pH (open diamonds); (A) $pH_0 = 6.9$; (B) $pH_0 = 7.3$; (C) $pH_0 = 7.6$; (D) $pH_0 = 8.1$.

Figure 2. Study of pH control strategy in STBR batch cultivations from pure glycerol: time of course of the concentrations of glycerol (closed circles), 1,3-propanediol (open triangles), dry biomass (closed squares) and pH (open stars); (A) uncontrolled; (B) controlled by pulses from 6.5 to 6.9; (C) controlled from 6.9 to 6; and (D) controlled from 6.9 to 6.5.

Figure 3. Results of the runs carried out at different stirring speed in STBR batch cultures using raw glycerol. Time of course of glycerol (closed circles), 1,3-propanediol (open triangles) and dry biomass (closed squares); (A) N = 100 rpm; (B) N = 150 rpm; (C) N = 200 rpm; (D) N = 300 rpm; and (E) N = 400 rpm.

Figure 4. Influence of gas supply during STBR batch cultivations from raw glycerol: time of course of the concentrations of glycerol (closed circles), 1,3-propanediol (open triangles), organic acids (open squares) and dry biomass (closed squares); (A) gasless batch; (B) air, 0.04 vvm; (C) air, 0.08 vvm; and (D) nitrogen, 1 vvm.



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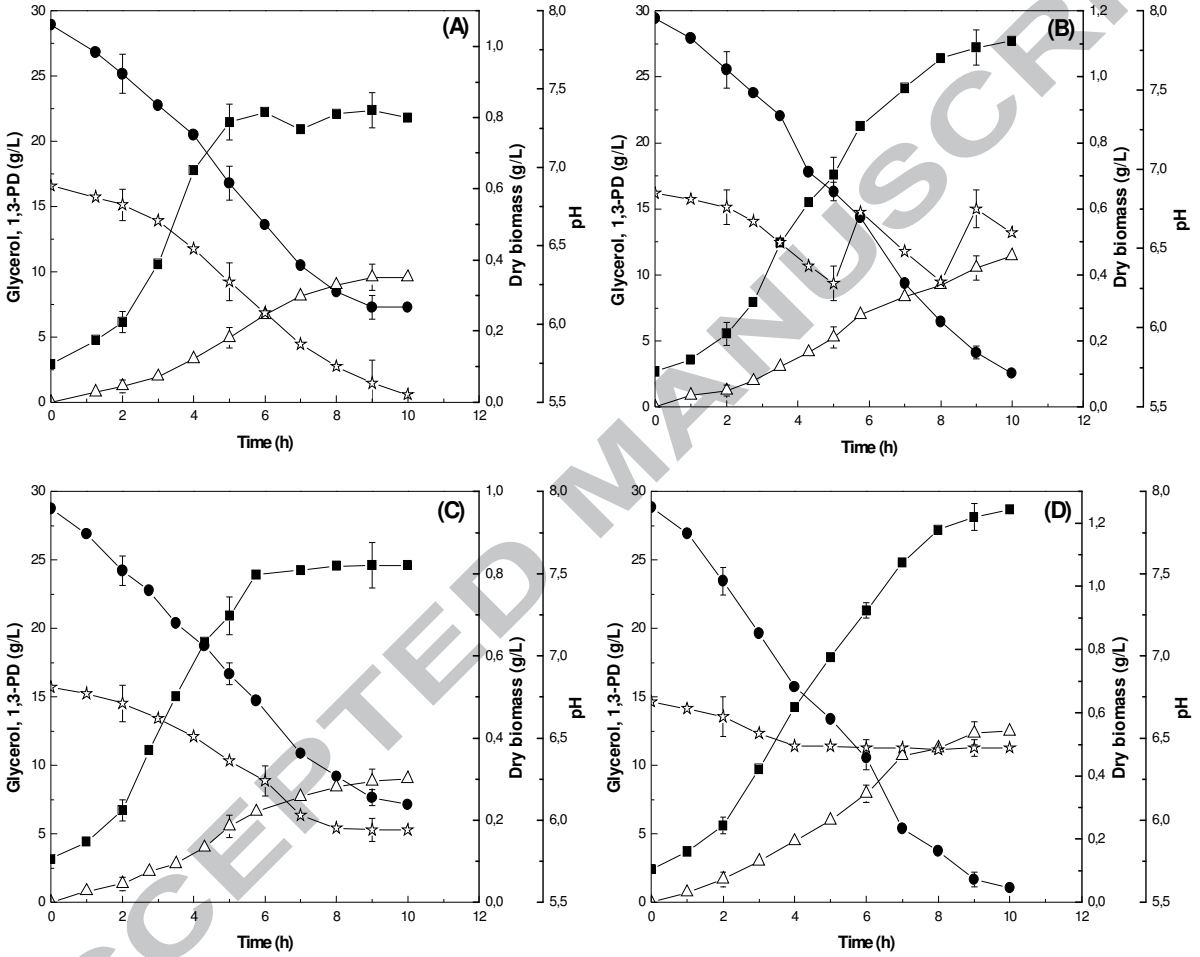

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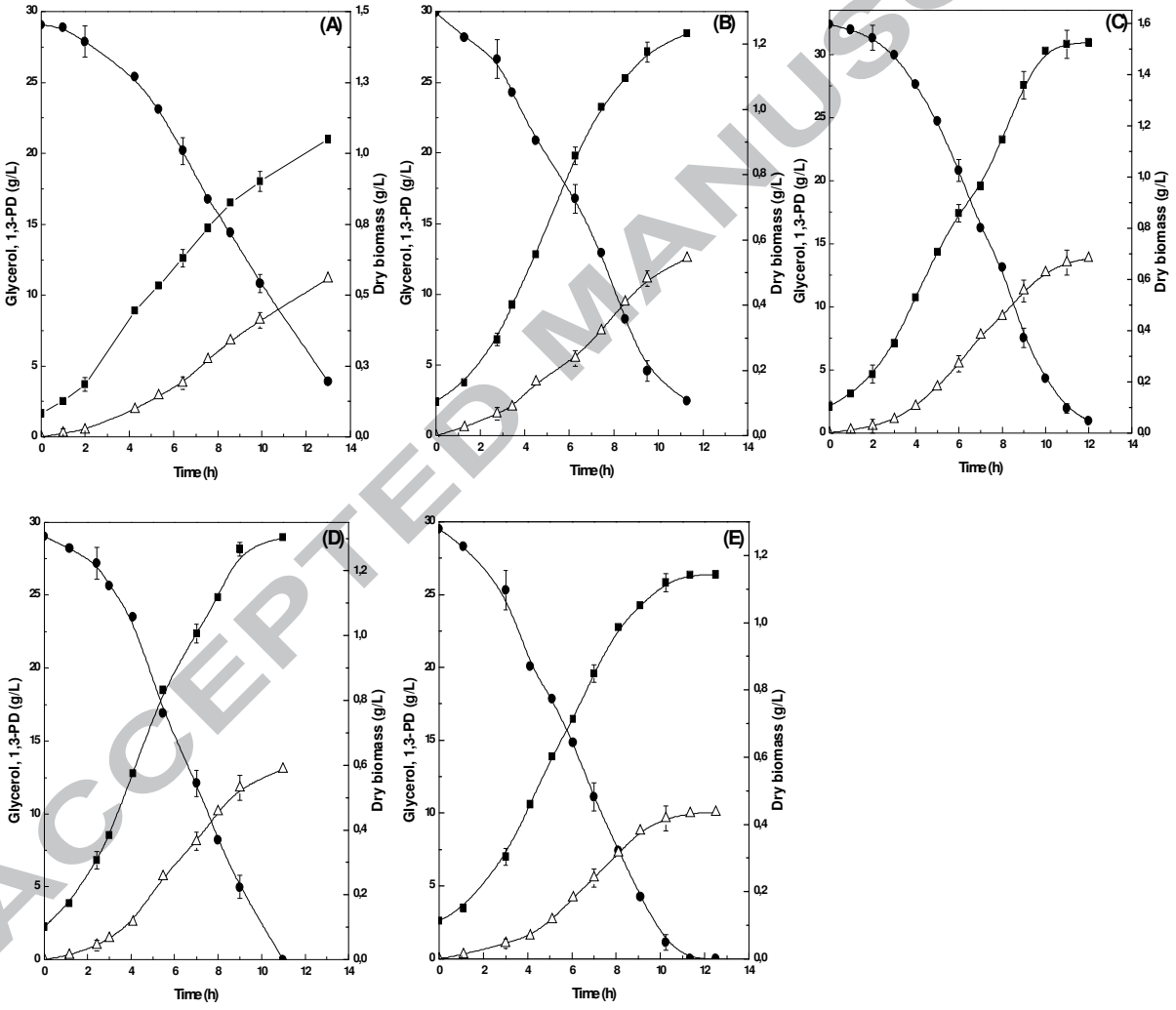

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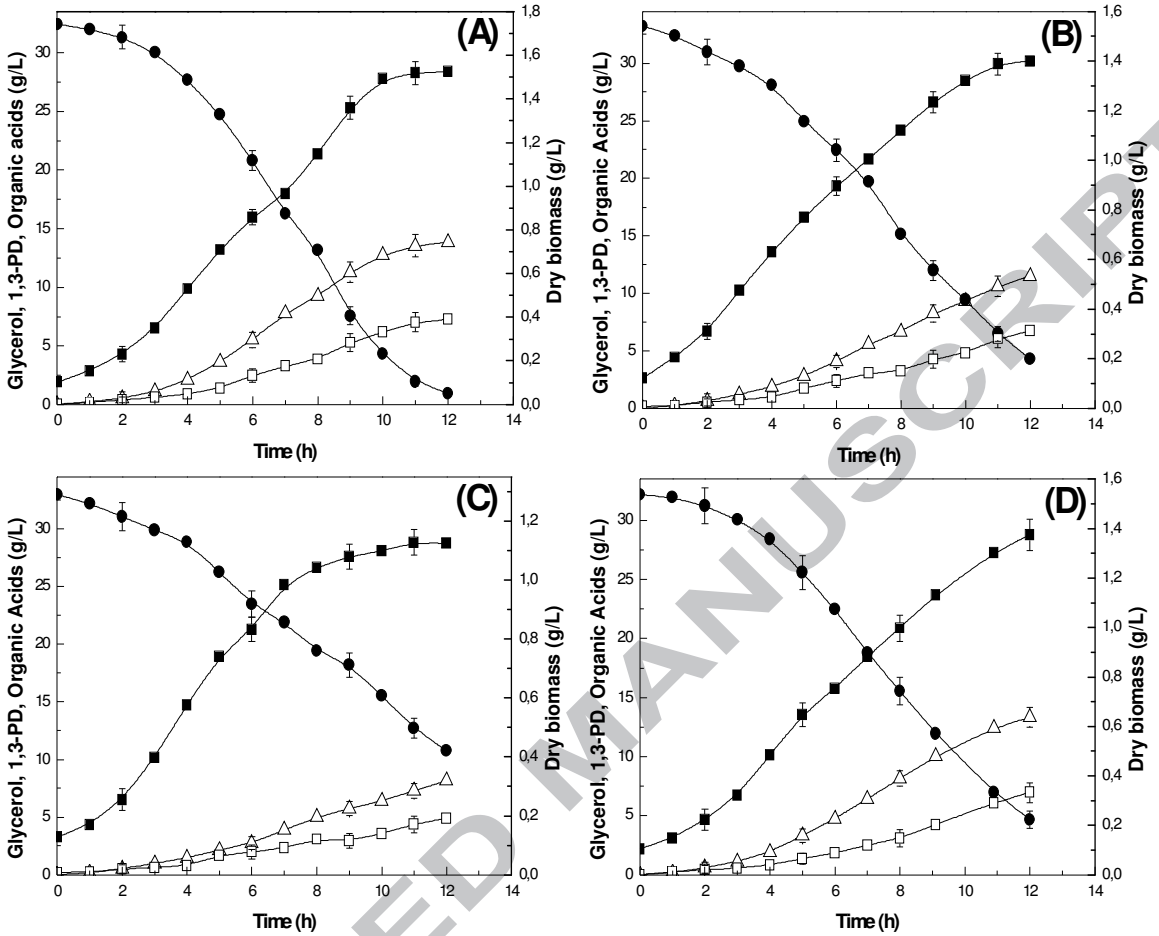

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Table 1. 1,3-propanediol production by different bacterial strains cultivated on substrates containing glycerol as sole or co-substrate in batch fermentations.

Glycerol type	Biocatalyst	Strain risk class*	Co-substrate	$Y_{P/G}$ (g/g)	$P_{P/G}$ [g/L·h)]	References
Pure	*K. pneumoniae*	2	-	0.75	1.53	(Zhang et al., 2007)
			-	0.23	0.77	(Zheng et al., 2008)
			-	0.51	1.70	(da Silva et al., 2014)
			-	0.35	1.30	(Durgapal et al., 2014)
	K. oxytoca	2	-	0.34	0.90	(Wojtusik et al., 2015)
	Klebiellasp.	2	-	0.36	0.18	(Yen et al., 2014)
	L. diolivorans	1	Glucose (3% w/v)	0.76	0.30	(Pflugl et al., 2012)
	L. reuteri	1	-	0.66	1.42	(Vieira et al., 2015)
	C. freundii	2	-	0.61	0.97	(Drozdzynska et al., 2014)
	H. alvei	2	-	0.45	0.28	(Drozdzynska et al., 2014)
	S. blattae	1	-	0.49	1.25	This work
Raw	*K. pneumoniae*	2	-	0.51	1.27	(Jalasutram et al., 2011)
			-	0.37	1.00	(Rossi et al., 2013)
	K. oxytoca	2	-	0.47	0.28	(Metsoviti et al., 2012)
	C. butyricum	2	-	0.58	N/A	(Dabrowski et al., 2012)
			-	0.56	0.76	(Chatzifragkou et al., 2012)
			-	0.54	1.55	(Szymanowska-Powałowska, 2014)
	S. blattae	1	-	0.45	1.19	This work

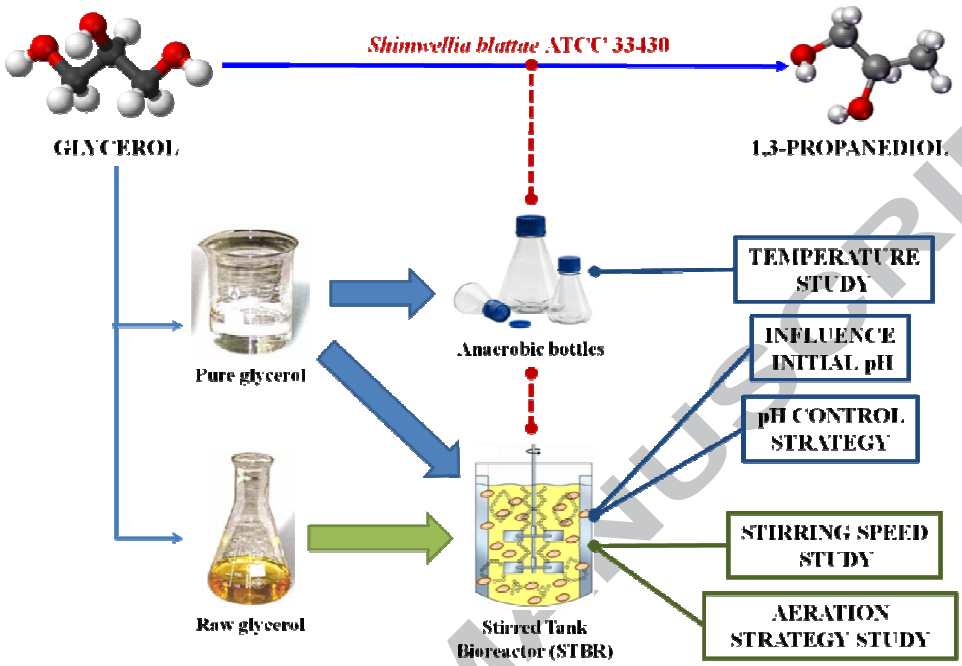
*According to Integrated Taxonomy Information System (ITIS)

Table 2. Overview of C_{PDO}, Y_{PG}, residual substrate and P_{PG} for the batch cultivations of *S. blattae* using pure glycerol.

Run Setup	T (ºC)	pH control strategy	pH_0	pH_F	Residual glycerol [% (w/w)]	C_{PDO} (g/L)	Y_{PG} (g/g)	P_{PG} [g/(L·h)]
Anaerobic bottle (10 mL)	33	Free	6.9	5.08	46.8	6.75	0.47	0.61
Anaerobic bottle (10 mL)	35	Free	6.9	5.04	38.4	8.75	0.46	0.76
Anaerobic bottle (10 mL)	37	Free	6.9	4.95	41.9	9.78	0.62	0.89
Anaerobic bottle (10 mL)	39	Free	6.9	4.94	40.6	9.52	0.62	0.86
Bioreactor (2 L)	37	Free	6.9	5.5	27.2	9.58	0.49	0.96
Bioreactor (2 L)	37	Free	7.3	5.5	28.8	9.41	0.46	0.88
Bioreactor (2 L)	37	Free	7.6	5.83	16.5	11.26	0.5	0.94
Bioreactor (2 L)	37	Free	8.1	5.67	6.68	10.21	0.41	0.64
Bioreactor (2 L)	37	Pulses	6.9	6.5	9.27	11.43	0.45	1.14
Bioreactor (2 L)	37	Controlled	6.9	6	28.9	9.04	0.51	0.9
Bioreactor (2 L)	37	Controlled	6.9	6.5	3.98	12.51	0.49	1.25

Table 3. Overview of residual substrate, C_{PDO}, Y_{PG} and P_{PG} for the batch cultivations of *S. blattae* using crude glycerol.

N (rpm)	Gas supply/Q_{Gas} (vvm)	Residual glycerol [% (w/w)]	C_{PDO} (g/L)	Y_{PG} (g/g)	P_{PG} [g/(L·h)]
100	-	13.6	11.16	0.45	0.86
150	-	8.54	12.55	0.48	1.11
200	-	-	13.84	0.44	1.15
300	-	-	13.08	0.45	1.19
400	-	-	9.98	0.34	0.88
200	Air/0.04	13.4	11.52	0.39	0.96
200	Air/0.08	32.6	8.13	0.37	0.68
200	Nitrogen/1	14.5	13.33	0.48	1.11



Shimwellia blattae, a wild type risk class 1 bacterium, produces 1,3-propanediol (PDO)

The influence of Temperature, pH and fluid dynamic conditions is determined

PDO production is not affected for the use of crude glycerol as raw material

Yield and productivity values are in line with published data from risk class 2 strains