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1,3-PROPANEDIOL: STATISTICAL OPTIMIZATION OF MEDIUM TO IMPROVE PRODUCTION BY *Clostridium beijerinckii* DSM 791

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ABSTRACT

Biodiesel is a promising alternative biofuel, and as its production increases, so does production of the principal co-product, glycerol. The present study is aimed at maximizing the glycerol consumption and 1,3-propanediol (1,3-PDO) production by *C. beijerinckii* DSM 791, using sequential experimental design methodology for optimization of nutrients concentration for the fermentation medium. Three sequential experimental designs were performed: Plackett-Burman, fractional factorial and central composite rotational. The following nutrients of the medium were evaluated: urea, yeast extract, $C_2H_3NaO_2$, KH_2PO_4, K_2HPO_4, $MgSO_4.7H_2O$, $MnSO_4.H_2O$, $FeSO_4.7H_2O$, glycerol, butyric acid, glucose and $CaCO_3$. Urea, KH_2PO_4, $MgSO_4.7H_2O$, $MnSO_4.H_2O$, $FeSO_4.7H_2O$, butyric acid, glucose and $CaCO_3$ were removed from the medium optimization since they had an insignificant statistical effect on 1,3-PDO production. The optimal concentrations of yeast extract, $C_2H_3NaO_2$, K_2HPO_4 and glycerol predicted by the optimization were as follows (g/l): 0.5, 0.005, 5 and 8, respectively, which were validated experimentally. Desirability function allows the maximization of the 1,3-PDO production and percentage glycerol consumption simultaneously, which resulted in yield of 0.58 mol/mol of 1,3-PDO and 100 % of glycerol consumption. The results showed that the use of sequential experimental design and the use of the desirability function led to the optimization of the 1,3-PDO fermentation by *C. beijerinckii* DSM 791.

Indexing terms/Keywords

1,3-propanediol; Glycerol; *Clostridium beijerinckii* DSM 791; Fractional factorial; Central composite rotatable design.

Academic Discipline And Sub-Disciplines

Biotechnology, Bioprocess Engineering, Design of experiments.

SUBJECT CLASSIFICATION

Biotechnology, Biochemistry, Bioprocess Engineering, Design of experiments, Statistical analysis.

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INTRODUCTION

In recent years, concern over the biological production of commercially important metabolites has been growing. This is mainly attributed to escalating global energy and environmental problems, which have led researchers worldwide to devise methods for producing almost everything in a “green” way [1,2]. Of these, the production of biofuels has attracted a great deal of attention [1]. Biodiesel is an alternative fuel that reduces net greenhouse effects and its use has become mandatory in many countries. The main by-product of the biodiesel plant is glycerol, a chemical compound of importance as an end product and as a starting material for other useful products [3].

The production of waste glycerol follows increasing biodiesel production; since the stoichiometry of the reaction dictates that for each 100 kg of biodiesel approximately 10 kg of glycerol is produced [4, 5]. The surplus of glycerol coming from biodiesel fabrication has increased enormously, representing about 65 % of the world’s glycerol production [3]. Compared to direct application and chemical transformation, microbial conversion of waste glycerol is a viable alternative without certain disadvantages, such as low product specificity, high energy input (pressure/temperature), intensive pretreatment requirements and/or release of environmentally toxic intermediate compounds [2, 4, 5]. In addition, compared to conventional biorefinery substrates, such as glucose and sucrose, waste glycerol presents a class of substrates that are inexpensive, sustainable, and not considered a suitable human food source [4, 5]. Moreover, bioprocess should be periodically reviewed and incorporate to technological innovations in order to increase their performance and lucrativeness [6].

Glycerol is the natural substrate for microbial production of 1,3-propanediol (1,3-PDO). First the glycerol is dehydrated to 3-hydroxypropionaldehyde (3-HPA) by glycerol dehydratase. Then the product of dehydration reaction, 3-HPA, is reduced to 1,3-PDO by an NAD-dependent oxidoreductase [7, 8]. Since 50% of the entire cost of microbial production of 1,3-PDO is due to the price of raw materials, waste glycerol from biodiesel production processes may be an interesting renewable carbon source for microorganisms that produce 1,3-PDO [9]. 1,3-PDO can be successfully produced fermentatively from glycerol by bacteria of *Enterobacteriaceae* and *Clostridiaceae* families, mainly *Klebsiella* spp., *Clostridium* spp. [5, 7, 10] and *E. coli* modified [11].

1,3-PDO is a bifunctional organic compound that could potentially be used for many synthesis reactions, in particular as a monomer for polycondensations to produce polyesters, polyethers and polyurethanes [7]. Products obtained by 1,3-PDO polymerization are characterized by good biodegradability, better specificity and higher industrial safety, in addition to being cheaper than those based on 1,2-propanediol, ethylene glycol or butanediol [4]. It has a number of other interesting applications in addition to that of polymer constituent, for example, synthesis of polytrimethylene terephthalate (PTT) that in turn can be used to make carpets (CorterraW, Shell), special textile fibers (SoronaW, DuPont), monofilaments, films, and nonwoven fabrics [12]. Furthermore, 1,3-PDO can improve the properties of solvents, adhesives, laminates, resins, detergents and cosmetics [13]. Therefore, this work is aimed at optimizing the fermentation medium to improve glycerol consumption and 1,3-PDO production by *C. beijerinckii* DSM 791 using sequential experimental design methodology.

MATERIALS AND METHODS

Microorganism and culture medium

C. beijerinckii DSM 791 was obtained from the Leibniz-Institut DSMZ – German Collection of Microorganisms and Cell Cultures and was maintained in microtubes at -80 °C on Reinforced Clostridial Medium (RCM) [14] with 30 % (v/v) of glycerol. Cellular growth was performed in two steps. First, the cells were cultured anaerobically in a rotary shaker (New Brunswick Scientific – Edison N. J., USA) at 37ºC, initial pH 6.5 and 80 rpm. The medium used for pre-culture and inoculum contained (g/l): peptone, 10; beef extract, 10; yeast extract, 3; NaCl, 3; cysteine, 0.5; $C_2H_3NaO_2$, 3; agar, 0.5; and glycerol, 5. The medium was reduced with nitrogen gas and sterilized before inoculation. Then the pre-culture, inoculum and fermentation medium were grown anaerobically in 100 mL flasks, containing 70 mL of medium, at 37 °C and 80 rpm. The pre-culture and inoculum times were respectively 16 h and 9 h, determinate in previous experiments. The inoculum culture was initialized with 10 % (v/v) from pre-culture medium and the fermentation medium with 20 % (v/v) from inoculum culture.

Optimization of the fermentation medium for 1,3-PDO production

A strategy of three sequential experimental designs was adopted to optimize the fermentation medium to improve 1,3-PDO production and glycerol consumption. The design experiment and the statistical treatment of the results were performed by the software package STATISTICA, version 6.0 (StatSoft, Inc.) including ANOVA, to obtain the impact and significance of each term and the interactions between the process variables and response. The fit quality of the polynomial model was expressed via the determination coefficient R^2, and its statistical significance was verified with the *F*-test using the same software program, considering a confidence level of 95 % or *p-value* less than 0.05 [15, 16].

In the first experimental design, a Plackett-Burman design was chosen to screen and identify variables that had significant influence. This design is based on the first order polynomial model [17]:

$$Y = \beta_0 + \sum \beta_i . X_i$$

where Y is the response, β_0 is the model intercept and β_i is the coefficient of linear equation, and X_i is the level of independent variable.

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Twelve components were selected for analysis: urea, yeast extract, $C_2H_3NaO_2$, KH_2PO_4, K_2HPO_4, $MgSO_4.7H_2O$, $MnSO_4.H_2O$, $FeSO_4.7H_2O$, glycerol, butyric acid, glucose and $CaCO_3$; in two levels, minimum and maximum, coded as "−1" and "+1", respectively, and three replicates of the center point, coded as "0" (Table 1) resulting in 19 runs performed at 37 °C and 80 rpm.

Table 1. Factors and levels used in the Plackett-Burman design for optimization of fermentation medium

Factor (g/l)	Code	Minimum -1	Center Point 0	Maximum +1
Urea	x1	0.50	1.50	3.00
Yeast extract	x2	0.50	1.50	3.00
$C_2H_3NaO_2$	x3	0.005	0.01	0.05
KH_2PO_4	x4	0.10	0.50	1.50
K_2HPO_4	x5	0.20	2.00	4.00
$MgSO_4.7H_2O$	x6	0.10	0.20	0.40
$MnSO_4.H_2O$	x7	0.005	0.01	0.02
$FeSO_4.7H_2O$	x8	0.01	0.30	0.50
Glycerol	x9	5.00	35.00	70.00
Butyric acid	x10	0.20	0.50	2.00
Glucose	x11	0.00	6.00	12.00
$CaCO_3$	x12	0.30	1.00	2.00

After the first screening experiments using Plackett-Burman design, a fractional factorial design was used to find significant factors affecting 1,3-PDO production. This design was used because it is accurate for estimating the main effects and interaction, with a reduced number of experiments if compared to a complete factorial design [18]. Yeast extract, $C_2H_3NaO_2$, K_2HPO_4, urea, $MgSO_4.7H_2O$ and glycerol were used as independent variables and the 1,3-PDO production was used as a dependent variable. Therefore, to analyze these six components the second experimental fractional factorial design (2^{6-2}) was performed with two levels, minimum and maximum, coded as "−1" and "+1", respectively, and three replicates of the center point (Table 2) resulting in 19 runs performed at 37 °C and 80 rpm.

Table 2. Factors and levels used in the fractional factorial (2^{6-2}) design for optimization of fermentation medium

Factor (g/l)	Code	Minimum -1	Center Point 0	Maximum +1
Urea	x1	0.00	0.50	1.00
Yeast extract	x2	0.50	1.50	3.00
$C_2H_3NaO_2$	x3	0.005	0.01	0.05
K_2HPO_4	x4	0.20	2.00	4.00
$MgSO_4.7H_2O$	x5	0.00	0.10	0.20
Glycerol	x6	5.00	10.00	20.00

Finally, a central composite rotatable design (CCRD) was carried out to optimize 1,3-PDO production using the response surface methodology. The concentrations of K_2HPO_4 and glycerol, ranging from 0.5 to 5.0 g/L and 2.0 to 12.0 g/L, respectively, were chosen as independent variables (Table 3). The experiment comprised 12 runs (3 replicate runs at the center point and 2 axial levels). The concentration of yeast extract and $C_2H_3NaO_2$ were maintained at 0.5 and 0.005 g/L, respectively, in accordance with the results obtained from the fractional factorial design performed at 37 °C and 80 rpm. 1,3-PDO production and percentage reduction of substrate (PRS) were used as response variables. The fundamental of this method, for quantitative variables, involves fitting first-order (linear) or second-order (quadratic) functions of the predictors to one or more response variables, and then examining the characteristics of the fitted surface to decide the appropriated action [18]. A quadratic polynomial equation was proposed to describe the mathematical relationship between the independent variables and response variables under the current conditions [15]:

$$Y = \beta_0 + \beta_1.x_1^2 + \beta_2.x_2^2 + \beta_3.x_1 + \beta_4.x_2 + \beta_5.x_1.x_2$$

where Y is the response, β_n are the coefficients and x_n the independent variables.

Table 3. Factors and levels used in the experimental CCRD for optimization of fermentation medium

Factor (g/l)	Code	Axial -1.41	Minimum -1	Center Point 0	Maximum +1	Axial +1.41
K_2HPO_4	x1	0.50	0.90	2.60	4.30	5.00
Glycerol	x2	2.00	3.50	7.00	10.50	12.00

The global desirability function (D) was performed to maximize the 1,3-PDO production and percentage reduction of substrate (PRS) simultaneously. This function consists in converting each response into a single *desirability* function (di) that ranges from 0 to 1 ≤ 0 ≤ d. For a function with two independent variables, a plot of composite *desirability* can be constructed as a function of filler alone, using the equation [19]:

$$D = (d_1.d_2)^{1/2}$$

The predicted values were validated experimentally in batch fermentation (three replicates), carried out in predicted conditions during 24 h.

Analytical methods

The concentrations of 1,3-PDO, glycerol and organic acids were analyzed by high-performance liquid chromatography (HPLC) equipped with a refractive index detector and UV detector. Samples were first centrifuged at 10.000 rpm for 10 minutes at 4 °C (Sigma Laborzentrifugen 2K15). The supernatants were filtered (Millex-HV, PVDF membrane, 0.2 µm pore size, 13 mm diameter - Millipore) for measurements of 1,3-PDO, glycerol and acids (lactic, acetic and butyric). The column used for separation was Hi Plex H, 300 x 7.7 mm (Agilent Technologies) at 45 °C. Analyses performed at a flow rate of 0.6 mL/min at a constant temperature of 45 °C, H_2SO_4 (0.5 mN) were the mobile phase and a wavelength of 210 nm. External standards were applied for identification and quantification of peak areas. The cell concentration (g/l) was determined using a linear equation derived from the relationship of cell dry weight (90 °C until constant weight) and the optical density (OD) at 600 nm.

RESULTS AND DISCUSSION

The Plackett-Burman design was the first step in the sequential strategy to select the factors to optimize the medium. The 1,3-PDO concentrations ranged from 0.34 g/l to 7.23 g/l (Table 4). In accordance with ANOVA (analysis of variance), using 95 % of confidence level (p ≤ 0.05) and standard error, the r squared was shown to be a good fit to the experimental results of 0.98 and the r squared adjusted 0.93. These results allowed made it possible to plot the Pareto chart (Fig 1), which provided important data about the statistical relevance of the factors. From this chart, one can see that urea, yeast extract, $C_2H_3NaO_2$, K_2HPO_4, $MgSO_4.7H_2O$, glycerol and glucose had significant relevance for 1,3-PDO production. On the contrary, KH_2PO_4, $MnSO_4.H_2O$, $FeSO_4.7H_2O$, butyric acid and $CaCO_3$ presented insignificant effect for 1,3-PDO production and then were removed from medium composition.

The glucose was eliminated from the medium composition since it presented the significant but negative effect for 1,3-PDO production and the interest of this work is to use glycerol. Even though the glycerol had a significant positive effect, the analysis range was decreased based on the residual glycerol results shown in Table 4, since the amount for residual glycerol reached was 68.18 g/l. The range for urea and $MgSO_4.7H_2O$ were reduced, since these nutrients presented a significant but negative effect. The other significant factors that presented a positive effect had the analysis range maintained and the significant factors with a negative effect had the analysis range decreased.

Nonetheless, the 1,3-PDO production can still be enhanced, as the center points are still lower than the other points, showing an increasing tendency in the direction of the maximum point. According to the results of the second experimental design, the fractional factorial (2^{6-2}), which are shown in Table 5, the 1,3-PDO production ranged from 2.32 g/l to 4.55 g/l. The ANOVA, using 95 % of confidence interval (p ≤ 0.05), of fractional factorial design results with replicated center points, the curvature in the tested region was significant with a *p-value* of less than 0.05, which suggests that a response surface study with a quadratic model is required to optimize 1,3-PDO production with the selected significant variables in this design. These results (Table 5) allowed made it possible the plotting of the Pareto chart (Fig 2), which provided important data about the statistical relevance of the factors, as well as of their interactions. From this chart, one can see K_2HPO_4 and glycerol had significant relevance for 1,3-PDO production. The K_2HPO_4 that presented a positive effect had the analysis range increased.

Even though the glycerol had a significant positive effect, the analysis range was decreased based on residual glycerol results shown in Table 5, since the amount for residual glycerol reached was 16.09 g/l. The urea and $MgSO_4.7H_2O$ was eliminated from the medium, since the minimum concentration analyzed was zero and these factors had a negative effect on 1,3-PDO production. The $C_2H_3NaO_2$ and yeast extract factors presented an insignificant effect on 1,3-PDO production, so these concentrations were kept to a minimum. The analysis of the Pareto chart also depicted significant curvature denoting that there exists a point of maximum 1,3-PDO productivity. Moreover, the values of the center points are close to the maximum obtained in the studied range, indicating the need to add axial points to the following experiment to optimize the 1,3-PDO production.

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Table 4. Matrix of experimental Plackett-Burman design and their corresponding results of 1,3-PDO production and residual glycerol (Res. gly.)

Run	x1	x2	x3	x4	x5	x6	x7	x8	x9	x10	x11	x12	1,3-PDO (g/l)	Res. gly. (g/l)
1	+1	-1	-1	-1	+1	-1	-1	+1	+1	-1	+1	-1	0.51	67.72
2	+1	+1	-1	-1	-1	+1	-1	-1	+1	+1	-1	+1	2.59	64.82
3	+1	+1	+1	-1	-1	-1	+1	-1	-1	+1	+1	-1	0.34	5.45
4	+1	+1	+1	+1	-1	-1	-1	+1	-1	-1	+1	+1	0.42	4.93
5	-1	+1	+1	+1	+1	-1	-1	-1	+1	-1	-1	+1	7.23	55.43
6	+1	-1	+1	+1	+1	+1	-1	-1	-1	+1	-1	-1	3.05	0.81
7	-1	+1	-1	+1	+1	+1	+1	-1	-1	-1	+1	-1	0.37	4.87
8	+1	-1	+1	-1	+1	+1	+1	+1	-1	-1	-1	+1	2.87	0.00
9	+1	+1	-1	+1	-1	+1	+1	+1	+1	-1	-1	-1	3.57	60.94
10	-1	+1	+1	-1	+1	-1	+1	+1	+1	+1	-1	-1	5.68	59.00
11	-1	-1	+1	+1	-1	+1	-1	+1	+1	+1	+1	-1	0.41	68.04
12	+1	-1	-1	+1	+1	-1	+1	-1	+1	+1	+1	+1	0.69	67.47
13	-1	+1	-1	-1	+1	+1	-1	+1	-1	+1	+1	+1	0.46	5.33
14	-1	-1	+1	-1	-1	+1	+1	-1	+1	-1	+1	+1	0.77	68.18
15	-1	-1	-1	+1	-1	-1	+1	+1	-1	+1	-1	+1	2.80	0.34
16	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	2.96	0.15
17 (PC)	0	0	0	0	0	0	0	0	0	0	0	0	0.62	34.69
18 (PC)	0	0	0	0	0	0	0	0	0	0	0	0	0.64	36.90
19 (PC)	0	0	0	0	0	0	0	0	0	0	0	0	0.64	36.42

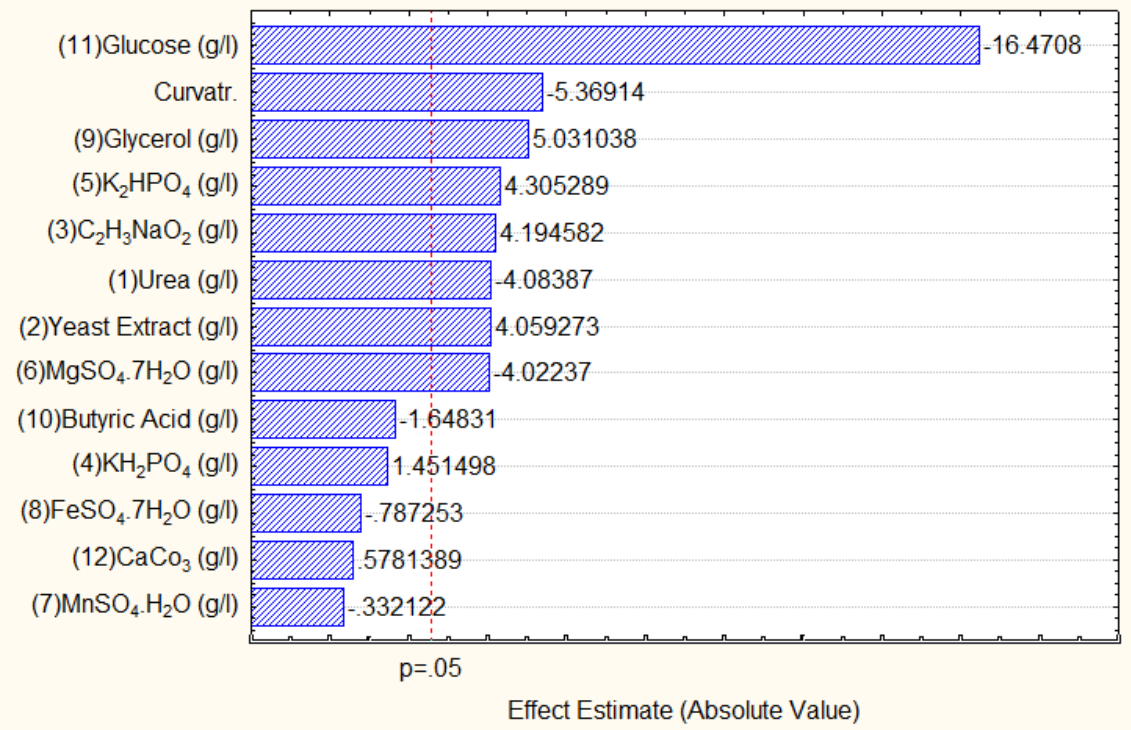

Fig 1: Pareto chart for 1,3-PDO production in the Plackett-Burman design

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Table 5. Matrix of experimental fractional factorial (2^{6-2}) design and their corresponding results of 1,3-PDO production and residual glycerol (Res. gly)

Run	x1	x2	x3	x4	x5	x6	1,3-PDO (g/l)	Res. gly. (g/l)
1	-1	-1	-1	-1	-1	-1	2.46	0.44
2	1	-1	-1	-1	1	-1	2.57	0.27
3	-1	1	-1	-1	1	1	3.35	13.37
4	1	1	-1	-1	-1	1	2.98	12.78
5	-1	-1	1	-1	1	1	2.64	14.24
6	1	-1	1	-1	-1	1	2.32	16.09
7	-1	1	1	-1	-1	-1	2.62	0.28
8	1	1	1	-1	1	-1	2.76	0.00
9	-1	-1	-1	1	-1	1	4.27	13.21
10	1	-1	-1	1	1	1	4.14	12.38
11	-1	1	-1	1	1	-1	2.89	0.10
12	1	1	-1	1	-1	-1	2.86	0.00
13	-1	-1	1	1	1	-1	2.86	0.00
14	1	-1	1	1	-1	-1	2.65	0.00
15	-1	1	1	1	-1	1	5.23	10.71
16	1	1	1	1	1	1	5.31	9.97
17 (PC)	0	0	0	0	0	0	4.41	6.11
18 (PC)	0	0	0	0	0	0	4.51	5.70
19 (PC)	0	0	0	0	0	0	4.55	5.57

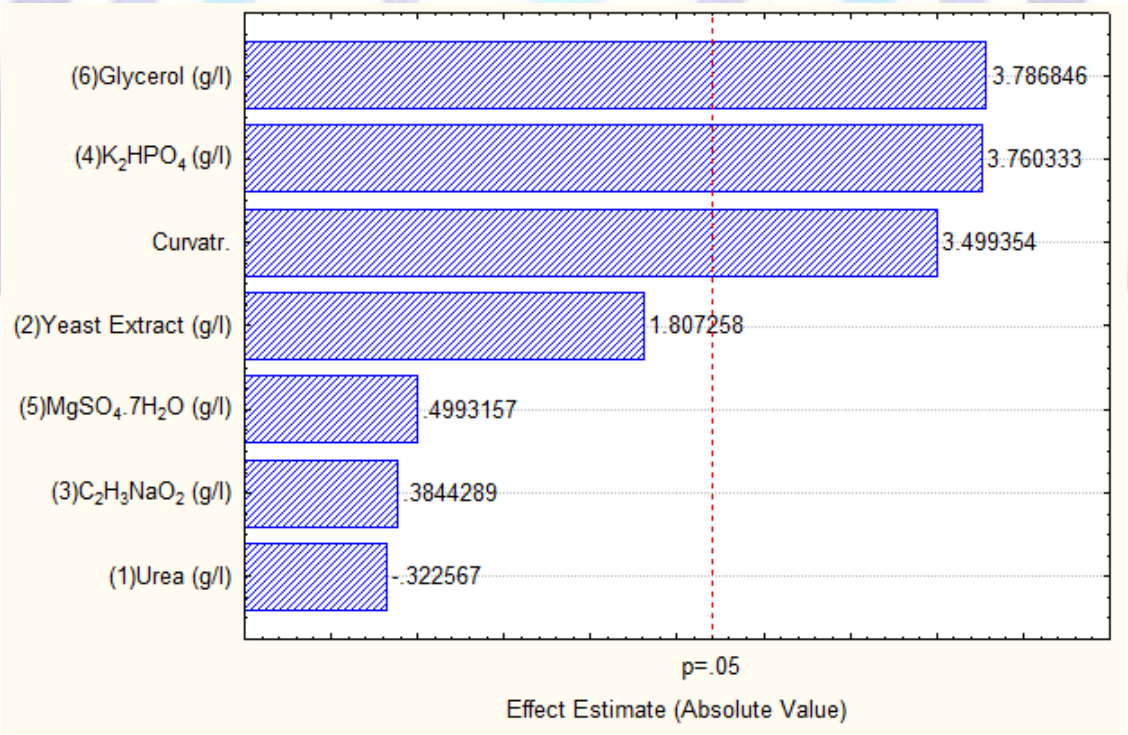

Fig 2: Pareto chart for 1,3-PDO production in the fractional factorial (2^{6-2}) design

Thus, the next step was to optimize the K_2HPO_4 and glycerol concentrations, evaluating the 1,3-PDO production and percentage reduction of substrate using the Central Composite Rotational Design (DCCR), since it allows the predicting of

the response surface with curvature plots and visualize of the maximum values of the dependent variables. The ANOVA of CCRD results were presented in Table 6 and 7, using 95 % of confidence interval ($p \leq 0.05$) and pure error, shows in both K_2HPO_4 and glycerol concentrations significant linear effects; this means the factors presented mutual interference in 1,3-PDO production and PRS (percentage reduction of substrate). The CCRD showed quadratic effect for glycerol ($p \leq 0,05$) on 1,3-PDO production and also for PRS, thus denoting a curvilinear plan, as can be observed in ANOVA.

Table 6. Analysis of variance (ANOVA) of factors for 1,3-PDO production

Factor	Sum of squares	Degrees of freedom	Mean Square	*F*	*p-value*
K_2HPO_4 (L)	0.407	1	0.407	85.906	0.003
Glycerol (L)	4.907	1	4.907	1035.532	0.001
Glycerol (Q)	1.358	1	1.358	286.589	0.001
1L by 2L	0.125	1	0.125	26.312	0.014
lack of fit	0.020	4	0.005	1.080	0.495
Pure error	0.014	3	0.005		
Total SS	6.831	11			

Table 7. Analysis of variance (ANOVA) of factors for PRS

Factor	Sum of squares	Degrees of freedom	Mean Square	*F*	*p-value*
K_2HPO_4 (L)	276.294	1	276.294	60.345	0.004
Glycerol (L)	2059.883	1	2059.883	449.901	0.001
Glycerol (Q)	310.569	1	310.569	67.832	0.004
1L by 2L	92.719	1	92.719	20.251	0.020
lack of fit	10.998	4	2.749	0.600	0.690
Pure error	13.736	3	4.579		
Total SS	2764.199	11			

Analyzing the data from Tables 6 and 7, the model adequately adjusts to the experimental points, representing the confidence of the results for both dependent variables analyzed. Considering the confidence level of 95 %, the models obtained were significant and were able to explain 99 % of variance (R^2), since the determination coefficient R^2 values (99 % to 1,3-PDO product and PRS), R adjust values (99 % to 1,3-PDO product and PRS), the *lack of fit* was insignificant (above 0,05) combined with the significant *F* and *p-values*. By means of ANOVA, for both response factors, significant effects quadratic and linear were found for the glycerol, while only a linear effect was found for K_2HPO_4, the quadratic term of K_2HPO_4 was removed from models because it presented insignificant effect. This means the glycerol concentration has more influence than K_2HPO_4 for both response factors analyzed (1,3-PDO production and PRS).

The response surface of the third experimental design is presented in Fig 3. The surfaces present the relation between dependent factors (Fig 3a: 1,3-PDO production and Fig 3b: PRS) and independent factors (glycerol and K_2HPO_4 concentrations). At the range studied, the higher production of 1,3-PDO was obtained at the higher level of glycerol and K_2HPO_4, presenting the maximum results of around 4 g/l to 1,3-PDO. The analysis of the PRS surface made it possible to observe the higher percentage of PRS was obtained at lower level of glycerol, while in the lower level of glycerol the influence of K_2HPO_4 level was the same.

The equations representing 1,3-PDO production and PRS, where x_1 is the K_2HPO_4 concentration (g/l) and x_2 is the glycerol concentration (g/l), follow:

$$\text{1,3-PDO (g/l)} = 3.43 - 0.45x_2^2 + 0.22x_1 + 0.78x_2 + 0.18x_1x_2$$

$$\text{PRS (\%)} = 90.88 - 6.82x_2^2 + 5.88x_1 - 16.05x_2 + 4.81x_1x_2$$

The global desirability value to reach the optimum for the two factors simultaneously was 0.93, meaning that the optimization by this function fulfills 93 % of the maximum obtainable for each dependent variable (response variable). The optimum concentration for K_2HPO_4 was 5 g/l and for glycerol was 8 g/l. The predicted values using the global desirability function and the experimental validation of the results in optimized conditions for 1,3-PDO production and PRS are presented in Table 8. It is observed that the experimental values are within the confidence limits −95 % and +95 %, showing that these experimental findings were in close agreement with the model prediction.

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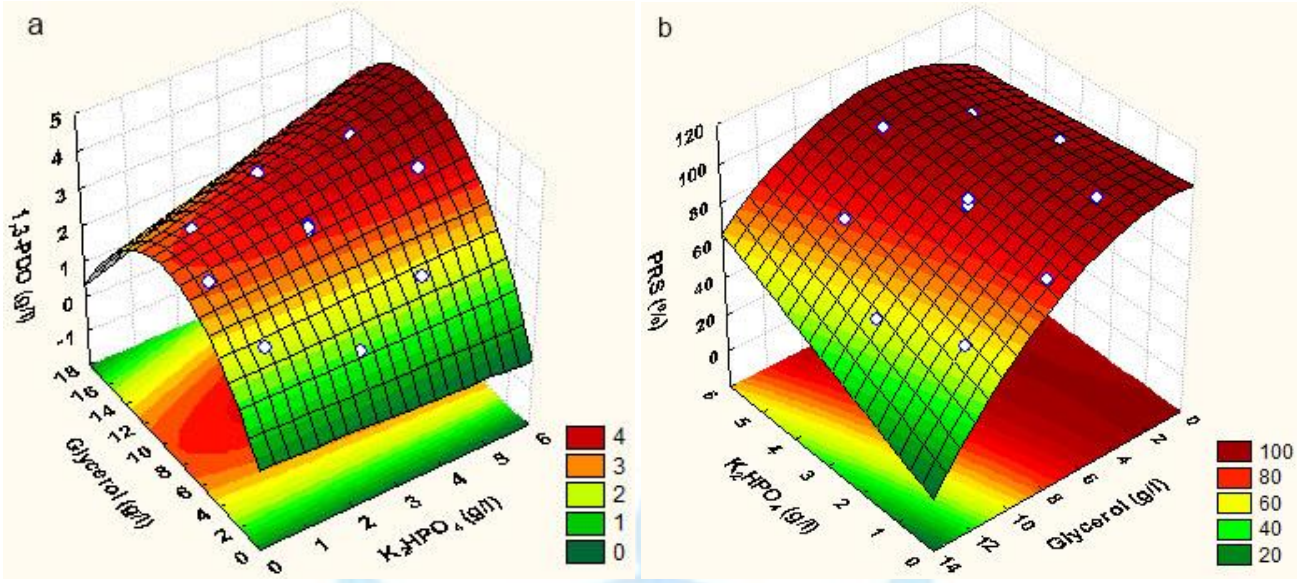

Fig 3: Response surface of K_2HPO_4 and glycerol concentrations for a: 1,3-PDO production and b: PRS

According to the results of Table 8 and Fig 4, the yield obtained was 0.58 mol/mol of 1,3-PDO per consumed glycerol in 12 h of fermentation without pH control. The efficiency of the glycerol fermentation by *C. beijerinckii* DSM 791 with the medium optimized in this study was 81 %, since Biebl et al. (1999) conclude the maximum yield for 1,3-PDO from glycerol by fermentation is 0.72 mol/mol of 1,3-PDO per consumed glycerol. It was demonstrated that *C. beijerinckii* DSM 791 was perfectly able to grow and produce 1,3-PDO on glycerol as the sole source of carbon and energy (Fig 4).

Table 8. Validation of the fermentation medium for improve glycerol consumption and 1,3-PDO production

Factor	Limits		Predict value	Experimental result
	-95 %	+95 %		
1,3-PDO (g/l)	3.85	4.14	3.99	3.85 ± 0.06
PRS (%)	91.70	100	96.18	100 ± 0.00

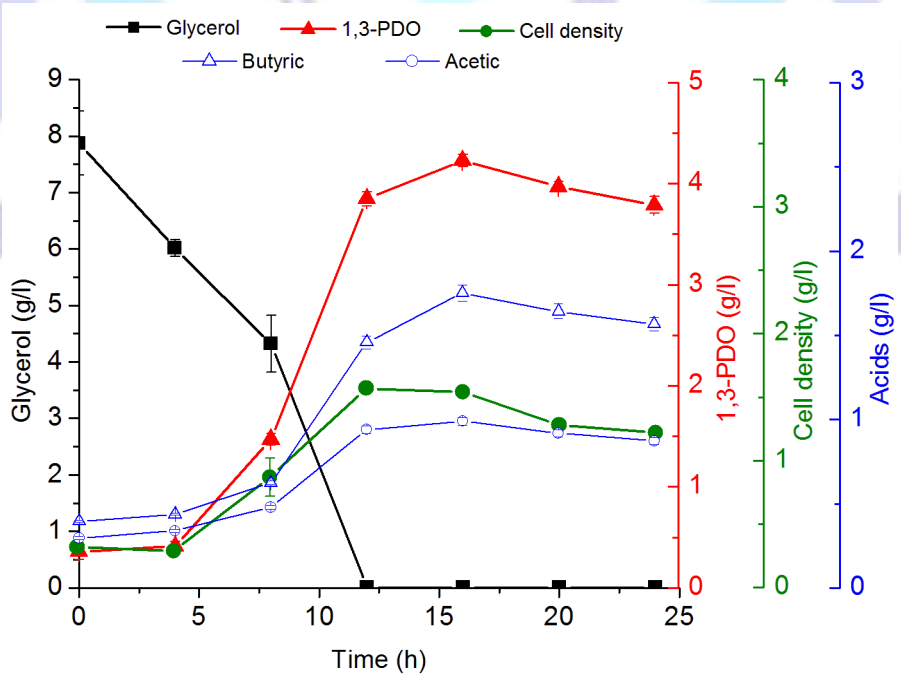

Fig 4: Kinetic profile using optimized conditions for improve glycerol consumption and 1,3-PDO production by *C. beijerinckii* DSM 791

The results of this study are comparable with those obtained by Gungormusler et al. (2010a), who obtained the yield of 0.58 mol/mol of 1,3-PDO per consumed glycerol in 24 h by *C. beijerinckii.* In another study, Gungormusler et al. (2010b) compared the 1,3-PDO production potential, without pH control, by different *Clostridium* spp. (*C. saccharobutylicum*, *C. acetobutylicum*, *C. pasteurianum*, *C. beijerinckii* and *C. butyricum*). Yields varied between 0.37 mol/mol to 0.54 mol/mol of 1,3-PDO per consumed glycerol while the PRS varied between 38 % and 93 % in 24 h of fermentation. Moon et al. (2011) optimized the medium for 1,3-PDO production from glycerol without pH control, by *C. pasteurianum* and obtained yield of 0.36 mol/mol of 1,3-PDO per consumed glycerol in 24 h.

The pH certainly influenced the metabolism of *Clostridium* spp. and some authors obtained higher yields of 1,3-PDO by controlling the pH during the glycerol fermentation. For example, Biebl et al. (1992) obtained 0.62 mol/mol of 1,3-PDO per consumed glycerol by *C. butyricum* and Otte et al. (2009) obtained 0.64 mol/mol of 1,3-PDO per consumed glycerol by *C. diolis*, controlling this important process variable.

CONCLUSIONS

The sequential experimental design strategy combined with the use of the desirability function for the optimization of the composition medium was shown to be an important tool for maximizing 1,3-PDO production and glycerol consumption by *C. beijerinckii* DSM 791, resulting in the yield of 0.58 mol/mol of 1,3-PDO per consumed glycerol. Among the evaluated factors, glycerol and K_2HPO_4 are of greater importance to 1,3-PDO production and percentage reduction of substrate by *C. beijerinckii* DSM 791. An optimum condition was successfully found and validated in this interval of study. The optimal concentrations for the medium compounds are 0.5 g/l of yeast extract, 0.005 g/l of $C_2H_3NaO_2$, 5 g/l of K_2HPO_4 and 8 g/l of glycerol.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical statement

This article does not contain any studies with human participants or animals performed by any of the authors. The authors confirm that principles of ethical and professional conduct have been followed in this research and in the preparation of this article.

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