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Improved 1,3-propanediol production with maintained physical conditions and optimized media composition: Validation with statistical and neural approach

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ABSTRACT

This work is aimed at assessing the use of response surface methodology (RSM) and artificial neural networks (ANNs) for modelling, and predicting, the optimum parameters for 1,3-Propanediol production by *Lactobacillus brevis* N1E9.3.3 from glycerol and glucose co-fermentation. A preliminary study of physical parameters was conducted using Plackett-Burman design to reduce the number of input variables up to seven; i) beef extract, ii) yeast extract, iii) $MgSO_4 \cdot 7H_2O$, iv) $MnSO_4 \cdot H_2O$, v) vitamin B_{12}, vi) glycerol and vii) glucose. The traditional RSM models were improved by ANN models between a 54.08% and 12.19% in terms of root mean square error (RMSE). This study suggested that RSM and ANN can be considered as effective tools to model and predict optimum parameters for 1,3-Propanediol production by *L. brevis* N1E9.3.3.

1. Introduction

1,3-Propanediol (1,3-PDO) is an important chemical compound used for different synthesis reactions, such as polycondensation reactions to produce polyesters, polyethers and polyurethanes [1,2]. The glycerol as the raw substrate is the only known natural source for 1,3-propanediol production and it is shown to be the best substrate, as the accumulation of glycerol was increasing in biodiesel industries, as the major by-product with a concentration of 10% (w/w) of biodiesel produced [3,4]. The industrialization of a polyester polytrimethylene terephthalate (PTT), competed with the commercial polyesters like polybutylene terephthalate (PBT) and polyethylene terephthalate (PET) due to the efficient supply of 1,3-propanediol, a monomeric subunit of PTT along with terephthalic acid or dimethyl terephthalate [5]. Two efficient processes for the production of 1,3-propanediol were developed in the past using acrolein and ethylene as the raw materials [6], later on, DuPont developed a bioprocess for 1,3-propanediol production using genetically engineered microorganisms [7].

Several microorganisms such us *Clostridium* [8], *Klebsiella* [9], *Lactobacillus* [10,11], *inter alia,* have been reported for production of 1,3-propanediol naturally from crude or pure form of glycerol. The kinetic behaviour of *Klebsiella* and *Clostridium* strains towards the substrate and products along with the genetic makeup of the particular operon favouring the dissimilation of glycerol towards 1,3-propanediol was investigated [1,12]. However, few strains of the genus *Klebsiella* reported for higher yields were pathogenic [10]; the maintenance of these cultures in an industrial scale would be hazardous. Hence, requirement of non-pathogenic strains which result in higher titers of 1,3-propanediol was a question to be addressed.

The members of genus *Lactobacillus* were well-known probiotic strains with industrial relevance. Few strains like *Lactobacillus reuteri* [13], *Lactobacillus diolivorans* [14], *Lactobacillus panis* [15] and *Lactobacillus brevis* [11] were found to produce 1,3-propanediol from glycerol. Though *L. brevis* was investigated to produce higher

Abbreviations: ANN, artificial neural network; APD, average percentage deviation; MLP, multi layer percepton; PBD, Plackett Burman design; RMSE, root mean square error; R^2, coefficient of determination; RSM, response surface methodology; 1,3-PDO, 1,3-propanediol.

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yields, only a few reports described the presence of 1,3-propanediol oxidoreductase gene and the heterologous expression studies of that particular gene [16]. Hence, detailed study on optimization of media components to increase the product concentration and yield would add relevance for utilization of the strain in an industrial scale production. The classical model to optimize experimental procedure is one-variable-at-a-time to monitor the influence of one factor at a time on an experimental response [17]. This method i) requires a lot of work, ii) does not allow determine cross effects of the parameters under investigation [18–20], iii) and increases the number of necessary experiments to conduct the research [17]. To solve these disadvantages, different analytical methods have been carried out by using multivariate statistic techniques [17], one of the most used option is the response surface methodology (RSM). RSM, is a method originally described by Box and Wilson in 1951 [21]. RSM is a mathematical and statistical method based on the fit of a polynomial equation [17] which enables to assess the effect of the independent variables and their interactions to improve and optimize the experimental process [18]. Response surface methodology generates a mathematical model, which can describe the process, for this reason, RSM models are applied in different fields from Food Technology [22] to Biorefinery [23], *inter alia*.

Nevertheless, RSM is not the only available tool for modelling and predicting production of compounds of interest. Artificial neural networks (ANNs) offer other attractive possibilities for providing non-linear behaviours for response surfaces [17]. For this reason, in the last three decades, ANNs have become one of the most used techniques for modelling and optimization, especially for non-linear problems [17,24]. Neural models are computational mathematical methods based on representative cells in biological neural system (neurons) [17–19] with deep and complex connections, forming a parallel network [25]. ANN learn in the similar way that human brain and presents benefits in learning process such as; i) can learn from imprecise data, ii) neural models can use experimental databases with noise and iii) can model non-linear behaviours [26]. The most used ANN model is the Multi Layer Perceptron (MLP) [24,27,28] where neurons are arranged in different layers; i) one input layer constituted by input neurons which collect input information (independent variables), ii) one, or more, intermediate layers associate with input neurons and iii), one output layer, associate to the last intermediate layer, to provide the predicted value [17]. MLP models allow modelling complex and highly non-linear behaviour through the different neural layers [29] and allow to predict an output on the basis of input data without the knowledge the relationship between them [30]. Due to that, ANN models are amply applied in different research fields such as, Hydrology [31] or Chemistry [32], *inter alia*.

Therefore, it seems clear that response surface models and neural models have a large scope of applications; however, their use as mathematical method to model 1,3-propanediol production has not been extensively studied. It is possible to find in literature a couple of papers relative to 1,3-propanediol. Kirilova et al. developed an ANN model used to biotransformation process of crude glycerol to 1,3-propanediol using bacteria *Pseudomonas denitrificans*1625 [33]. Their ANN model presents an RMSE ≈ 0.78 g/L, for production ranging between zero and ≈4 g/L [33]. Other interesting study was developed by Hongwen et al. to optimize the process for key enzymes accumulation of 1,3-propanediol production from *Klebsiella pneumoniae* [1]. The ANN model presented good results with and average relative error of 9.43% and a maximum relative error of 14.0% [1].

The aim of this paper was used *L. brevis* N1E9.3.3 isolated from soil samples in a municipal dumping yard through onsite enrichment technique which has shown significant 1,3-propanediol yields with the pure and crude form of glycerol under alkaline conditions. 1,3-PDO titers were optimized using uniform design and response surface methodology. Then, with the data obtained from various experimental runs, RSM model and the ANN model were developed to improve fermentation media composition.

2. Materials and methods

2.1. Experimental methodology

The 1,3-propanediol producing *L. brevis* N1E9.3.3 strain was isolated from onsite enriched soil from the municipal dumping yard in southern India as reported earlier [11]. The inoculum was prepared in MRS broth and incubated in an orbital shaker at 37 °C, for 16 h at 200 rpm. Five percent (v/v) of the inoculum was used to inoculate production media having composition of (per liter distilled water): 10 g bacterial peptone, 10 g meat extract, 5 g yeast extract, 30 g glucose, 20 g glycerol, 5 g sodium acetate, 2 g K_2HPO_4, 2.6 g sodium citrate dehydrate, 1.17 g $(NH_4)_2HPO_4$, 0.2 g $MgSO_4{\cdot}7H_2O$, 0.05 g $MnSO_4{\cdot}H_2O$, 4 mg vitamin B_{12} and 4 mg $CoCl_2$. The initial pH of the production media was adjusted to 8.5 using 5N NaOH as the value was observed to be optimum. The incubation was carried out at 37 °C, 72 h at 200 rpm with a working volume of 100 ml [11]. The aforementioned physical conditions were similar in all the experiments carried out in this work unless or until stated in particular.

2.2. Analytical methods

The post fermentation analysis of glycerol and secondary metabolites like 1,3-propanediol, lactic acid, acetic acid and ethanol was performed by HPLC (Shimadzu prominence UFLC) method using Rezex-ROA Organic acid column 300 × 7.8 mm (Phenomenex), coupled with RI detector with 0.01N H_2SO_4 as the mobile phase with a flow rate of 0.6 ml/min with column temperature 65 °C [11].

2.3. Plackett-Burman design

In preliminary study physical parameters were optimized using one factor at a time approach as described earlier [11]. With these conserved factors, the effect of each component in the production media on 1,3-propanediol production was analysed using Plackett-Burman design (PB). The experimental design is a factorial design; screening n variables, in this study eleven factors, coded between −1 and +1 for real values; i) beef extract [3,10] (g/L), ii) yeast extract [2,5] (g/L), iii) peptone [3,10] (g/L), iv) sodium acetate [2,5] (g/L), v) K_2HPO_4 [1,3] (g/L), vi) sodium citrate [1,3] (g/L), vii) ammonium hydrogen orthophosphate [0.50, 1,50] (g/L), viii) $MgSO_4{\cdot}7H_2O$ [0.05, 0.2] (g/L), ix) $MnSO_4{\cdot}H_2O$ [0.02, 0.05] (g/L), x) $CoCl_2{\cdot}H_2O$ [1,4] (mg/L) xi) vitamin B_{12}[1,4] (mg/L) along with 20 g/l glucose and glycerol respectively. The PB design is based on first order polynomial Eq. (1).

$$Y = \beta_0 + \sum_{i=1}^{N} \beta_i X_i \tag{1}$$

In the equation; Y is the response (1,3-PDO concentration g/l), β_o is the model constant, β_i is the linear coefficient, X_i is the level of independent variable [34]. The effect of each individual variable on 1,3-propanediol production was determined using Eq. (2).

$$E(X_i) = \frac{\sum_{i=1}^{N} M^{i+} - \sum_{i=1}^{N} M^{i-}}{N} \tag{2}$$

Where $E(X_i)$ is the calculated response of variable either in negative or positive, $\sum M^{i+}$ corresponds with the sum of high level