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Research Article

THE OPTIMIZATION OF MEDIUM CONDITIONS FOR PRODUCTION OF D-LACTIC ACID FROM SYNTHETICALLY WHEY BY *KLUYVEROMYCES LACTIS Y-8279* USING RSM

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Abstract

The present work was performed to optimize the production of lactic acid (POLA) by *Kluyveromyces lactis Y-8279* in artificially prepared neutralizing whey in batch experiments. Response surface methodology (RSM) which is a combination of Placket Burmann (PB), Steepest Ascent (SA) and Central Composite Designs (CCD) programs was successfully applied to obtain the maximum POLA. In the first step PBD was utilized to determine the most effectively factors on the POLA. The lactose concentration, yeast extract concentrations, pH and the medium temperature were determined as the most significant factors. Subsequently, the optimum combinations of the selected factors were explored by SA and CCD. The optimum values for lactose concentration, yeast extract concentrations, pH and medium temperature were found to be 18.8 g/L, 0.94 g/L, 5.2 and 32.4 °C, respectively. Experiments carried out at the optimum conditions revealed that the maximum lactic acid concentration of 1.4 g/L in 24 h of fermentation time. Furthermore *K. lactis* yeast strain was rarely used to product the D-lactic acid and the using it are discussed.

Keywords: *Kluyveromyces lactis*; RSM; Lactic acid; Optimization

Introduction

The use of microorganisms for industrial purposes has getting more attention by developing technologies. Recently, microorganisms are not only used for production fine bio-chemical like organic acids and alcohols but also used for wastewater treatment plants. One of them *Kluyveromyces* species has been the most widely used yeast strains for ethanol fermentation from cheese whey due to galactose fermenting capability of this yeast strain. *Kluyveromyces* cells are known to possess a lactose carrier protein (lactose permease) on their cell membrane that mediates the transport of lactose across the cell membrane (Dıckson and Barr 1983). Lactic acid is considered important ingredient for many foods, industrial, pharmaceutical and cosmetic applications. It is widely used as natural preservative and as an acidulant. Many species have been used for lactic acid production however; *K. marxianus*, *S. cerevisiae* and *K. lactis* yeast and some lactic acid bacteria (LAB) are the most frequently used (Tango and Ghaly (1999), Marta et al., 2000, Plessas et al., 2008).

The proposed mixed starter cultures of *K. marxianus* and *L. bulgaricus* ör *L. helveticus* can be successfully used to enhance lactic acid production from cheese whey. The POLA was used from the synthetic whey. Whey is a by-product of the dairy industry whose major components are lactose, proteins, and mineral salts (Siso, 1996). Being an expensive process with high investment and operational costs, treatment of whey seems to be a major problem for medium and small-scale cheese-making plants. Nevertheless, biological treatment of whey offers a favorable process because composition of whey is very suitable for some microorganism growth (Hwang and Hansen, 1997). Some of variable programs were used to optimize the independent parameters at industrial areas, biological areas and others. RSM is one

of them. It is an effective statistical technique commonly used for optimization of multi variable systems, particularly. It uses quantitative data in experimental design to determine and simultaneously solve multivariate equations in order to optimize processes or products (Giovanni, 1983). Identifying and fitting from experimental data an appropriate response surface model requires some use of statistical experimental design fundamentals, regression modeling techniques, and optimization methods. All three of these topics are usually combined into Response Surface Methodology (RSM). The RSM is also extremely useful as an automated tool for model calibration and validation especially for modern computational multi-agent large-scale social-networks systems that are becoming heavily used in modeling and simulation of complex social networks (Kathleenand et al., 2004). In fact, the relationship between the response and the independent variables is usually unknown in a process; therefore the first step in RSM is to approximate the function (response) through analyzing factors (independent variables). Usually, this process employs a low-order polynomial equation in a pre-determined region of the independent variables. If there is a curvature in the response, then a polynomial of higher degree, such as a second-order model, must be used to approximate the response, which is later analyzed to locate the optimum values of independent variables for the best response value (Ismail and et al., 1999, Prapullaz and at al., 1992). In some studies RSM was used to achieved collection of statistical techniques useful for developing, improving, and optimizing of β-galactosidase production using *K. lactis* NRRL Y-8279, lactose utilization in deproteinated whey, medium components for increased production of C-phycocyanin from *Phormidium ceylanicum*, xylitol production by *Candida guilliermondii* FTI 20037 (Ismail and et al., 1999, Seval and Yekta 2008, Aktas and et al., 2006, Aktas 2003).

This study was carried out to optimize the production of lactic acid in synthetically prepared neutralizing whey in batch experiments. Our findings in this study can be thought of as a new approach for producing free lactic acid by *K. lactis* yeast culture. To our knowledge some of bacteria and yeast were always used to product the lactic acid but *K. lactis* was used rarely to product the lactic acid on synthetic whey. Simulated whey was selected as the model media because it is a common dairy pollutant and has serious negative impact on environment. The investigation of the fermentation conditions was explored by a multi-step optimization procedure with aid of Design-Expert 6.0 trial version. PBD was applied for elimination of the significant parameters, while SA was used to locate the optimum region and finally CCD utilized to determine the optimum conditions for the maximum lactic acid concentration.

Materials and Methods

Yeast was provided by NRRL (Northern Region Research Laboratories) culture collection (Peoria,IL, USA). Lactose, KH_2PO_4, $(NH_4)2SO_4$, NaCl, $CaCl_2.2H_2O$, FeCl2. $6H_2O$ and $MgSO_4$. $7H_2O$, sulphuric acid and sodium hydroxide were purchased from Merck (Darmstadt, Germany). Agar, peptone, yeast and malt extracts were purchased from Oxoid (Hampshire, UK).

Yeast strain and maintenance

K. lactis strain was used in this work. First, lyophilized yeast was re-activated in 0.5 ml yeast malt extract medium (both at 3 g/L concentration) for 2– 3 min then culture was aseptically speared on solid agar slants combination of 3 g/L yeast extract, 3 g/L malt extract, 5 g/L peptone, 10 g/L glucose and 20 g/L agar in distilled water previously autoclave at 121 °C for 15 min. The inoculated solid medium was incubated at 30 °C for 4 days for appropriate growth and stored at 5 °C for further uses. The solid medium culture was transferred monthly for maintenance (Myers and Montgomery 1995).

Inoculum development and culture media

The solid cultures incubated at 30 °C were used for inoculation. Inoculums was prepared in 250-mL Erlenmeyer flasks containing 100 ml of inoculums medium consisting of 50 g/L lactose, 3 g/L yeast extract and 5 g/L peptone in distilled water previously autoclaved at 120 °C for 15 min. Incubation was carried out with constant shaking at 130 rpm at 30 °C for 24 h in a Jeio-Tech orbital shaking incubator, model SI-600R (Seoul, Korea). The inoculum was transferred aseptically into the fermentation medium at 5% (v/v).

The medium of fermentation was prepared from lactose, medium temperature, yeast extract and pH prepared as concentrations described in Table 3; the medium also contained the following chemicals at pH 5 and 130 rpm; KH_2PO_4 1 g/L, %65 $NH_4(SO_4)$,%35 $NH_4(PO_4)$ 6 g/L ,$CaCl_2$ 0.05 g/L, $MgSO_4$ $7H_2O$ 0.2 g/L, $FeCl_3$ $6H_2O$ 0.574 g/L, $ZnSO_4$ $7H_2O$ 0.532 g/L, $CuSO_4$ $5H_2O$ 0.112 g/L, $MnSO_4$ H_2O 0.056 g/L, Na_2MO_4 $2H_2O$ 0.014 g/L, yeast extract 1.5 g/L in distilled water. pH adjustment was made using either 0.5 N sulphuric acid or 0.5 N sodium hydroxide. The prepared fermentation medium was sterilized at 121 °C for 30 min.

Batch fermentation

For the effect of operational conditions, batch runs were performed in 250-mL Erlenmeyer flasks containing 100 ml of culture medium. Flasks were plugged with cotton stoppers and aerated through a silicone tube immersed in the medium. The batch runs started after the aseptic addition of inoculums to the fermentation medium with 5% (v/v) inoculum ratios. The runs were carried out in a temperature controlled orbital shaker (Jeio-Tech model of SI-600R, Seoul, Korea) at 150 cycles per min, which enabled adequate aeration and homogenization. All runs were performed for 24 h.

Analysis

Fermentation medium (5 ml) was removed aseptically and centrifuged at 5000 rpm for 10 min and the supernatant was removed for microbial concentration. Remaining materials was washed twice with distilled water and centrifuged, then diluted to original volume (5 ml) with distilled water for use in microbial concentration analysis. Microbial concentration was monitored spectrophotometrically with a Labomed, Inc UV–VIS spectrophotometer. Absorbance of prepared of microbial concentration solution was measured at 500 nm and was estimated from a microbial concentration dry weight vs. absorbance calibration. All experiments was determined like ın absorbance-calibration curve. The lactic acid concentration in the supernatant was determined using a Dıonex HPLC system with an HyperREZ XP Carbohydrate H^+) Column (300x7,7 mm), 0.005 M H_2SO_4 at 0,6 mL/min was used as an eluent, detection was RI 101 , and column temperature was 65 $^{\circ}C$.

Experimental design

RSM is a collection of mathematical and statistical techniques that are useful for the modeling and analysis of problems in which a response of interest is influenced by several variables and the objective is to optimize this response (Myers and Montgomery 1995). In order to ascribe the effect of factors at first on PBD Table 1 after than steepest-ascent Table 2 and a central composite design (CCD) Table 3 with four factors was performed. PBD design was applied using Design- Expert 6.0 (trial version). Twelve experiments were carried out with 7 factors and 4 dummy factors to find theirs affect to the responses POLA. The second program Steepest-Ascent was applied to maximum curve for POLA of *K. lactis* after PBD was applied. The method of steepest ascent is a procedure for moving sequentially along the path of steepest ascent that is, in the direction of the maximum increase in the response. Further studies for the optimization involved experiments carried out along the path of steepest ascent, which means, the direction at right angles to the contour lines representing equal yield, that shows the relative amounts by which the factors have to vary in order to attain a maximum increase of (Rodrigues and et al., 2006). The total number of experiments with four factors was $20 = 2k + 2^k + 6$, where k is the number of factors. Thirty experiments were augmented with six replications at the center points to evaluate the pure error. The first four columns of Table 3 show run number and experimental conditions of the runs arranged by CCD. Performance of the process was evaluated by analyzing the response, which was the yeast concentration after 24 h 130 rpm in the fermentation medium. In the optimization process the response can be related to chosen factors in quadratic models. A quadratic model given as

$$\eta = \beta_0 + \sum_{i=1}^{3}(\beta_{ii}) + \sum_{i=1}^{3}(\beta_{ii}).(\chi_i^2) + \sum_{i=1}^{2}\sum_{j=i+1}^{3}\beta_{ij}\chi i\chi i \qquad (1)$$

where η is the response, β_0 is the constant coefficient, χ_i (i = 1–3) are non-coded variables, β_i are the linear, and are the quadratic, and β_{ij} (i and j = 1–3) are the second-order interaction coefficients. Data were processed for Eq. (1) using the Design-Expert 6.0 program (trial version) including ANOVA to obtain the interaction between the process variables and the response. The quality of fit of the polynomial model was expressed by the coefficient of determination R^2 and its statistical significance was checked by the F-test in the same program.

Determination of maximum points.

The second-order model obtained from CCD studies, Eq. 2, is adequate for the optimal points. A general mathematical expression, Eq. 2, was used to locate the stationary points (Rodrigues and et al., 2006, Şahan and et al., 2010, Ramani 2014). Writing the second-order model in matrix notation, we have

$$y = \beta_0 + x^1 b + x^1 B x_s \qquad (2)$$

where,

$$x_s\,(\text{Stationary points}) = \begin{bmatrix} X_1 \\ X_2 \\ X_k \end{bmatrix}, \qquad b = \begin{bmatrix} \beta_1 \\ \beta_2 \\ \beta_k \end{bmatrix}, \quad \text{and}$$

$$B = \begin{bmatrix} \beta_1 & \frac{\beta_{12}}{2} & \frac{\beta_{1k}}{2} \\ & \frac{\beta_{22}}{2} & \frac{\beta_{2k}}{2} \\ sym & & \beta_{kk} \end{bmatrix} \qquad (3)$$

where b is a (kx1) vector of the first order regression coefficient and B is a (kxk) symmetric matrix whose main diagonal elements are the pure quadratic coefficients (β_{ii}) and whose off-diagonal elements are one half of the mixed quadratic coefficients (β_{ij}, $i \neq 1$). The stationary points (x_s) are the solution of Eq. 3

$$x_s = -\frac{1}{2} B^{-1} b$$

Results and Discussion

The objective at the onset of this experiment was to find the best set of operating conditions for POLA of *K. lactis*. The experiments were carried out in random order as required in many design procedures. In the experimental design, the optimum conditions sought were the operating conditions for maximizing POLA of *K. lactis***.** Analysis of the optimum conditions was carried out sequentially through PBD design to CCD of RSM.

Plackett–Burman Design

Culture conditions play an important role on growth and utilization of nutrients in all biological treatment systems. Thus, PBD design was successfully applied to investigate significant medium parameters (Ramani, 2014). The effects of independent 7 variables, which are medium temperature, pH, lactose, ammonia, yeast extract, NaCl, and $MgSO_4$ concentrations, on the growth conditions of *K. lactis* and utilization of lactose as well, were investigated through PBD method (Dickson and Barr 1983). Table 1 indicates the levels of the variables and their effects on the response**.** Based on the analysis of the variance (ANOVA), data not shown, lactose, yeast extract pH and medium temperature determined relatively the most effective parameters on the response. A first-order model evaluated form the software was fitted to data obtained from the experiment. The obtained first order model is given in Equation 4.

y = +5.49 + 1.16 Lactose + 0.43 Total Amonia + 2.26 Yeast Exract + 0.31 $MgSO_4$ - 0.48 NaCl + 3.81 pH - 1.29 Medium Temperature.
(4)

Path of steepest ascent

PBD is a valuable tool for screening parameters that significantly affect the response, but it is unable to predict the optimum region of the parameters. Based on the obtained firs order model equation and the four important medium parameters, the steepest ascent method was applied to find adequate direction of changing parameters (Jack and et al., 2004). The method of steepest ascent is a procedure for moving sequentially in the direction of the maximum increase in the response. Of course, if minimization is desired, then we call this technique the method of steepest descent (Montgomery 2009). The way of the steepest ascent was determined to find the proper direction of changing variables by increasing the concentration of lactose (X_1), yeast extract (X_2) concentrations, pH (X_3) and decreasing the medium temperature (X_4). Seven experiments were conducted to locate the plateau of the response. The path of the steepest ascent and the obtained results are given in Table 2. It was obtained that the highest response was 39.19 g/L wet microorganism when the lactose concentration, yeast extract concentration, pH and the medium temperature were 17 g/L, 0.91 g/L, 7, 26 ºC, respectively. It was concluded that the optimum point was in the region.

Effects of factors and their interactions on POLA

The effects of parameters on the response are valuable for regression analysis because the positive sign increases the response, while the negative sign decreases the response (Celevi and et al., 2007). The interaction between pH and medium temperature, the interaction between medium temperature and lactose concentration and interaction between pH and lactose concentration have positive effects but interaction between medium temperature and yeast extract have negative effects.

The results of experiments were expounded by Pareto analysis, ıt will be more significant information. The following equation was used for calculation of percentage of factors on POLA(Haaland 1989, Kesraoui and et al., 2008).

$$Pi = \frac{bi^2}{\sum bi^2} x100 \quad (i \neq 0) \qquad (5)$$

As shown in Fig. 1. Pareto graphic analysis was shown. Among variables, quadratic effect of initial concentration of dye (0.13 %), quadratic effect of pH (3.5 %), temperature (0.38 %) and quadratic effect of adsorbent amount (0.1 %), are effective parameters on POLA.

Central Composite Design

Based on the steepest ascent results the boundaries of the optimum point were determined to be in the region of 14-20 g/L of lactose and 0.5-1.30 g/L of yeast extract concentrations 5.50-8.50 pH and 23-29 ºC of medium temperature.

Thirty trials were performed to locate optimum conditions for maximum response. The experiments were carried out in random order as suggested in many design program (Jack and et al., 2004). In the experimental design, optimum conditions meant to be the operating conditions for maximizing the POLA. Table 3 shows experimental conditions for batch runs and the results (responses) in terms of the POLA.

Fig. 2a shows lactose concentration and pH effects on the POLA. As observed in Fig. 2a, the POLA has strongly been affected by both of them. As clearly being seen form Fig. 2a any increasement in medium temperature by 27 ºC and in lactose concentration by 18.8 was increase the POLA while higher values have negative effects on the response.

Fig. 2b represents lactose concentrations and temperature effect on the POLA at the fixed pH and yeast extract. As seen from Fig. 2 b) a curvature occurred in the response and the maximum POLA was obtained. Incensement in the lactose concentration by 18-19 g/L was increase the POLA while the same response was obtained in pH like in Fig. 2a In a work that like ours; optimum pH was obtained 7.0 for production of acetic acid and glycerol from salted and dried whey (Şahan et al., 2010).

Fig. 3a represents medium temperature pH effect on the POLA at the fixed lactose concentration of 16 g/Land yeast extract 0.90 g/L. A curvature occurred in the response and the POLA was obtained. Increasement in the pH by 5.0-6.0 was increase the POLA while increasement in the temperature 26-27 ºC was upset too. The temperature is the most effective on the production of glucose and xylose monosaccharide (Hye and at al., 2011).

Fig. 3b show that medium temperature and yeast extract concentration effect on the POLA at the fixed lactose concentration of 17 g/L and pH 7.0. A curvature occurred in the response and the POLA rate was obtained. Increasement in the yeast extract concentration by 1-1.5 g/L was increase the POLA but its concentration effect is rarely explored. The yeast extract concentration was calculated 1.5-2.0 g/L for optimization of D-lactic acid production by Lactobacillus coryniformis [24]. In some work, yeast extract is important parameters on butyric and lactic acid production (Chun-Huim and et al., 2009, Naveena and at al., 2005).

RSM was applied to build up an empirical model for POLA by *K. lactis* in terms of operational parameters of lactose, yeast extract concentrations, pH and medium temperature. A quadratic equation POLA was obtained through Design Expert 6.0 as in Equation 6.

The optimum levels of the selected variables were obtained by solving the regression equation and by analyzing the response Surface contour and surface plots (Niraj and et al., 2009, Abdelhay and at al.,2008).

$$POLA = +1.04 + 0.048X_1 - 0.25X_2 + 0.081X_3 + 0.045X_4 - 0.33X_1^2 + 0.10X_2^2 - 0.22X_3^2 - 0.33X_4^2 - 0.068X_1X_2 - 0.041X_1X_3 + 0.036X_1X_4 + 0.00081X_2X_3 - 0.065X_2X_4 - 0.054X_3X_4 \quad (6)$$

Figure 4 shows observed POLA versus the predicted ones from empirical model (Equation 6). from the figure, the predicted empirical model fairly represents POLA in a wide range of operating factors.

Table 1 The factors effects to response with PBD program and corresponding results (the response).

Run	Lactose (g/L)	Total ammonia (g/L)	Yeast Extract (g/L)	MgSO4 (g/L)	NaCl (g/L)	pH	Temperature (Co)	dummy	dummy	dummy	dummy	Biomass (g/L)
1	25.00	0.00	0.00	0.00	0.15	7.00	40.00	-1.00	1.00	1.00	-1.00	6.22
2	5.00	0.00	1.50	0.40	0.15	3.00	40.00	1.00	-1.00	1.00	-1.00	0.96
3	5.00	5.00	0.00	0.00	0.00	7.00	40.00	1.00	-1.00	1.00	1.00	3.52
4	25.00	5.00	1.50	0.00	0.15	7.00	20.00	1.00	-1.00	-1.00	-1.00	14.85
5	25.00	0.00	1.50	0.40	0.00	7.00	20.00	-1.00	-1.00	1.00	1.00	14.93
6	5.00	5.00	1.50	0.00	0.15	3.00	20.00	-1.00	1.00	1.00	1.00	2.50
7	5.00	5.00	1.50	0.40	0.00	7.00	40.00	-1.00	1.00	-1.00	-1.00	11.97
8	25.00	5.00	0.00	0.40	0.15	3.00	40.00	-1.00	-1.00	-1.00	1.00	1.23
9	25.00	5.00	0.00	0.40	0.00	3.00	20.00	1.00	1.00	1.00	-1.00	1.39
10	25.00	0.00	1.50	0.00	0.00	3.00	40.00	1.00	1.00	-1.00	1.00	1.25
11	5.00	0.00	0.00	0.40	0.15	7.00	20.00	1.00	1.00	-1.00	1.00	4.24
12	5.00	0.00	0.00	0.00	0.00	3.00	20.00	-1.00	-1.00	-1.00	-1.00	2.72

Table 2 Experimental design of steepest ascent and corresponding response

Run	Lactose (g/L)	Temperature (Co)	Yeast Extract (g/L)	pH	Biomass g/L
0	15.0	30.00	0.75	5	3.98
0 +1Δ	15.5	29.00	0.79	5.5	4.61
0 + 2Δ	16.0	28.00	0.83	6	20.39
0 + 3Δ	16.5	27.00	0.87	6.5	33.86
0+ 4Δ	17.0	26.00	0.91	7	39.19
0 + 5Δ	17.5	25.00	0.94	7.5	33.40
0 + 6Δ	18.0	24.00	0.10	8	28.55

Table 3 Experimental CCD runs and corresponding results (the responses)

Run	*X 1 Lactose (g/L)*	*X 2 Temperature (Co)*	*X3 Yeast Extract (g/L)*	*X4 pH*	*y1 POLA (g/L)*
1	17.00	26.00	1.70	7.00	0,650
2	17.00	26.00	0.90	10.00	0,840
3	20.00	29.00	0.50	5.50	0,500
4	17.00	26.00	0.90	7.00	0,990
5	17.00	26.00	0.90	7.00	2,540
6	14.00	29.00	0.50	8.50	0,370
7	17.00	26.00	0.90	7.00	0,160
8	17.00	20.00	0.90	7.00	0,000
9	20.00	23.00	0.50	5.50	0,390
10	23.00	26.00	0.90	7.00	0,010
11	17.00	26.00	0.10	7.00	2,790
12	17.00	26.00	0.90	4.00	0,010
13	20.00	29.00	0.50	8.50	0,620
14	17.00	26.00	0.90	7.00	0,870
15	20.00	29.00	1.30	8.50	0,099
16	20.00	23.00	1.30	5.50	0,006
17	14.00	23.00	0.50	8.50	0,029
18	11.00	26.00	0.90	7.00	0,000
19	17.00	26.00	0.90	7.00	0,150
20	14.00	29.00	1.30	5.50	0,003
21	14.00	23.00	0.50	5.50	0,000
22	20.00	29.00	1.30	5.50	0,015
23	14.00	23.00	1.30	5.50	0,011
24	14.00	29.00	0.50	5.50	0,001
25	14.00	23.00	1.30	8.50	0,077
26	20.00	23.00	1.30	8.50	0,002
27	17.00	32.00	0.90	7.00	0,000
28	20.00	23.00	0.50	8.50	0,003
29	17.00	26.00	0.90	7.00	1,520
30	14.00	29.00	1.30	8.50	0,001

Table 4 Coded values of independent variables

Variables	Symbols	Coding				
		−1.682	**−1**	**0**	**1**	**+1.682**
Lactose concentration (g/L)	X_1	11	14	17	20	23
Temperature °C	X_2	20	23	26	29	32
Yeast Extract (g/L)	X_3	0.10	0.50	0.90	1.30	1.70
pH	X_4	4.00	5.50	7.00	8.50	10.0

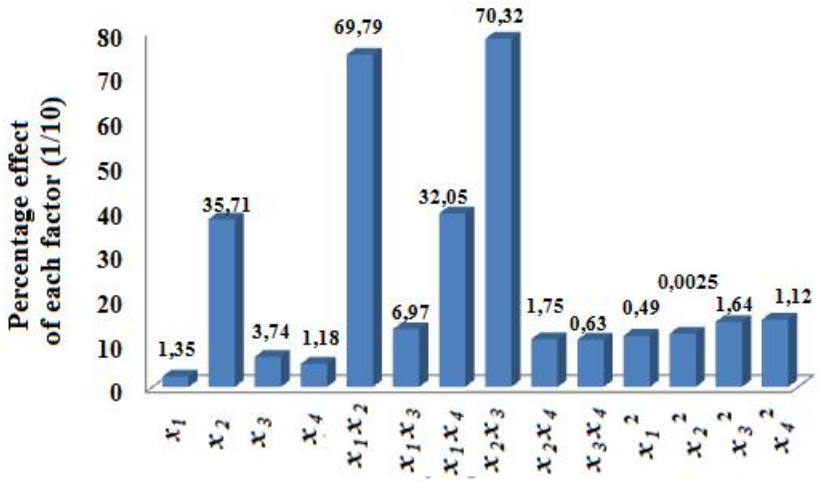

Figure 1. Pareto graphic analysis.

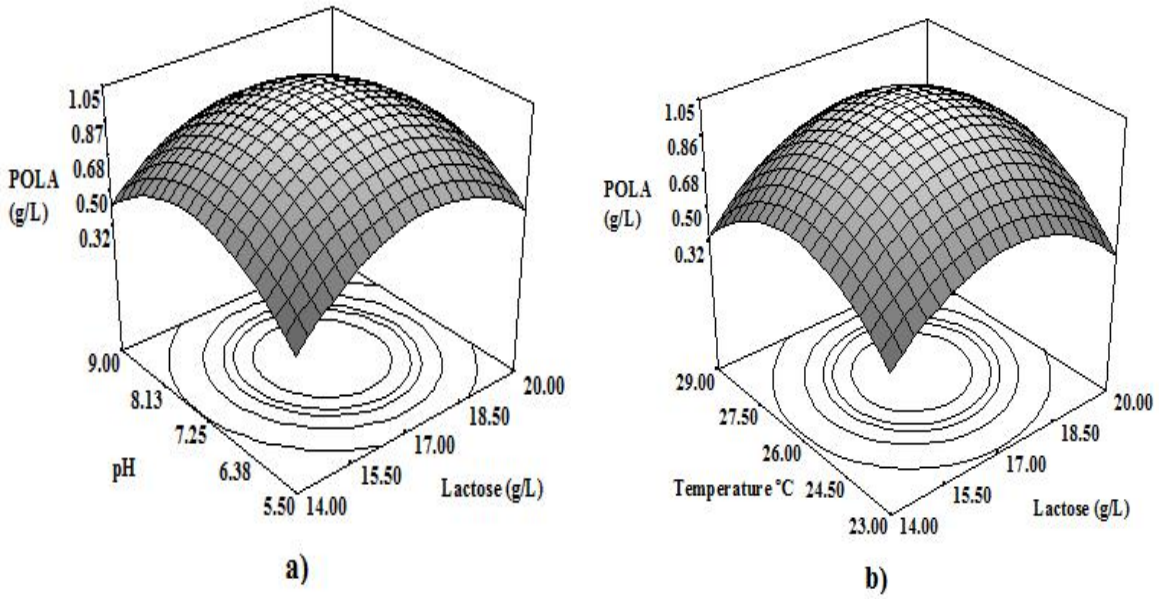

Figure 2. Response surface plot and the corresponding contour plot representing the effects of parameters **a)** The effect lactose concentrations and pH on POLA at fixed medium temperature 26 ºC and yeast extract 0.90 g/L **b)** The effects of lactose concentrations and temperature on POLA at fixed pH 7.0 and yeast extract 0.90 g/L

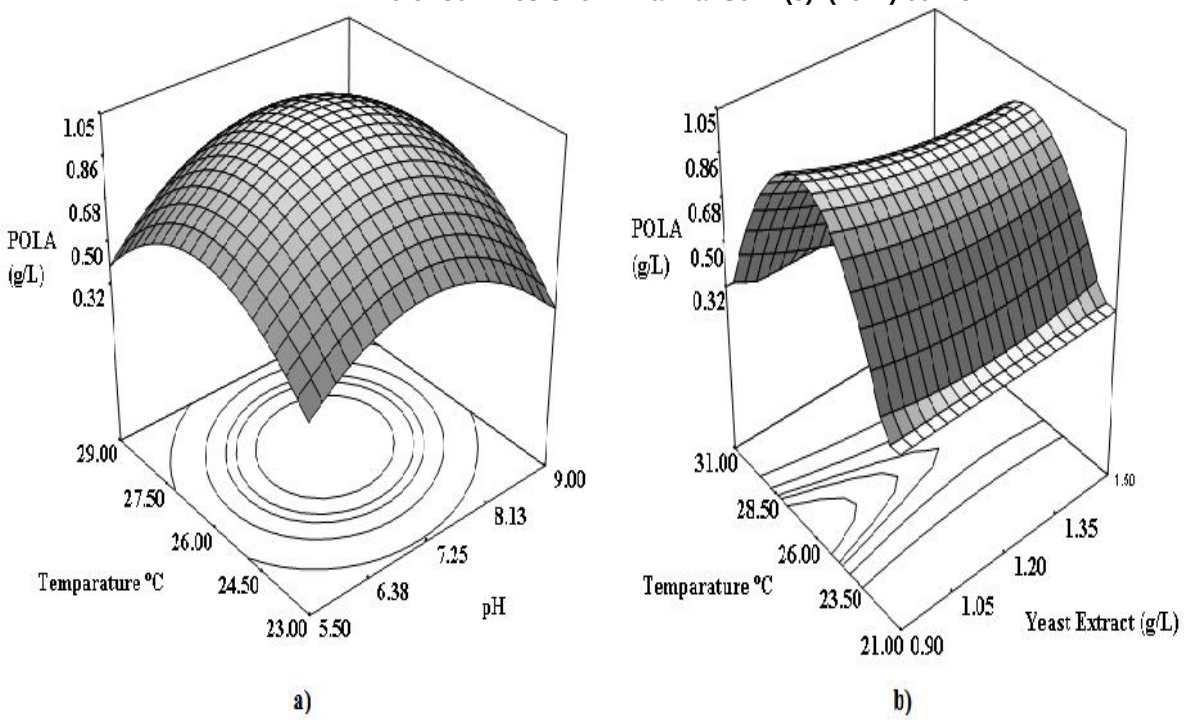

Figure 3. Response surface plot and the corresponding contour plot representing the effects of parameters **a)** The effect of pH and temperature on POLA at fixed lactose concentrations 17.0 g/L and yeast extract 0.90 g/L **b)** the effects of yeast extract and temperature on POLA at fixed lactose concentrations 17.0 g/L and pH 7.0

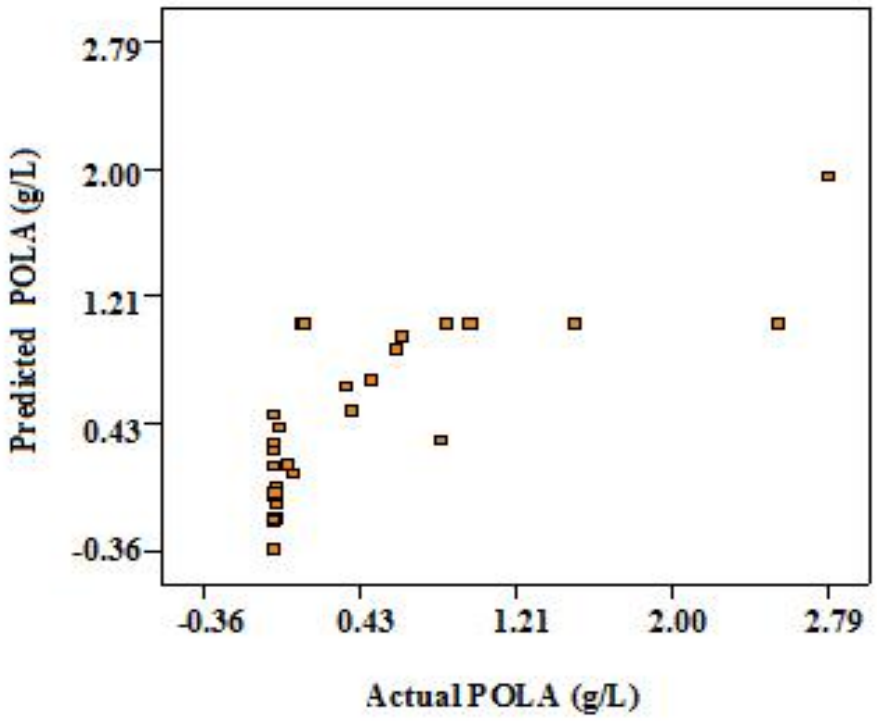

Figure 4. The actual and predicted values of POLA.

Conclusion

The result show that RSM was applied successfully to optimize of POLA with *K. lactis* artificially prepared whey in batch experiments. It was obtained that lactose and yeast extract concentration, pH and medium temperature were the most effective factors for POLA. Only 49 experimental trials were required. An empirical model to simulate percent POLA with *K. lactis* was developed (factors) by RSM (Placket Burman, Steepest-Ascent and CCD) and an ANOVA tests was performed. Optimum values for POLA with *K. lactis* lactose, yeast extract, pH and medium temperature were found to be 18.8 g/L, 0.94 g/L, 5.2 and 27 °C for 24 h fermentation time respectively, under the constraints. The maximum POLA was achieved 1.4 g/L by *K. lactis* yeast strain. To our knowledge some of yeasts strain and bacteria were always used to product the lactic acid but *K. lactis* was used rarely to product the lactic acid on synthetic whey.

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