Digest Journal of Nanomaterials and Biostructures Vol. 8, No. 3, July - September 2013, p. 1023 - 1035

DETECTİNG OPTIMUM AND COST-EFFICIENT OF MICROBIAL GROWTH RATE AND LACTOSE CONSUMPTION OF KLUYVEROMYCES LACTIS Y-8279 USING RSM

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Exploring optimum and cost-efficient medium composition for microbial growth rate and lactose consumption of *Kluyveromyces Lactis Y-8279* yeast culture growing on whey was studied by applying a multi-step response surface methodology (RSM). RSM is a combination of Plackett Burman (PB), Steepest-Ascent (SA) and Central Composite Design (CCD) Medium temperature, pH, yeast extract and lactose concentrations were evaluated the most effective operational factors through seven factors by applying PBD. These four factors were selected for SA program and SA experiments were carried out to find approximately maximum microbial concentration with lactose, pH, yeast extract and medium temperature. Then CCD was carried out with these factors (lactose, medium temperature, pH and yeast extract). Based on ANOVA test the optimum operational conditions of maximum growth rate were obtained as 18.8 g/L, 0.94 g/L, 5.2 and 27 ^{o}C, lactose concentration, yeast extract concentration, pH, and medium temperature, respectively. And then optimum operational conditions of lactose consumption were obtained as 13 g/L, 0.91 g/L, 8 and 27.5 ^{o}C, lactose concentration, yeast extract concentration, pH, and medium temperature, respectively too.

(Received May 8, 2013; Accepted July 26, 2013)

Keywords: Kluyveromyces Lactis; Response surface methodology (RSM); Lactose; Optimization

1. 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 lactic acid, fumaric acid, citric acid, ethanol, methanol, 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. Ethanol fermentation of cheese whey was realized by different methods including micro-aeration in batch fermentations [1]. *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 [2]. The growth of microorganisms on dairy waste has received considerable attention because of the possibility of producing single cell protein for feed and simultaneous utilization of the waste which is a serious environmental nuisance. Rapid formation of an appropriate amount of cell mass is of great importance for the process [3]. Whey is a by-product of the dairy industry whose major components are lactose (44-52 g/L), proteins (6-8 g/L) and mineral salts (4-9 g/L) [4-5].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 [13].

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Response surface methodology (RSM) 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 [14]. 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 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 [15]. 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 [16-17]. RSM has been successfully applied by Hwang and Hansen for modeling and optimization in anaerobic bioconversion of complex substrates.[13] while applied by Aktas et al. for developing, improving, and optimizing of lactose utilization in whey permeate.[7] utilized by Ismail et al. for optimization of butylgalactoside synthesis by β-galactosidase from *Aspergillus oryzae* [16], utilized by Prapulla et al. for maximization of lipid production by *Rhodotorula gracilis*.[17] In some other investigations, xylitol production by *C. guilliermondii* [9], medium composition to increased production of C-phycocyanin from *P. ceylanicum.*[8] lipase production from *Candida* sp. [20], κ-carrageenase production by *Pseudomonas elongata* [10], and chitosanase production from *Microbacterium* sp.[11] were effectively studied by RSM.

The objective of present study was to treat whey as a dairy wastewater, whose sole carbon source was lactose, in batch fermentation experiments. The scope of the study included the investigation of the impact of the fermentation conditions such as pH, medium temperature, medium composition on growth of *K. Lactis Y- 8279* yeast culture. 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 maximum microbial growth and lactose consumption of *K. Lactis Y- 8279* yeast culture. Simulated whey was selected as the model media because it is a common dairy pollutant and has serious negative impact on environment.

2. Materials and methods

2.1 Material

K. Lactis Y- 8279 was kindly donated by NRRL (Northern Region Research Laboratories) culture collection (Peoria,IL, USA). Lactose, KH_2PO_4, $(NH_4)2SO_4$, NaCl, $CaCl_2.2H_2O$, $FeCl_2.6H_2O$ 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).

2.2 Yeast strain and maintenance

In this paper, *K. Lactis Y- 8279* strain was used. 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 spreated 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 ^oC for 15 min. The inoculated solid medium was incubated at 30 ^oC for 4 days for appropriate growth and stored at 5 ^oC for further uses. The solid medium culture was transferred monthly for maintenance [18].

2.3 Inoculum development

Four-day-old solid cultures incubated at 30 ^oC 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 ^{0}C for 15 min. Incubation was carried out with constant shaking at 130 rpm at 30 ^{o}C for 24 h in a Jeio-Tech orbital shaking incubator, model SI-600R (Seoul, Korea). The inoculums were transferred aseptically into the fermentation medium at 5% (v/v).

2.4 Culture medium

Fermentation medium 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 ^{o}C for 30 min.

2.4 Experimental set-up and procedure

For the investigation of 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.

2.5 Analysis

Fermentation medium (5 milliliter) 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 lactose concentration in the supernatanat was determined using a Dıonex HPLC system with an HyperREZ XP Carbohydrate H^+) Column (300x7,7 mm), deionized water at 0,6 mL/min was used as an eluent, detection was RI 101 , and column temperature was 75 ^{o}C .

2.6 Experimental design

Response surface methodology 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 these responses [19]. In order to ascribe the effect of factors at first on PB Table 1 after than steepest-ascent Table 2 and a CCD Table 3 with four factors was performed. PB 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 (microbial growth rate and lactose consumption). The second program SA was applied to maximum curve for microbial growth rate of *K. Lactis Y- 8279* after PB was applied. The method of SA 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 responses [20]. 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.

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

Run	Laktose(g/L)	Total amonia (g/L)	Yeast Extract (g/L)	$MgSO_4$ (g/L)	NaCI (g/L)	pH	Temperature (C^0)	D	D	D	D	Culture Concentration (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	2.507
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. For maximum curve of the microbial growth rate of K. Lactis in Steepest-Ascent runs and corresponding results (the response)

Run	Lactose (g/L)	Temperature (C0)	Yeast Extrac (g/L)	pH	Response (Culture Concentration (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 central composite design (CCD) runs and corresponding results (the responses)

Run	X_1 Lactose (g/L)	X_2 Temperature (C^0)	X_3 Yeast Extract (g/L)	X_4 pH	y_1 Microbial growth rate	y_2 Lactose Consumption (%)
1	17.00	26.00	1.70	7.00	0.08	25.53
2	17.00	26.00	0.90	10.00	0.13	34.58
3	20.00	29.00	0.50	5.50	0.37	34.42
4	17.00	26.00	0.90	7.00	0.75	69.80
5	17.00	26.00	0.90	7.00	0.72	68.80
6	14.00	29.00	0.50	8.50	0.30	76.29
7	17.00	26.00	0.90	7.00	0.63	73.83
8	17.00	20.00	0.90	7.00	0.01	39.61
9	20.00	23.00	0.50	5.50	0.34	33.63
10	23.00	26.00	0.90	7.00	0.53	50.52
11	17.00	26.00	0.10	7.00	0.44	57.23
12	17.00	26.00	0.90	4.00	0.97	34.58
13	20.00	29.00	0.50	8.50	0.58	59.77
14	17.00	26.00	0.90	7.00	0.73	69.80
15	20.00	29.00	1.30	8.50	0.58	69.83
16	20.00	23.00	1.30	5.50	0.33	44.65
17	14.00	23.00	0.50	8.50	0.35	28.29
18	11.00	26.00	0.90	7.00	0.26	69.72
19	17.00	26.00	0.90	7.00	0.69	73.83
20	14.00	29.00	1.30	5.50	0.38	28.76
21	14.00	23.00	0.50	5.50	0.14	30.66
22	20.00	29.00	1.30	5.50	0.83	38.79
23	14.00	23.00	1.30	5.50	0.32	17.03
24	14.00	29.00	0.50	5.50	0.56	40.73
25	14.00	23.00	1.30	8.50	0.27	49.62
26	20.00	23.00	1.30	8.50	0.19	62.83
27	17.00	32.00	0.90	7.00	0.36	13.51
28	20.00	23.00	0.50	8.50	0.07	48.58
29	17.00	26.00	0.90	7.00	0.76	73.33
30	14.00	29.00	1.30	8.50	0.15	76.29

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^0	X_2	20	23	26	29	32
Yeast Extrac (g/L)	X_3	0,10	0.50	0,90	1.30	1.70
pH	X_3	4.00	5.50	7.00	8.50	10.0

Table 5. Analysis of variance (ANOVA) of second-order polynomial model for the μ obtained through CCD

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	1.479	14	0.106	4.215	0.0045	significant
$\mathbf{X_1}$	0.069	1	0.069	2.766	0.1170	
$\mathbf{X_2}$	0.009	1	0.009	0.343	0.5670	
$\mathbf{X_3}$	0.240	1	0.240	9.590	0.0074	
$\mathbf{X_3}$	0.259	1	0.259	10.322	0.0058	
$\mathbf{X_1^2}$	0.161	1	0.161	6.411	0.0230	
$\mathbf{X_2^2}$	0.339	1	0.339	13.522	0.0022	
$\mathbf{X_3^2}$	0.042	1	0.042	1.657	0.2175	
$\mathbf{X_4^2}$	0.458	1	0.458	18.256	0.0007	
$\mathbf{X_1X_2}$	0.048	1	0.048	1.896	0.1887	
$\mathbf{X_1X_3}$	0.002	1	0.002	0.081	0.7801	
$\mathbf{X_1X_4}$	0.065	1	0.065	2.584	0.1288	
$\mathbf{X_2X_3}$	0.024	1	0.024	0.952	0.3446	
$\mathbf{X_2X_4}$	0.001	1	0.001	0.043	0.8377	
$\mathbf{X_3X_4}$	0.003	1	0.003	0.104	0.7518	

$R^2 = 0.80$ (Microbial growth rate)

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 a 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

$$\eta = \beta_0 + \sum_{i=1}^{3} \beta_{ii}\chi + \sum_{i=1}^{3} \beta_{ii}\chi_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij}\chi_i\chi_j \quad \text{(a)}$$

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. (a) 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.

3. Result and discussions

The objective at the onset of this experiment was to find the best set of operating conditions for microbial growth rate of *K. Lactis Y- 8279*. 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 microbial growth rate of *K. Lactis Y- 8279 1.* Analysis of the optimum conditions was carried out sequentially through PB design to CCD of RSM

3.1 Plackett–Burman design (PB)

Culture conditions play an important rol on growth and utilization of nutrients in all biological treatment systems. Thus, PB design was successfully applied to investigate significant medium parameters [21]. 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 Y- 8279* and utilization of lactose as well were investigated through PB method [2]. Table 1 indicates the levels of the variables and their effects on the response, microbial growth rate**.** 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 Equation1.

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

3.2 Path of steepest ascent

PB 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 [22]. 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 14.9 g/L wet microorganism when the lactose concentration, yeast extrac 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.

3.3 Central Composite Design (CCD)

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-.50 pH and 23-29 ºC of medium temperature.

Thirty trials were performed to locate optimum conditions for maximum microbial growth rate and lactose consumption of *K. Lactis Y- 8279* yeast culture. The experiments were carried out in random order as suggested in many design program [8]. In the experimental design, optimum conditions meant to be the operating conditions for maximizing microbial growth rate and lactose consumption. Table 3 shows experimental conditions for batch runs and the results (responses) in terms of microbial growth rate and lactose consumption.

Any increment in medium pH towards to 5.2 and in lactose concentration to 18.8 g/L significantly increased the μ up to 0.73 1/h in Figure 1, but higher values have negative effects on the response. In a study carried out by [24] μ was found to be 0.125 1/h for *C. Utilis* on sucrose which was the sole carbon source in the media and the optimum value was 15 g/L. Figure 2 represents medium temperature and lactose concentrations effect on the microbial growth rate at

the fixed pH 7 and yeast extract 0,9 g/L curvature occurred in the response and the maximum growth rate was obtained 0.70 1/h. Increasement in the lactose concentration by 18.8 g/L was increase the microbial growth rate while the same response was obtained in temperature like in Figure 3. [25] reported 0.70 1/h for *lactobacillus helveticus* in whey permeate/ yeast extract media. The specific growth rate for *C. intermedia* was found comparatively low. Figure 4 show that combined influence of the medium temperature and pH on the μ at the fixed lactose concentration 17 g/L and yeast extract 0,9 g/L. As shown in Fig. 4, μ has strongly been affected by both factors

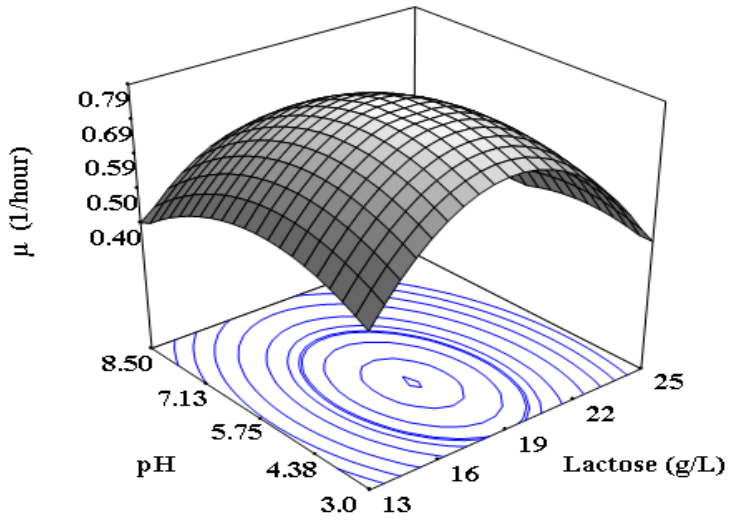

Fig.1. Percent microbial growth rate as a function of pH and lactose (medium temperature 26 ºC and yeast extract 0.9 g/L.

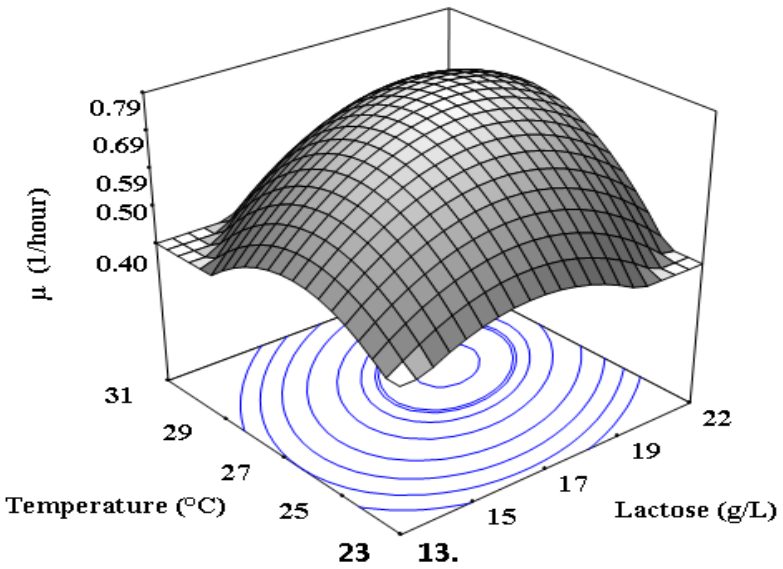

Fig.2. Percent microbial growth rate as a function of medium temperature and lactose. (pH 7 and yeast extract 0.9 g/L)

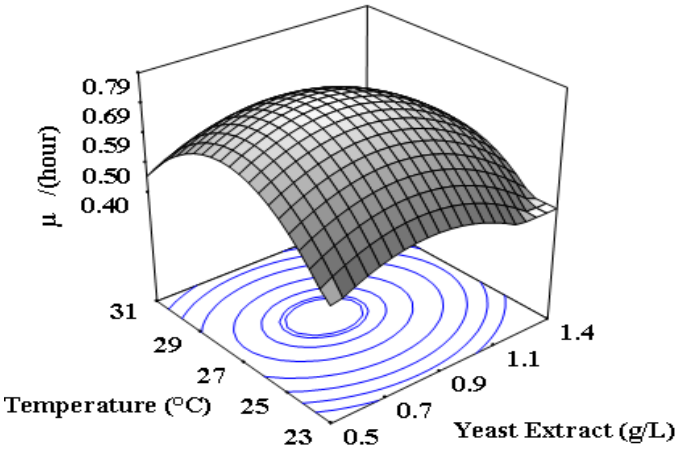

Fig.3. Percent microbial growth rate as a function of medium temperature yeast extract (pH 7 and lactose 17 g/L)

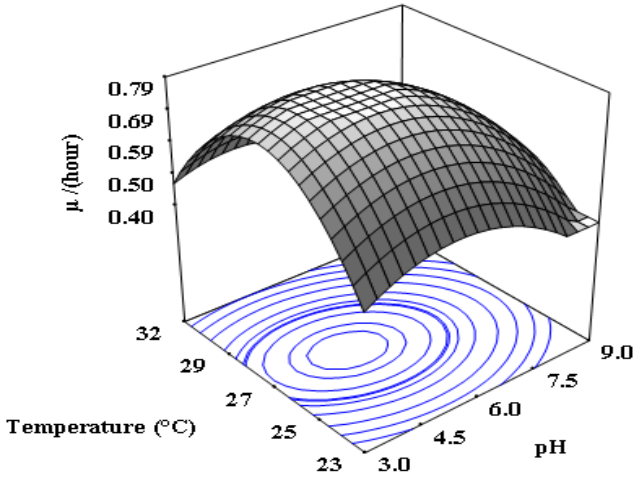

Fig.4. Percent microbial growth rate as a function of medium temperature and pH (lactose 17 g/L and yeast extract 0.9 g/L).

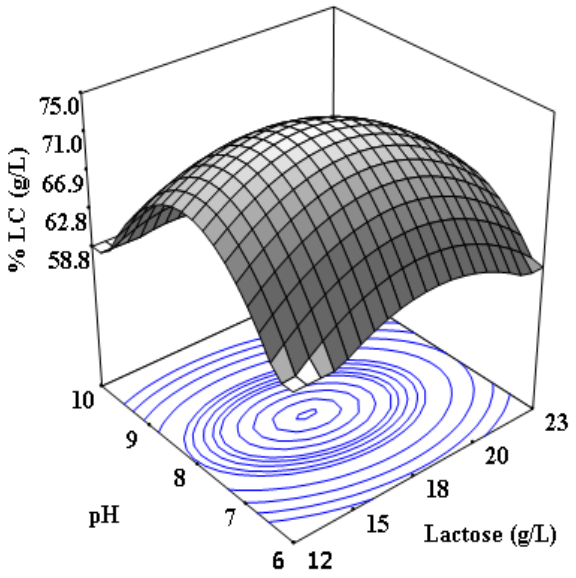

Fig.5. Percent lactose consumption as a function pH and lactose (medium temperature 26 °C and yeast extract 0.9 g/L)

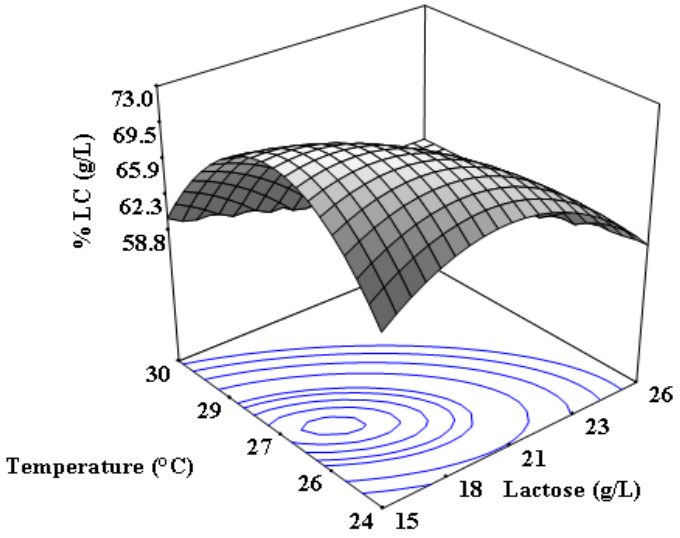

Fig.6. Percent lactose consumption as a function medium temperature and Lactose (pH 7.00 and yeast extract 0.9 g/L)

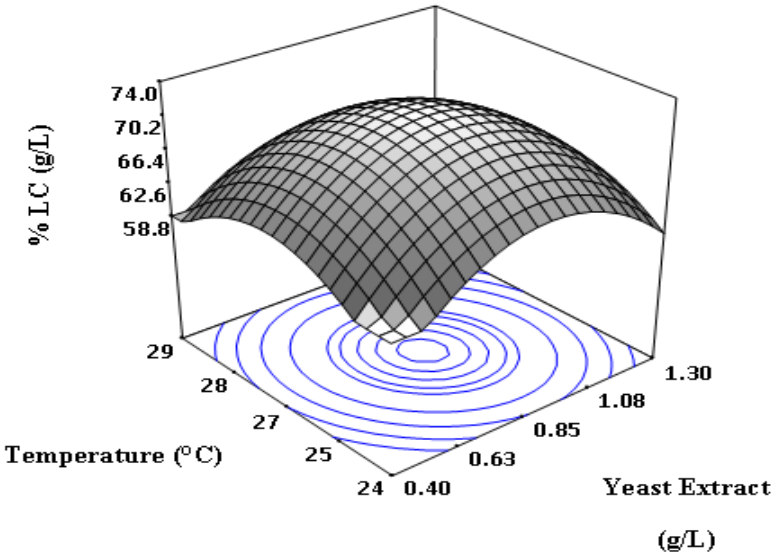

Fig.7. Percent lactose consumption as a function medium temperature and yeast extract (lactose 17 g/L and pH 7.00)

Maximum % LC was obtained 71 (%) at pH and lactose consentration respectively in Fig. 5. Further increase or decrease in the concentration of either of these lead to the decrease in the lactose consumption. Figure 6 represents the interaction between temperature and lactose concentration. A maximum of 66 % LC was obtained respectively while Figure 6 presents the interaction between lactose concentration and the medium temperature where a maximum of 42.9 % of lactose consumption and medium temperature was obtained at 18.8 g/L and 27 °C. The medium temperature was calculated 31 ºC for optimization of lactose utilization in deproteinated whey by [7].

The influence of medium temperature and yeast extract was showed in Fig. 7. Maximum lactose consumption was obtained 71 % in this figure [24]. Fig. 8 represents medium temperature

and pH effect on % LC at the fixed yeast extract 0,9 g/L and lactose concentration 17 g/L curvature occurred in the response and % LC was obtained 74 respectively.

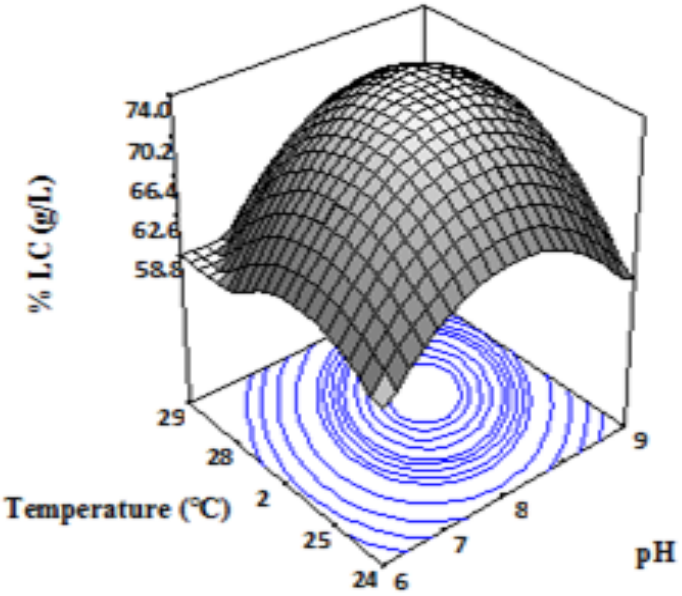

Fig.8. Percent lactose consumption as a function pH and medium temperature (yeast extract 0.9 g/L and lactose 17 g/L)

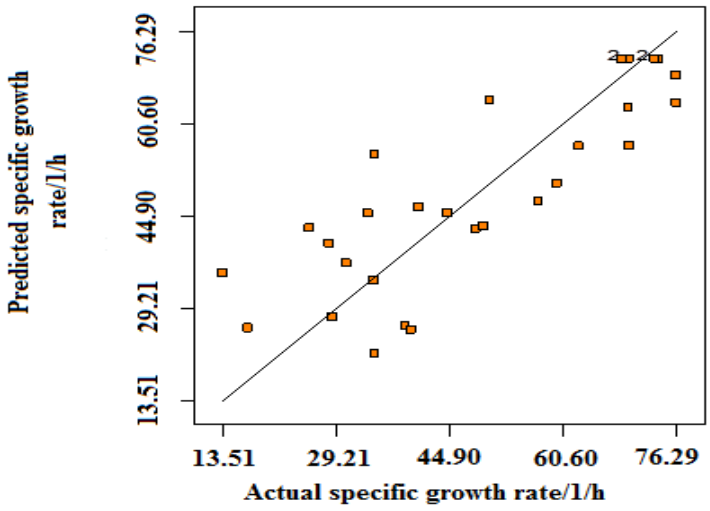

Fig. 9. The actual and predicted values of microbial growth rate (R^2 = 80%)

However, due to the complex nature of biological processes, it is very difficult to predict distinctively the effects of all parameters, which may have multiple interactions. Therefore, RSM was applied to build up an empirical model for modeling microbial growth rate and lactose consumption of *K. Lactis Y- 8279* in terms of operational parameters of lactose, yeast extract concentrations, pH and medium temperature. A quadratic equation microbial growth rate and lactose consumption was obtained through Design Expert 6.0 as in equations 2 and 3.
The optimum levels of the selected variables were obtained by solving the regression equation and by analyzing the response Surface contour and surface plots [23].

$$y_1 = +71.57 + 0.27\,X_1 - 1.17X_2 + 8.45\,X_3 + 2.39\,X_4 - 1.93\,X_1^2 - 6.61\,X_2^2 - 8.31\,X_3^2 - 10.32X_4^2 + 2.75X_1\,X_2 - 1.49\,X_1\,X_3 - 5.21X_1\,X_4 + 3.49X_2\,X_3 \quad (2)$$

$$y_2 = +0.72 + 0.054\,X_1 - 0.019X_2 - 0.1X_3 + 0.1\,X_4 - 0.077X_1^2 - 0.11X_2^2 - 0.039X_3^2 - 0.13X_4^2 + 0.055X_1X_2 - 0.011\,X_1X_3 + 0.064X_1X_4 + 0.039X_2X_3 - 0.0083X_2X_4 + 0.0131X_3X_4 - 1.91X_2X_4 + 4.7691X_3X_4 \quad (3)$$

ANOVA results of the quadratic model in Table 5 indicated that the model equation derived by RSM of Design Expert 6.0 could adequately be used to describe *K. Lactis Y- 8279* microbial growth rate and lactose consumption in a wide range of operating conditions, although the microbial growth rate and lactose consumption was determined by three independent factors and their interaction effects. For the models, there was no lack of fit and a fair coefficient of determination (R^2 80%) and (R^2 72%) were observed.

Fig. 9 shows the μ evaluated from Eq. 6 versus the observed ones. The figure proves that the predicted μ is well in agreement with the observed ones. The correlation coefficient (R^2), 0.80 shows that the suitability between predicted and observed microbial growth rate.

4. Conclusions

RSM was applied successfully for the optimization of growth conditions of *K. Lactis Y- 8279* artificially prepared whey in batch experiments. It was obtained that lactose concentration; yeast extract concentration; pH and medium temperature were the most effective factors on *K. Lactis Y- 8279* culture. Only 49 experimental trials were required. An empirical model to simulate percent microbial growth rate of *K. Lactis Y- 8279* was developed (factors) by RSM (PB, SA and CCD) and an ANOVA tests was performed. Optimum values for microbial growth rate of *K. Lactis Y- 8279* lactose, yeast extract, pH and medium temperature were found to be 18.8 g/L, 0.94 g/L, 5.2 and 27 ^oC and optimum values for lactose consumption of *K. Lactis Y- 8279* lactose, yeast extract, pH and medium temperature were found to be 13 g/L, 0.73 g/L, 8.12 and 27.5 ^oC and respectively, under the constraints. The models which we found were used in some industrial and others areas where fermentation medium used for example waste water, whey and other wastes for production of ethanol.

Acknowledgements

This research was supported by Yüzüncü Yıl University Research Fund with grant no. 2008-FBE-D140. We are indebted to NRRL for supplying the culture free of charge.

NOMENCLATURE

ANOVA : Analysis of variance
β_0 : Constant coefficient of Equation a
β_i : Linear coefficient of Equation a
β_{ii} : Quadratic coefficient of Equation a
β_{ij} : Interaction coefficient of Equation a
CCD : Central Composite Design
PB : Plackett Burmann
LC : Lactose Consumption
R^2 : Coefficient of determination
RSM : Response surface methodology
y_1 : Microbial growth rate
y_2 : Lactose Consumption (%)
X_i : Independent variable
X_1 : Lactose concentration (g/L) Equation b
X_2 : Temperature Equation b
X_3 : Sodium chloride concentration (g/L) Equation b

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