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Optimization of a Modified GS Medium for a Probiotic Strain (*L. acidophilus* ATCC4356)

Pedram, N., Ataei, S.A.[*]

Biotechnology group, Chemical Engineering Department, Shahid Bahonar University of Kerman, Kerman, Iran

Abstract

Probiotics are defined as living microorganisms with beneficial effects on the host. Probiotics, according to the least negative effect on the body, are considered as a good alternative to chemical drugs. Lactobacillus acidophilus is used as a probiotic that is able to reduce cholesterol level in the blood. The effect of various concentrations of carbon, nitrogen and phosphorus for enhancing the biomass production of *Lactobacillus acidophilus* was examined. The response surface methodology based on Box–Wilson CCD was applied to explore the optimal medium composition. Glucose, yeast extract, K_2HPO_4 and K_2HPO_4 were selected as dependent variables. All experiments were run at 37°C for 31h under stationary conditions. By solving the regression equation and analyzing the response surface carton, optimal concentrations of the components were determined as glucose (5-8.75 $g.l^{-1}$), yeast extract (36.75-39 $g.l^{-1}$), K_2HPO_4 (0.1-0.2125 $g.l^{-1}$) and K_2HPO_4 (0.3925-0.7075 $g.l^{-1}$) ($P<0.05$). Validation experiment confirmed that the optimized medium was comparable to the MRS medium (the most common medium for Lactobacillus acidophilus strain) in biomass production, having the advantages of economy and practicality.

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Correspondence to:
Ataei S.A. (PhD)
Assistant Prof.
Tel: +989131405823
Fax: +983412118298
Email address:
Ataei@uk.ac.ir

1. Introduction

High serum cholesterol concentration is associated with the development of coronary heart disease, a major cause of death worldwide. Reduction of serum cholesterol can lower the incidence of coronary thrombosis in hypercholesterolemic individuals [1].

Many studies have reported the ability of probiotics such as *Lactobacillus (L.) acidophilus* and *Bifidobacteria* in reducing cholesterol or in removing it from the growth medium [2]. Probiotics are feed-microbial supplements, which beneficially affect the host because they improve the balance of its intestinal bacterial ecosystem [3]. Thus both types of bacteria (*L. acidophilus* and *Bifidobacteria*) may have the potential to reduce serum cholesterol concentration in humans. However, the ability to assimilate cholesterol from the medium varies significantly amongst different bacterial strains [4].

Among the lactic acid bacteria, *L. acidophilus* has attracted much research attention for its potential role in promoting human health [1]. *L. acidophilus* is the most common probiotic used commercially in the dairy industry in the production of probiotic fermented milk, and especially yoghurt. The ability to decrease cholesterol uptake may be influenced by numerous factors such as kind of medium and presence of bile salts, as well as the viability and number of bacterial cells [5]. As Lactobacilli are fastidious with respect to nutrient requirements, they require a rich medium is required for better growth [6].

Many factors such as carbon, nitrogen and phosphorus sources are important variables affecting the growth of microbes. Currently, there are several media to cultivate *L. acidophilus*. In spite of the differences among the medium compositions and concentrations, the biomass density of *L. acidophilus* cultivated in the MRS medium is not very high, so it is

difficult to achieve more biomass, and it is better to use the best medium for cultivation. Response Surface Methodology (RSM), an experimental strategy for seeking the optimum conditions for a multi-variable system described by Box and Wilson, is a much more efficient technique for medium optimization. RSM consists of a group of mathematical and statistical procedures that can be used to study relationships between one or more responses and a number of independent variables. This experimental methodology generates a mathematical model that is able to accurately describe the overall process. This method has been successfully applied to the optimization of medium composition, conditions of enzymatic hydrolysis, and parameters of food preservation and fermentation processes.

In this research, the concentrations of medium components are considered as the independent variables; each variable is referred to some base values, and varies in a certain pattern [3]. Identifying the key components of a medium plays a major role in the commercialization of fermented products. It has large effects on the products` concentration, yield and volumetric productivity [7]. The aim of this study was to determine the optimal modified GS culture medium compositions for the growth of *L. acidophilus* ATCC 4356.

2. Materials and Methods

2.1. *Microorganism*

L. acidophilus ATCC 4356 (PTCC 1643) was obtained from the Persian type culture collection. The strain was grown in GS medium broth at 37ºC and stored in slant.

2.2. *Culture media*

GS medium components included: 30 $g.l^{-1}$ glucose, 30 $g.l^{-1}$ yeast extract, 0.6 $g.l^{-1}$ $MgSO_4.7H_2O$, 0.03 $g.l^{-1}$ $MnSO_4.H_2O$, 1 $g.l^{-1}$ Sodium acetate, 0.5 $g.l^{-1}$ K_2HPO_4, 0.5 $g.l^{-1}$ KH_2PO_4, and 0.03 $g.l^{-1}$ $FeSO_4.7H_2O$ [8].

Seed culture was prepared by growing *L. acidophilus* in 50 ml of GS broth at 37°C. After 16h of cultivation, 5% of the seed culture was transferred into each fermentation medium, which contained components according to the experimental design, and sterilized at 121°C for 15min in tubes. The cultures were incubated at 37°C for 31h. Biomass was measured by the cell dry weight method [9].

2.3. *Optimization of culture medium*

Sources of carbon, nitrogen and phosphorus were chosen to optimize the culture medium to produce more microbial mass. A response surface design appropriate for running the process was identified. Box-Behnken design was employed for the experimental design, which was randomized with the analysis of the results and process optimization.

2.4. *Experimental design*

An interiorly augmented Box-Behnken design was used to allocate factor level combinations in this experiment. The response denoted by Y was considered as the cell dry weight. The aim of the experiment was to determine the factor-level combination that maximizes the Y value. The response was assumed to be under the influence of four factors, including glucose, yeast extract, K_2HPO_4 and KH_2PO_4. Table 1 shows the unit and range of levels for each factor. For statistical design and analysis of the experiment, the factor levels were coded as follows:

$$X_1 = (Glucose - 12.5)/ 7.5 \quad \text{(Eq. 1)}$$
$$X_2 = (Yeast\ extract - 30)/ 9 \quad \text{(Eq. 2)}$$
$$X_3 = (K_2HPO_4 - 0.55)/ 0.45 \quad \text{(Eq. 3)}$$
$$X_4 = (KH_2PO_4 - 0.55)/ 0.45 \quad \text{(Eq. 4)}$$

Factor–level combination arrangement and observed responses are presented in Table 2.

$$Y = = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j \quad \text{(Eq. 5)}$$

Where, Y represents the response variable, β_0 is the interception, β_i denotes linear coefficients, β_{ij} denotes interaction coefficients, and Xi, X_j are the coded values of the factors chosen as the result of the design analysis.

Table 1. Experimental range and levels of the test variables

X_n	Independent variables	Low level ($g.l^{-1}$)	Central level ($g.l^{-1}$)	High level ($g.l^{-1}$)
X_1	Glucose	5	12.5	20
X_2	Yeast extract	21	30	39
X_3	K_2HPO_4	0.1	0.55	1
X_4	KH_2PO_4	0.1	0.55	1

2.5. *Statistical analysis*

Data were analyzed using the Minitab software version 10. The model permitted evaluation of the effects of linear, quadratic and interactive terms of the independent variables (Glucose, yeast extract, K_2HPO_4 and KH_2PO_4).

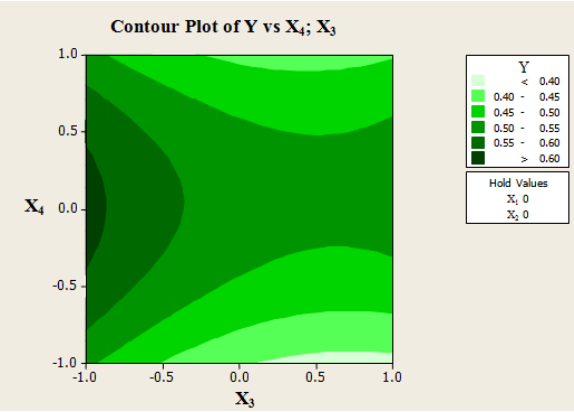

Figure 1. Response surface contour plot showing combined effect between K2HPO4and KH2PO4 on biomass with the other two variables, yeast extract and glucose, at their zero levels.

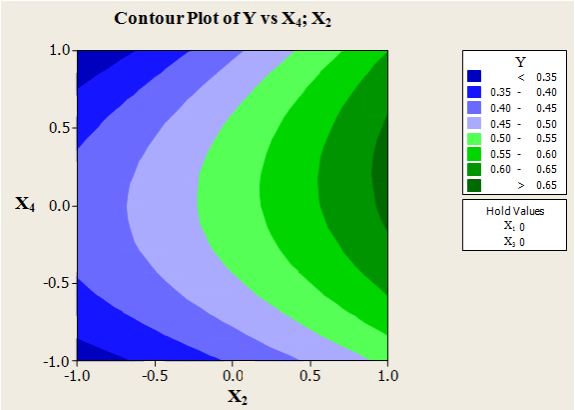

Figure 2. Response surface contour plot showing combined effect between yeast extract and KH2PO4 on biomass with the other two variables, K2HPO4 and glucose, at their zero levels.

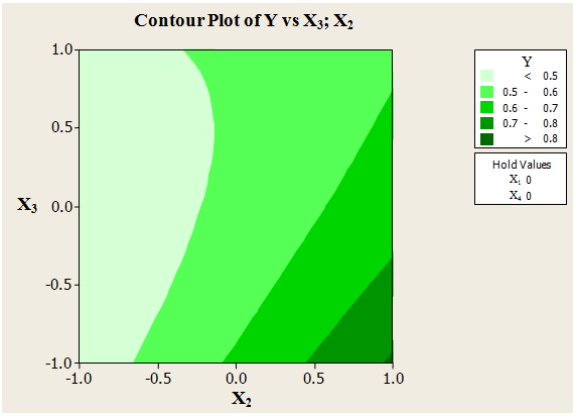

Figure 3. Response surface contour plot showing combined effect between K_2HPO_4 and yeast extract on biomass with the other two variables, KH_2PO_4 and glucose, at their zero levels.

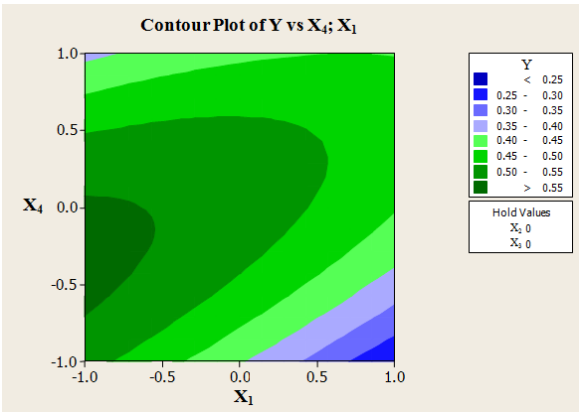

Figure 4. Response surface contour plot showing combined effect between KH_2PO_4 and glucose on biomass with the other two variables, K_2HPO_4 and yeast extract, at their zero levels.

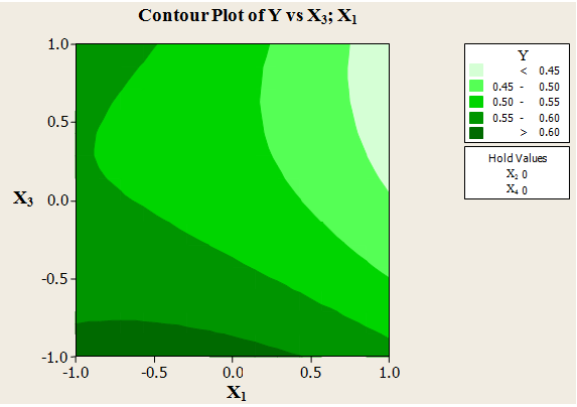

Figure 5. Response surface contour plot showing the effect of interaction between K_2HPO_4 and glucose on biomass with the other two variables, yeast extract and KH_2PO_4, at their zero levels.

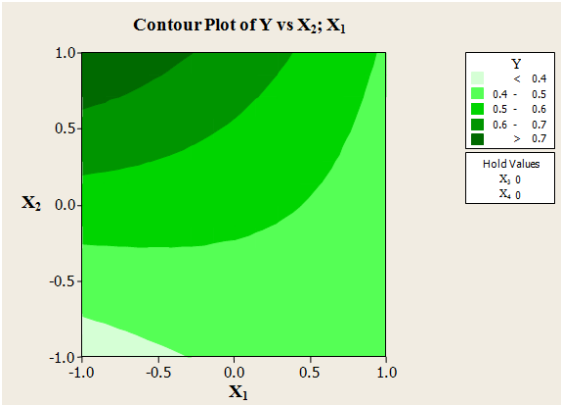

Figure 6. Response surface contour plot showing the effect of interaction between yeast extract and glucose on biomass with the other two variables, K_2HPO_4 and KH_2PO_4, at their zero levels.

3. Results and discussion

Optimization of the medium components was carried out using a Box–Wilson CCD. The experimental design and the results are shown in Table 2. The statistical significance of the second-order model equation was checked by F-test (ANOVA) (Table 3). The fit value, termed R^2 (determinant coefficient), of the polynomial model was calculated to be 0.974, indicating that 97.4% of the variability in the response could be explained by the second-order polynomial prediction equation given below:

$$Y = 0.5267 - 0.0510\ X_1 + 0.1216\ X_2 - 0.0487\ X_3 + 0.0410\ X_3^2 - 0.1050\ X_4^2 - 0.0997\ X_1 X_2 + 0.0826\ X_1 X_4 - 0.0625\ X_2 X_3 \quad \text{(Eq. 6)}$$

The ANOVA results showed that this model is appropriate. They also suggested that the biomass density of *L. acidophilus* was primarily determined by the linear and quadratic terms of the glucose, yeast extract, K_2HPO_4 and KH_2PO_4 of the model.

It was evident from the calculated response surface that *L. acidophilus* reached its maximum at a combination of the coded level (-1 to -0.5) X_1, (0.75 to 1) X_2, (-0.75 to -1) X_3 and (-0.35 to 0.35) X_4. This is a reconfirmation that the fitted surface had a maximum point, which was (5-8.75 $g.l^{-1}$) glucose, (36.75-39 $g.l^{-1}$) yeast extract, (0.1-0.2125 $g.l^{-1}$) K_2HPO_4 and (0.3925-0.7075 $g.l^{-1}$) KH_2PO_4. The resulting contour plots, showing the effect of glucose, yeast extract, K_2HPO_4, KH_2PO_4 concentrations on the biomass growth, are illustrated in Figures 1-6.

The response surface methodology proved to be a powerful tool in optimizing the fermentation medium for *L. acidophilus*. In the present study, the experimental results clearly showed that the biomass density of *L. acidophilus* is dependent mainly on the value of glucose, yeast extract, K_2HPO_4 and KH_2PO_4. It is also evident from the experimental results that the yeast extract had significant positive effect on growth.

The reason for higher concentration of yeast extract benefiting the growth of *L. acidophilus* could be because it neutralized some proteins, produced by *L. Acidophilus* during the incubation.

The optimized fermentation medium for incubating *L. acidophilus* ATCC 4356 composed was of 6.9±1.9 $g.l^{-1}$ glucose, 0.6 $g.l^{-1}$ $MgSO_4.7H_2O$, 0.03 $g.l^{-1}$ $MnSO4.H_2O$, 1.0 $g.l^{-1}$ sodium acetate, 0.03 $g.l^{-1}$ $FeSO_4.7H_2O$, 38±1.75 $g.l^{-1}$ yeast extract, 0.16±0.05 $g.l^{-1}$ K_2HPO_4 and 0.55±0.16 $g.l^{-1}$ KH_2PO_4 at the initial medium (pH of 6.5).

Table 2. Experimental design for optimization of a modified GS medium for a probiotic strain

Runs	Blocks	X_1	X_2	X_3	X_4	Y
1	1	-1	0	1	0	0.56
2	1	1	0	1	0	0.42
3	1	-1	0	0	-1	0.51
4	1	1	0	0	1	0.45
5	1	0	-1	-1	0	0.42
6	1	1	-1	0	0	0.46
7	1	0	1	-1	0	0.83
8	1	0	0	-1	1	0.53
9	1	0	-1	0	-1	0.33
10	1	-1	0	-1	0	0.61
11	1	-1	0	0	1	0.38
12	1	0	1	0	1	0.58
13	1	0	0	1	1	0.46
14	1	1	0	-1	0	0.37
15	1	0	-1	0	1	0.29
16	1	0	0	1	-1	0.56
17	1	-1	1	0	0	0.35
18	1	1	1	0	0	0.47
19	1	0	0	-1	-1	0.51
20	1	0	1	1	0	0.61
21	1	0	0	0	0	0.49
22	1	1	0	0	-1	0.64
23	1	0	0	0	0	0.54
24	1	0	-1	1	0	0.45
25	1	0	1	0	-1	0.51
26	1	0	0	0	0	0.55
27	1	-1	-1	0	0	0.38

4. Conclusion

L. acidophilus (ATCC4356), as a probiotic, has the potential to reduce cholesterol level in the blood. By employing the optimum experimental conditions, an enhancement in biomass production was achieved and the optimal GS modified medium compositions for the growth of *L. acidophilus* were determined. Based on our results, the optimized GS medium is comparable to the MRS medium- the most common medium for *L. acidophilus* strain- in biomass production.

Table 3. Analysis of variance for optimization of a modified GS medium for a probiotic strain (P<0.05)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	14	0.264152	0.264152	0.018868	21.77	0.000
Linear	4	0.145496	0.168664	0.042166	48.64	0.000
Square	4	0.059649	0.074795	0.018699	21.57	0.000
Interaction	6	0.059008	0.059008	0.009835	11.35	0.002
Residual Error	8	0.006935	0.006935	0.000867		
Lack-of-Fit	6	0.004868	0.004868	0.000811	0.79	0.654
Pure Error	2	0.002067	0.002067	0.001033		

Total	22	0.271087

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