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Screening of Nutrient Parameters for Lactic Acid Production by *Lactobacillus rhamnosus* 1-7 in Molasses Fermentation Using Plackett-Burman Design

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Abstract

Lactic acid is one of the functional and valuable compounds utilized in food, pharmaceutical and chemical industries while Poly lactic acid (PLA) is a biodegradable polymer that has a variety of applications. In recent years, microbial conversion of renewable raw materials has become an important objective in industrial biotechnology. Sugarcane molasses can be considered as potential renewable raw materials in PLA production. It is available in many countries as byproduct of sucrose production, which can be used as substrate for lactic acid fermentation. The objective of this study is to screen and analyses the important nutrient constituents was carried out using Plackett-Burman experimental design for lactic acid production by *Lactobacillus rhamnosus* 1-7 grown in molasses fermentation. Plackett-Burman experimental design was used to evaluate ten medium components added to molasses. Five variables namely Meat extract (10g/L), Calcium Carbonate (5g/L), Yeast extract (5g/L), Peptone (10g/L), and Di-potassium hydrogen phosphate (2g/L) of 10 variables the effect increased lactic acid production. The concentrations of these five components as well as the molasses were further optimized using the response surface method.

Keywords: Lactic acid, *Lactobacillus rhamnosus*, molasses, poly lactic acid, renewable raw material

Introduction

Lactic acid has numerous applications in the food, chemical, textile, pharmaceutical, and other industries. Recently, there has been a great demand for lactic acid, as it can be used as a monomer for the production of the biodegradable polymer Poly Lactic Acid (PLA), which is an alternative to synthetic polymers derived from petroleum resources. Molasses is a by-product of the sugar manufacturing process. It is also used as an animal feed as well as for ethanol and yeast production. The most abundant sugar is sucrose, the high concentration of which raises the viscosity of the liquid. However, among the genus *Lactobacillus* has appeared commonly in many investigations on the production of lactic acid (Sateesh *et al.*, 2013). Recently, the use of statistical experimental designs for screening instead of classical experimental design has become more popular in biotechnology. The Plackett–Burman statistical method offers a design where n variables are studied in n+1 experimental runs. These experimental designs are available in multiples of four runs and hence they are excellent screening methods, as the number of experimental runs required are very few, leading to saving of time, chemicals, glassware and manpower. Experimental design and data analysis using appropriate software makes the analysis easier as observed in the present study (Naveena *et al.*, 2005). Applying Plackett-Burman design to screen the medium components for lactic acid production is no exception. In the present study, it was described screening and analysis of important nutrient constituents was carried out using Plackett-Burman experimental design for production of lactic acid by *Lactobacillus rhamnosus* 1-7 grown in molasses fermentation.

Materials and Methods

Microorganisms, Medium and Culture Conditions

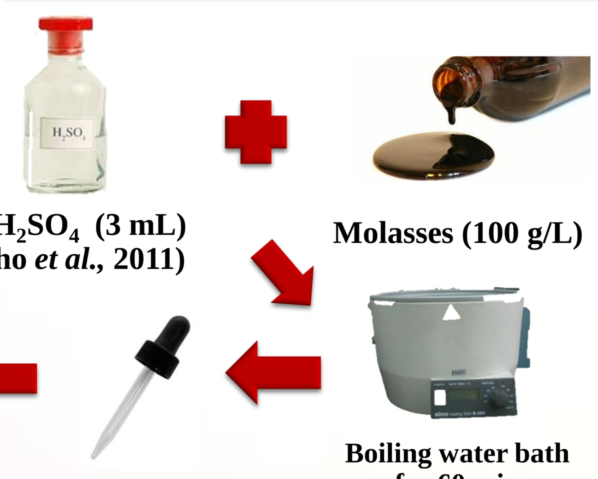
Seed culture *L. rhamnosus* 1-7

MRS medium (37 °C, 24 h)

10% inoculum

Molasses medium (37 °C, 12 h)

Substrate (Molasses Preparation)



20% H_2SO_4 (3 mL) (Coelho *et al.*, 2011)

Molasses (100 g/L)

Boiling water bath for 60 min

4.0 M KOH (pH 6.5)

Plackett-Burman Experimental Design

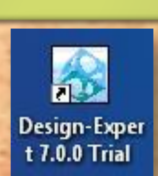
Table 1: Variables and levels used in Plackett-Burman design

No	Variables	Codes	Range and levels	
			-1	+1
A	Peptone (g/L)	X_1	0	10
B	Yeast extract (g/L)	X_2	0	5
C	Meat extract (g/L)	X_3	0	10
D	Sodium acetate (g/L)	X_4	0	5
E	Di-potassium hydrogen phosphate (g/L)	X_5	0	2
F	Tween 80 (g/L)	X_6	0	1
G	Di-ammonium hydrogen citrate (g/L)	X_7	0	2
H	Magnesium sulfate (g/L)	X_8	0	0.1
I	Manganese sulfate (g/L)	X_9	0	0.05
J	Calcium carbonate (g/L)	X_{10}	0	5

Determination of pH and Titratable Acidity (% Lactic Acid)

Titratable acidity (% Lactic Acid)

Statistical Analysis by Design Expert version 7.0



Results and Discussion

Table 2: Plackett-Burman design (real and coded values) with the respective results

Run	Independent variables[a]										Response
	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	Lactic acid (%)
1	10(1)[b]	5(1)	0(-1)	5(1)	2(1)	1(1)	0(-1)	0(-1)	0(-1)	5(1)	1.33
2	0(-1)	5(1)	10(1)	0(-1)	2(1)	1(1)	2(1)	0(-1)	0(-1)	0(-1)	1.59
3	10(1)	0(-1)	10(1)	5(1)	0(-1)	1(1)	2(1)	0.1(1)	0(-1)	0(-1)	1.59
4	0(-1)	5(1)	0(-1)	5(1)	2(1)	0(-1)	2(1)	0.1(1)	0.05(1)	0(-1)	1.41
5	0(-1)	0(-1)	10(1)	0(-1)	2(1)	1(1)	0(1)	0.1(1)	0.05(1)	5(1)	1.15
6	0(-1)	0(-1)	0(-1)	5(1)	0(-1)	1(1)	2(1)	0(-1)	0.05(1)	5(1)	0.80
7	10(1)	0(-1)	0(-1)	0(-1)	2(1)	0(-1)	2(1)	0.1(1)	0(-1)	5(1)	0.97
8	10(1)	5(1)	0(-1)	0(-1)	0(-1)	1(1)	0(-1)	0.1(1)	0.05(1)	0(-1)	1.24
9	10(1)	5(1)	10(1)	0(-1)	0(-1)	0(-1)	2(1)	0(-1)	0.05(1)	5(1)	1.33
10	0(-1)	5(1)	10(1)	5(1)	0(-1)	0(-1)	0(-1)	0.1(1)	0(-1)	5(1)	1.24
11	10(1)	0(-1)	10(1)	5(1)	2(1)	0(-1)	0(-1)	0(-1)	0.05(1)	0(-1)	1.50
12	0(-1)	0(-1)	0(-1)	0(-1)	0(-1)	0(-1)	0(-1)	0(-1)	0(-1)	0(-1)	0.88

[a] X_1= Peptone, X_2= Yeast extract, X_3= Meat extract, X_4= Sodium acetate, X_5= Di-potassium hydrogen phosphate, X_6= Tween 80, X_7= Di-ammonium hydrogen citrate, X_8= Magnesium sulfate, X_9= Manganese sulfate, and X_{10}= Calcium carbonate
[b](-1) and (1) are coded levels

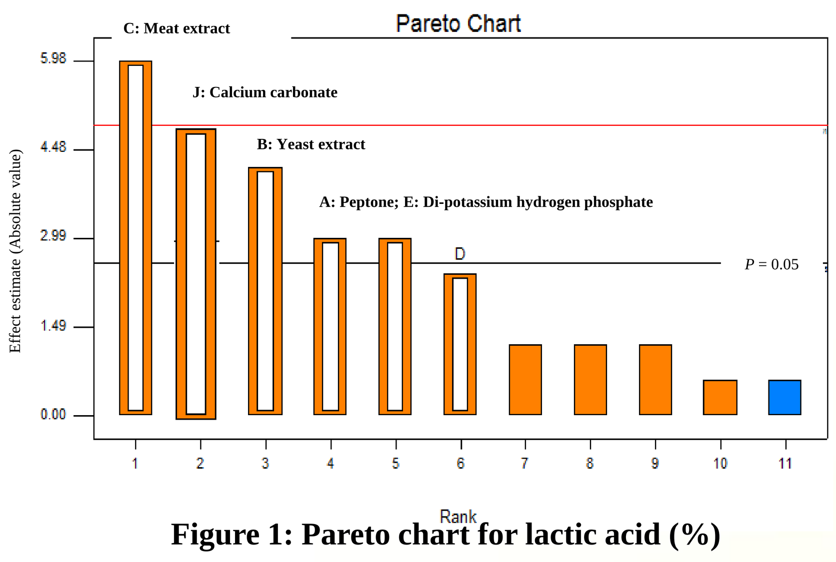


Figure 1: Pareto chart for lactic acid (%)

Table 3: Regression analysis output of the Plackett-Burman design for lactic acid (%) production *by L. rhamnosus* 1-7

Variables	Sum of Squares	Mean Square	*F*-value	*P*-value
Model	0.727717	0.121286	16.60714	0.0037*
A-Peptone	0.065208	0.065208	8.928571	0.0305*
B-Yeast extract	0.127807	0.127807	17.5	0.0086*
C-Meat extract	0.260831	0.260831	35.71429	0.0019*
D-Sodium acetate	0.041733	0.041733	5.714286	0.0624
E-Di-potassium hydrogen phosphate	0.065208	0.065208	8.928571	0.0305*
J-Calcium carbonate	0.166932	0.166932	22.85714	0.0050*

Conclusion

Plackett-Burman experimental design was used to evaluate ten medium components added to molasses (Peptone, Yeast extract, Meat extract, Sodium acetate, Di-potassium hydrogen phosphate, Tween 80, Di-ammonium hydrogen citrate, Magnesium sulfate, Manganese sulfate, and Calcium carbonate). Five variables namely Meat extract (10g/L), Calcium carbonate (5g/L), Yeast extract (5g/L), Peptone (10g/L), and Di-potassium hydrogen phosphate (2g/L) of 10 variables significant increased percentage of lactic acid. The concentrations of these five components as well as the molasses were further optimized using the response surface method. This experimental design helps efficiently screen the important factors among a great deal of variables and statistically analyze the results.

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