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# Optimization of lactic acid production from tea waste by *Lactobacillus plantarum* MTCC 6161 in solid state fermentation by central composite design.

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**Abstract:** The present study investigates the potential use of tea waste for the production of lactic acid using *Lactobacillus plantarum* MTCC 6161.The tea waste comprise of tannin, which acts as a carbon source, which upon microbial activity gets converted to glucose. In this study, initially the effects of four process variables namely yeast extract concentration, moisture content, substrate concentration, and $(NH_4)_2SO_4$ concentration were studied using fractional factorial design **(**FFD**)**. From FFD it was found out that yeast extract concentration, moisture content and substrate concentrations had statistically significant effect on lactic acid concentration in the final product. Consequently, these variables were optimized through response surface methodology. A central composite design was used for this purpose. A quadratic model was proposed to predict the lactic acid concentration. Maximum lactic acid concentration was achieved when amount of yeast extract , amount of substrate (tea waste) , and moisture content were 0.2 g, 11 g and 80% respectively. Corresponding lactic acid concentration was estimated to be 13.82 g/L. The results thus suggest that tea waste, otherwise waste, can be used to produce lactic acid.
**Keywords:** Tea waste, *Lactobacillus plantarum*, solid state fermentation(SSF), fractional factorial design(FFD), response surface methodology(RSM) .

## INTRODUCTION

The tea waste comprises of moisture (6.80%), total ash (6.25%), total extract (36.90%), tannin (10.25%) and caffine (2.02%)[1,2]. The source of carbon in the teawaste is the tannin component. This tannin component could be broken down to fermentable sugars by the microbial tannase activity[3]. Tannase (tannin acyl hydrolase) is an inducible extracellular microbial enzyme. Tannase catalyses the hydrolysis of ester and depside bonds in hydrolysable tannin such as tannic acid releasing glucose and gallic acid.[4,5,6] *Lactobacillus plantarum* shows excellent tannase activity[7,8].

In the present work, *Lactobacillus plantarum* was used to convert the tannin content of tea waste to fermentable sugars and then to lactic acid by solid state fermentation. Solid state fermentation (SSF) is the cultivation of the microorganism under controlled conditions in solid state, which has advantages like low capital investment, better product recovery, and less water output[8]. Initial screening of the process variables affecting the yield of lactic acid by *Lactobacillus plantarum* was done by employing fractional factorial design.

After choosing the important variables, optimization was carried out using response surface methodology. Response surface methodology (RSM) is a powerful technique to achieve global optimization. In this work, central composite design of experiments with 6 center points were carried out.

## MATERIALS & METHODS

### Media preparation for culturing microorganism

*Lactobacillus plantarum* MTCC 6161 was procured from Institute of microbial technology, Chandigarh and maintained on appropriate growth medium (MRS) with composition of peptone-10 g/L, beef extract-10 g/L, yeast extract-5 g/L, glucose-20 g/L, Tween 80 ml, $Na_2HPO_4$-2g/L, sodium acetate-5 g/L, tri-ammonium citrate -2 g/L, $MgSO_4.7H_2O$-0.2 g/L, $MnSO_4.4H_2O$-0.2 g/L, agar-15g/L, distilled water-1.0 L. Adjust the pH of the medium to 6.2-6.6. After inoculation the culture flask was maintained at 30 ºC for 24 h.

### Solid state fermentation

Effective removal of caffeine was done by hot water treatment for about 15 min and oven dried overnight. Solid state fermentation (SSF) was conducted in 250 ml conical flasks containing 5g tea waste and required amount of water was added. The concentrated hydrolyzate was supplemented with $(NH_4)_2SO_4$(0.5g/5g) and yeast extract (0.5g/5g). The flasks were autoclaved at 121 °C for 15 min. After cooling, the sterilized solid medium was inoculated with 2ml suspension, mixed thoroughly and incubated at 35 °C for 24 h.

### Extraction and estimation of lactic acid

After fermentation, the complete lactic acid produced in each flask was extracted and clarified by squeezing through dampened cheese cloth[9], followed by cold centrifugation at 5000 rpm for 20 min and the supernatant was used for the estimation of lactic acid[10].

### Fractional factorial design

In a factorial experimental design the explanatory variables are varied simultaneously in order to determine the effect of their variation on the response variable[11,12,13]. Here amount of yeast extract, amount of substrate, moisture content and ammonium sulphate were taken as explanatory variables. The response variable was the lactic acid concentration. In this work, yeast extract was varied from 0.2 to 2 g, amount of substrate was varied from 1 to 10 g, moisture content was varied from 20 to 80 % and ammonium sulphate was varied from 0.2 to 2 g. Plackett-Burman factorial design was employed for screening of important variables that affect the yield of lactic acid.

### Response surface methodology

Central composite design with 6 center points and hence a total of 20 experiments were carried out. For statistical calculations, the variables $X_i$ were coded as $x_i$ according to the following relationship.

$$x_i = \frac{(X_i - X_o)}{\Delta X} \quad \text{.........(1)}$$

Codified mathematic model employed for the central composite design was:

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \beta_{ij} x_i x_j \quad \text{.........(2)}$$

where,

Y - response variable (lactic acid concentration in the final product).

$x_i$ - explanatory variable i

$\beta$ - regression coefficient.

## RESULTS & DISCUSSION

### Fractional Factorial Analysis

Experimental results are shown in **table 1.** The results were analyzed using Minitab 15. The pareto chart is shown in **Fig. 1.** From the figure it can be seen that the calculated t-value was greater than the table value (2.26) for three variables namely substrate concentration, moisture content, and yeast concentration. This observation suggests that these variables have statistically significant effect on lactic acid yield. Concentration of ammonium sulphate did not affect the lactic acid concentration significantly.

**Table 1: Plackett-Burman factorial design for lactic acid production in solid state fermentation by *Lactobacillus plantarum* using tea waste as carbon source**

| Amount of yeast extract (g) | Amount of substrate concentration (g ) | Moisture content (%) | Ammonium sulphate (g) | Lactic acid concentration (g/L) |
|---|---|---|---|---|
| 2 | 1 | 80 | 0.2 | 7.49 |
| 2 | 11 | 20 | 2 | 11.99 |
| 0.2 | 11 | 80 | 0.2 | 18 |
| 2 | 1 | 80 | 2 | 5.99 |
| 2 | 11 | 20 | 2 | 11.99 |
| 2 | 11 | 80 | 0.2 | 14.99 |
| 0.2 | 11 | 80 | 2 | 18 |
| 0.2 | 1 | 80 | 2 | 5.25 |
| 0.2 | 1 | 20 | 2 | 6.75 |
| 2 | 1 | 20 | 0.2 | 4.5 |
| 0.2 | 11 | 20 | 0.2 | 14.99 |
| 0.2 | 1 | 20 | 0.2 | 5.99 |
| 1.1 | 6 | 50 | 1.1 | 4.40 |
| 1.1 | 6 | 50 | 1.1 | 3.15 |
| 1.1 | 6 | 50 | 1.1 | 4.30 |

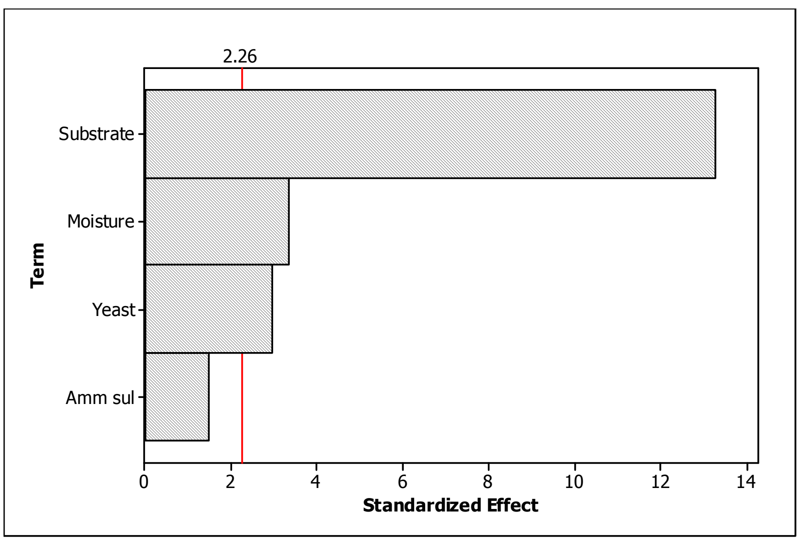


**Figure 1. Pareto Chart of the Standardized Effects**

**Table 2: Lactic acid concentrations at various process conditions.**

| Amount of yeast Extract (g) | Amount of substrate Concentration (g/) | Moisture content(%) | Concentration of lactic acid produced (g/ l) |
|---|---|---|---|
| 0.2 | 1 | 20 | 6.46 |
| 2.0 | 1 | 20 | 4.63 |
| 0.2 | 11 | 20 | 10.85 |
| 2.0 | 11 | 20 | 11.57 |
| 0.2 | 1 | 80 | 8.96 |
| 2.0 | 1 | 80 | 7.06 |
| 0.2 | 11 | 80 | 14.23 |
| 2.0 | 11 | 80 | 13.75 |
| 0.2 | 6 | 50 | 9.23 |
| 2.0 | 6 | 50 | 8.35 |
| 1.1 | 1 | 50 | 2.63 |
| 1.1 | 11 | 50 | 8.21 |
| 1.1 | 6 | 20 | 4.91 |
| 1.1 | 6 | 80 | 6.41 |
| 1.1 | 6 | 50 | 4.90 |
| 1.1 | 6 | 50 | 4.90 |
| 1.1 | 6 | 50 | 5.10 |
| 1.1 | 6 | 50 | 4.40 |
| 1.1 | 6 | 50 | 4.80 |
| 1.1 | 6 | 50 | 3.90 |

**Table 3: Results of regression analysis for the production of lactic acid**

| | Full model | | | | Reduced model | | | |
|---|---|---|---|---|---|---|---|---|
| Term | Coef | SE Coef | T | P | Coef | SE Coef | T | P |
| Constant | 4.790 | 0.153 | 31.40 | 0.000 | 4.845 | 0.154 | 31.374 | 0.000 |
| Y | -0.438 | 0.140 | -3.12 | 0.011 | -0.438 | 0.146 | -3.009 | 0.010 |
| S | 2.887 | 0.140 | 20.57 | 0.000 | 2.887 | 0.146 | 19.825 | 0.000 |
| M | 1.199 | 0.140 | 8.54 | 0.000 | 1.199 | 0.146 | 8.233 | 0.000 |
| $Y^2$ | 3.814 | 0.268 | 14.25 | 0.000 | 3.980 | 0.257 | 15.463 | 0.000 |
| $S^2$ | 0.443 | 0.268 | 1.66 | 0.128 | | | | |
| $M^2$ | 0.688 | 0.268 | 2.57 | 0.028 | 0.855 | 0.257 | 3.320 | 0.006 |
| YS | 0.497 | 0.157 | 3.17 | 0.010 | 0.497 | 0.157 | 3.17 | 0.010 |
| YM | -0.157 | 0.157 | -1.00 | 0.341 | | | | |
| SM | 0.078 | 0.157 | 0.50 | 0.628 | | | | |

**Table 4: ANOVA analysis for the production of lactic acid.**

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Regression | 6 | 145.94 | 145.94 | 24.32 | 236.44 | 0.000 |
| Linear | 3 | 58.90 | 66.67 | 22.22 | 216.03 | 0.000 |
| Square | 2 | 84.44 | 78.38 | 39.19 | 380.96 | 0.000 |
| Interaction | 1 | 2.59 | 2.59 | 2.59 | 25.22 | 0.000 |
| Residual Error | 11 | 1.13 | 1.13 | 0.10 | | |
| Lack-of-Fit | 7 | 0.86 | 0.86 | 0.12 | 1.84 | 0.290 |
| Pure Error | 4 | 0.27 | 0.27 | 0.07 | | |
| Total | 17 | 147.07 | | | | |

## Response surface methodology (RSM)

After identifying the factors that had great influence on the lactic acid production for this organism, optimization of these variables was carried out by employing response surface methodology. Experimental conditions and corresponding lactic acid yields are given in table 2. Experimental results are fitted to the eqn. 2 and the regression coefficients are shown in table 3 along with their corresponding t and p-values. Regression coefficients with p-values less than 0.05 are considered to be statistically significant at 95% confidence level. Accordingly substrate concentration (amount of tea dust) had both linear and quadratic effect while yeast extract and moisture content were not significant. It can be seen from the table 3 that regression coefficients of the factors $S^2$, YM and SM were not statistically significant (p-value > 0.05) at 95% confidence level. Therefore, these three terms were removed from the regression equation and the analysis was repeated. Results are shown in the table 3. High $R^2$ value (0.9867) confirms that the model proposed is fitting the experimental data very closely. ANOVA analysis of the model is shown in table 4. The p-value for all the sources namely the regression, linear terms, square terms, and interaction terms included in the reduced model were found less than 0.05. The p-value for the lack of fit was greater than 0.05 confirming that the fit is good. Substituting the regression coefficients in equation 2 we got the following expression:

$$LA = 4.845 - 0.438\,Y + 2.887\,S + 1.199\,M + 3.98\,Y^2 + 0.855\,M^2 + 4.97\,YS \quad .....(3)$$

Maximum lactic acid concentration was achieved when the values of Y, S and M were 2 g, 11 g/L and 80% respectively.

Lactic acid was produced by *Lactobacillus plantarum* in solid state fermentation using teawaste as carbon source. The effect of process variables namely amount of yeast extract, amount of substrate, moisture and ammonium sulphate had been studied by employing Plackett-Burman factorial design. It was found that amount of yeast extract, amount of substrate and moisture had statistically significant effect on lactic acid production. Subsequently optimization of process variables was carried out by central composite design. A quadratic equation was proposed to explain the variation in lactic acid concentration in terms of the process variables. The proposed equation could explain about 98.67% variation in lactic acid concentration leaving only 1.33% to residuals. Maximum yield was obtained when amount of yeast extract, amount of substrate and moisture were at 2g, 11 g/L and 80% respectively.

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