Papers published:


Papers presented at symposia:

- Presented a paper entitled “Lactic acid production of crude date juice” at Regional Symposium on Microbial Biotechnology, at Ahmedabad, Gujarat, India on 24 January 2005.

- Presented a paper entitled “Screening of media components for lactic acid production by statistical design” on Science Day celebration at Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar, Gujarat, India 28th February, 2006.


Application of Response Surface Methodology for Optimization of Lactic Acid Production Using Date Juice

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Received: March 7, 2006
Accepted: May 4, 2006
by applying the second-order polynomial model. The analysis was done using Design-Expert version 7.0.

MATERIAL AND METHODS

Organism
A homofermentative bacterial isolate, *Lactobacillus* sp. KCP01, has been employed for lactic acid production using date juice in the present study [3]. The organism was grown on MRS medium and maintained in 50% glycerol as a pure culture and stored below 0°C.

Inoculum Preparation
The inoculum was prepared by transferring 100 μl of glycerol stock to 100 ml of sterile lactobacillus MRS broth (pH 7.0; Hi-Media, India), in a 250-ml Erlenmeyer flask and incubated at 40°C for 24 h. Cells were harvested in a sterile centrifuge tube by centrifugation at 9,000 rpm for 10 min. The pellet obtained was resuspended in sterile distilled water to an optical density of 1.0 at 660 nm. One ml of thus prepared inoculum was transferred to 100 ml of production media.

Media Preparation and Lactic Acid Production
Forty g of thoroughly washed seedless dates were minced in distilled water, and the final volume was made up to 300 ml and boiled for 10 min. This was followed by filtration to obtain clear date juice [16, 22]. Crude juice of dates was further diluted with distilled water to obtain a final concentration of 5% reducing sugar. The juice was used as the production medium along with the significant components (Na-acetate, peptone and K₂HPO₄). The medium was adjusted to pH 7.0 and sterilized by autoclaving at 121°C for 15 min. After inoculation, the flasks were incubated at 40°C under static condition. Samples were collected at 48 h. Cells were harvested by centrifugation at 9,000 rpm for 10 min, and clear supernatant was subjected to lactic acid estimation.

Analysis of Media Constituents and Estimation of Lactic Acid
Reducing sugar from date juice was estimated by the dinitrosalicylic acid method [13]. Lactic acid production was confirmed by paper chromatography, HPLC, and enzymatic tests [16], and routine estimations were carried out by a colorimetric method [10].

Factorial Design and Analysis of Results
Design-Expert version 7.0 (State-Ease Inc., Minneapolis, U.S.A.) was used for experimental design (Central Composite Design, CCD), regression and graphical analysis of the data obtained. Four independent variables, including date juice, sodium acetate, peptone and K₂HPO₄ were studied at five different levels (Table 1).

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Levels</th>
<th>Values are expressed as g/l.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date juice (sugar)</td>
<td>-α</td>
<td>0.0 8.0 15.0 20.0 25.0 30.0</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>-α</td>
<td>0.0 2.0 4.0 6.0 8.0 10.0</td>
</tr>
<tr>
<td>Peptone</td>
<td>-α</td>
<td>0.0 2.0 4.0 6.0 8.0 10.0</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>-α</td>
<td>0.0 2.0 4.0 6.0 8.0 10.0</td>
</tr>
</tbody>
</table>

A set of 36 experiments was performed. The minimum and maximum ranges of variables were used, and the full experimental design with respect to their coded values is listed in Table 2. The data on lactic acid production obtained from RSM were subjected to the analysis of variance (ANOVA). The results of RSM were used to fit a second-order polynomial [Eq. (1)].

\[
Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{33}X_3^2 + \beta_{44}X_4^2 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{14}X_1X_4 + \beta_{23}X_2X_3 + \beta_{24}X_2X_4 + \beta_{34}X_3X_4
\]

where \(Y\) is the response variable (dependent variable), \(\beta_0\) is the intercept (constant), \(\beta_{11}, \beta_{22}, \beta_{33}, \beta_{44}\) and \(\beta_{ij}\) are linear coefficients, \(\beta_{12}, \beta_{13}, \beta_{14}, \beta_{23}, \beta_{24}, \beta_{34}\) are interaction coefficients, \(\beta_{11}, \beta_{12}, \beta_{13}, \beta_{14}, \beta_{22}, \beta_{23}, \beta_{24}, \beta_{33}, \beta_{34}\) are squared coefficients, and \(A, B, C, D\) are levels of independent variables. Statistical significance of the above model equation was determined by Fisher's test value, and the proportion of variance explained by the model was given by the multiple coefficient of determination, R squared (\(R^2\)) value. Later, an experiment was run using the optimum values for variables given by response optimization to confirm the predicted value and the lactic acid production was confirmed.

RESULTS AND DISCUSSION

Among the fifteen variables (date juice, peptone, beef extract, yeast extract, K₂HPO₄, KH₂PO₄, MgSO₄·7H₂O, MnSO₄·H₂O, sodium acetate, sodium sulfate, tri-sodium citrate, sodium succinate, tween-80, FeSO₄ and NaCl), significant components were screened out using fractional factorial Plackett-Burman design [19]. This experiment suggested four components, date juice, peptone, K₂HPO₄, and sodium acetate, as having significance of more than 95% confidence level [3]. In the present study, these variables were selected for further optimization by CCD of response surface methodology. The results of the CCD experiments for studying the effect of independent variables are presented in Table 2. CCD is based on three basic points with respect to concentration of components; full factorial points \(2^k\) where \(k\) is the number of variables, center points \(n_0 (n_0>1)\) and two axial points for each variable (\(\alpha = 2^{1/k}, =2\) for \(k=4\)).
Increased concentration of sodium acetate did not have any significant effect, unless peptone was present at concentration above 15.0 g/l (Fig. 1D). Elevated levels of sodium acetate along with increased concentration of K$_2$HPO$_4$ resulted in higher lactic acid production (Fig. 1E). The concentration of K$_3$HPO$_4$ had no significant effect; however, it resulted in a higher production of lactic acid with increased concentration of peptone (Fig. 1F). By applying multiple regression analysis on the experimental results, a second-order polynomial model (Eq. 2) was derived explaining the role of each variable and their interaction in lactic acid production.
Statistical screening of medium components by Plackett–Burman design for lactic acid production by *Lactobacillus* sp. KCP01 using date juice

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Received 25 September 2005; received in revised form 12 November 2005; accepted 16 November 2005

Available online 4 January 2006

Abstract

Statistical screening of media components for production of lactic acid by *Lactobacillus* sp. KCP01 using date juice as a sugar source was carried out by Plackett–Burman design. Date juice at 5% sugar concentration when used alone showed 2.6 g/l of lactic acid production. Increase in lactic acid production (15.1 g/l) was observed with supplementation of salts and organic nitrogen sources of MRS medium and after optimization of pH and temperature using date juice as a C-source. Plackett–Burman design showed peptone, K$_2$HPO$_4$, sodium acetate and date juice as significant components influencing the lactic acid production.

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Keywords: Date juice; Lactic acid; *Lactobacillus* sp.; Plackett–Burman; Statistical screening method

1. Introduction

Lactic acid is a valuable industrial chemical that is being used as acidulant, preservative for beverages and in food stuffs applications. Crude lactic acid is also used in leather, textile and laundry industries. Calcium lactate is employed in baking powders, as an animal feed supplement and as a means for providing Ca source in pharmaceutical preparations (Litchfield, 1996). Lactic acid can be obtained on industrial scale either by fermentation or chemical synthesis. Today lactic acid has become the most demanding monomer as it can be readily polymerized to biodegradable polymer-polylactide. Polylactide is used for the preparation of scaffolds for biocompatible artificial organs, self-dissolving sutures and as a means for sustained drug release (Datta et al., 1995; Gross and Kalra, 2002).

Date is a berry fruit of the palm tree (*Phoenix dactylifera*), mainly grown in Arabian countries and desert region of other tropical countries, including India. They are nutritionally rich in carbohydrates, minerals and vitamins (Morton, 1987; Kamel, 1979). Date can be a good source of sugar and its use as raw material can result in relatively cheaper fermentation process as it does not require any special treatment like acid hydrolysis, steam explosion or enzymatic treatment to release sugars in fermentable form. Considering this fact, date juice has been employed for the production of citric acid (Roukas and Kotzekidou, 1991) alcohol (Mehaia and Cheryan, 1991) and antibiotic oxytetracyclin (Abou-Zeid et al., 1993). Dates have also been used to produce single cell protein and biomass for use as dairy starter culture and baker's yeast (Kamel, 1979; Khan et al., 1995; Nancib et al., 1997, 1999).

Productivity of microbial metabolites can be increased by manipulating nutritional requirements, physical parameters and genetic make up of the producing strain (Greasham, 1983). Development of economical medium requires selection of carbon, nitrogen, phosphorous, potassium and trace element sources. Nutritional requirement can be manipulated by the conventional or statistical methods. Conventional method involves changing one independent variable at a time while keeping the others at fixed values.
level. However, statistical method offers several advantages over conventional method being rapid and reliable, short lists significant nutrients, helps understanding the interactions among the nutrients at various concentrations and reduces the total number of experiments tremendously resulting in saving time, glassware, chemicals and manpower (Srinivas et al., 1994; Carvalho et al., 1997). Initial screening of the ingredients is done to understand the significance of their effect on the product formation and then a few better ingredients are selected for further optimization (Greasham, 1983; Naveena et al., 2005; Ganapathy et al., 1998).

Different types of statistical methods are available for such optimization experiments (Thomas, 1977; Deming and Morgan, 1987; Demain et al., 1989). Multi factorial designs will be difficult, as large number of variable are to be screened, in terms of number of experiments \(2^k\), where \(k\) is the total number of variables. Hence two level fractional factorial designs like Plackett–Burman will be of choice that screens \(k\) variable in just \(k + 1\) experiments. Moreover, the design is orthogonal in nature and thus gives pure effect of each variable not confounded with interactions among variables.

Lactic acid production has been carried out with date juice by fermentation using *Lactobacillus casei* subsp. *rhamnosus* with yeast extract and \((NH_4)_2SO_4* supplementation (Nancib et al., 2001). Ammonium sulfate when used alone considering higher cost of yeast extract required supplementation of B vitamins for optimum lactic acid production (Nancib et al., 2005). Thus, there remains scope for screening of medium components by suitable statistical design, which may identify the significant ingredients influencing lactic acid production using date juice as a sugar source. The aim of this work was to use the date juice and screen suitable medium components for lactic acid production by *Lactobacillus* sp. KCP01 using Plackett–Burman design.

2. Methods

2.1. Organism

A local isolate of homofermentative, lactic acid producing bacterial strain was identified as *Lactobacillus* sp. KCP01 by morphological and biochemical tests according to Bergey's Manual of Determinative Bacteriology (Holt et al., 1994). It was grown on MRS medium and maintained in 50% glycerol and stored below 0 °C.

2.2. Inoculum preparation

The inoculum was prepared by transferring 100 µI glucose stock to 100 ml of sterile lactobacillus MRS broth (Hi-Media, India) in 250 ml Erlenmeyer flask and incubated at 37 °C for 24 h. Cells were harvested in a sterile centrifuge tube (15 ml) by centrifugation at 9000 g for 10 min. The pellet obtained was resuspended in sterile distilled water to adjust the optical density to 1.0 at 660 nm. One milliliter of thus prepared inoculum was transferred to 140 ml of production media.

2.3. Media preparation and lactic acid production

For preparation of date juice, 40 g of thoroughly washed seedless dates were weighed and minced in distilled water and final volume was made up to 300 ml. This juice was boiled for 10 min so as to flocculate the insoluble material that settled rapidly upon cooling to room temperature. This was followed by filtration to obtain clear date juice (Nancib et al., 2005; Samuel and Leç, 1980). Crude juice of dates was further diluted with distilled water to get final concentration of 5% reducing sugar. The juice alone was used as production medium or with the nutrient supplement of MRS medium, except the recommended concentration of sugar source.

The MRS medium used for lactic acid production contained (g/l): peptone, 10; beef extract, 10; yeast extract, 5; K_2HPO_4, 2; sodium acetate, 5; tri-sodium citrate, 2.15; MgSO_4·7H_2O, 0.2; MnSO_4·4H_2O, 0.05; Tween-80, 1 and date juice to give 5% reducing sugar. The pH of the medium was adjusted to 7.0 and sterilized by autoclaving at 121 °C for 15 min. After inoculation, the flasks were incubated at 40 °C under static condition. Samples were collected at every 24 h up to two days for initial studies and at 48 h during media component screening studies. Cells were harvested by centrifugation at 9,000 g for 10 min and clear supernatant was subjected to lactic acid estimation.

2.4. Analysis of media constituents and lactic acid estimation

Reducing sugars from date juice was estimated by dinitro salicylic acid method (Miller, 1951). Lactic acid production was confirmed by paper chromatography, HPLC and enzymatic tests (Nancib et al., 2002) while routine estimations were carried out by colorimetric method (Kimberley and Taylor, 1996). Glucose, fructose and protein were estimated colorimetrically whereas free fatty acids were estimated by titrimetric method (Sadasivam and M.Nickam, 1996). Sugars were identified by ascending paper chromatography using the solvent system: butanol:ethanol:water in the ratio of 40:11:19 (v/v) and developing with silver nitrate reagent (2.5 ml of saturated AgNO_3 in 50 ml acetone), dried and dipping in NaOH reagent (16 % NaOH in 800 ml of 0.5 N methanol).

2.5. Screening of important nutrient components

The present study was aimed at screening of the important medium components with respect to their main effects and not the interaction effects between various medium constituents and hence, Plackett–Burman design was used (Plackett and Burman, 1944). Including date juice, a total
of fifteen components [variables, \( k = 15 \)] were selected for the study with each variable represented at two levels, high concentration (+) and low concentration (−) and four dummy variables in 20 trials as shown in Tables 1 and 2. The number of positive signs and negative signs per trial are \((k + 1)/2\) and \((k − 1)/2\), respectively. Each column should contain an equal number of positive and negative signs. Thus, each row represents a trial run and each column represents an independent (assigned) or dummy (unassigned) variable. The effect of each variable was determined by following equation:

\[
E(x_i) = 2 \left( \frac{\sum M^+_i - \sum M^-_i}{N} \right)
\]

where \(E(x_i)\) is the concentration effect of the tested variable, \(M^+_i\) and \(M^-_i\) are the lactic acid production from the trials where the variable \((x_i)\) measured was present at high and low concentrations, respectively; and \(N\) is the number of trials (20). Experimental error was estimated by calculating the variance among the dummy variables as follows:

\[
V_{\text{eff}} = \sum (E_d)^2 / n
\]

where \(V_{\text{eff}}\) is the variance of the concentration effect, \(E_d\) is the concentration effect for the dummy variable and \(n\) is the number of dummy variables. The standard error (SE) of the concentration effect was the square root of the variance of an effect and the significance level \((p\text{-value})\) of each concentration effect was determined using student’s \(t\)-test:

\[
t(x_i) = \frac{E(x_i)}{SE}
\]

where \(E(x_i)\) is the effect of variable \(x_i\).

### 3. Results and discussion

#### 3.1. Date juice and lactic acid production

Dates are reported to be rich in carbohydrates (predominantly glucose and fructose) along with range of minerals and vitamins but low in protein content (1.5–3%, w/w) (Kamel, 1979; Nancib et al., 2001). In the present study, we selected two varieties of dates (black and brown, with black costing double than brown date) available in the local market. Analysis of the dates for their nutrient contents revealed presence of both glucose and fructose, with non detectable sucrose (Table 3). Both the varieties showed approximately 40 g of reducing sugars/100 g of dates.
Interestingly, comparatively higher protein content was found than reported previously (Kamel, 1979). Considering the nutrient composition and cost, brown date juice alone having 5% reducing sugar was used for lactic acid production, but very low production (2.6 g/l) was obtained. Lactic acid bacteria are reported to be fastidious and have complex nutrient requirements (Chapin, 1993; Fitzpatrick and O’Keeffe, 2001). Thus, palm dates, though found to be rich in easily extractable sugar source compared to other raw materials, must be treated prior with enzyme, or by other means to release the sugar in utilizable and fermentable form by the organism.

Supplementation of other salts and organic nitrogen sources along with the optimization of initial pH (7.0) and temperature (40 °C) increased production of lactic acid to 15.1 g/l (data not shown). Thus, though date juice when supplemented with other salts and organic nitrogen sources, proved to be a potential raw material, considering the number and amount of other organic nitrogen sources added; and their cost, it was thought necessary to have screening of media components using an appropriate statistical design to identify the significant components. Apart from MRS medium, reports have shown use of other media for lactic acid production (Srivastava et al., 1992; Hongo et al., 1986). Ingredients of these additional media were also considered and 15 components were selected for further screening as shown in Table 1.

### Table 3
Concentration of major nutrients in 100 g of two date varieties

<table>
<thead>
<tr>
<th>Type of analysis</th>
<th>Black date</th>
<th>Brown date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (g)</td>
<td>21.75</td>
<td>21.308</td>
</tr>
<tr>
<td>Fructose (g)</td>
<td>17.63</td>
<td>18.668</td>
</tr>
<tr>
<td>Sucrose</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Total sugar (g)</td>
<td>39.40</td>
<td>40.00</td>
</tr>
<tr>
<td>Free fatty acid (g)</td>
<td>3.07</td>
<td>2.71</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>7.29</td>
<td>6.254</td>
</tr>
<tr>
<td>ND: Not detected</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 4
Lactic acid production obtained and calculated t-value, probability and confidence level as per Plackett-Burman design

<table>
<thead>
<tr>
<th>Components</th>
<th>LA produced (g/l)</th>
<th>Effect</th>
<th>Standard error</th>
<th>t-Value</th>
<th>P</th>
<th>Confidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>7.6333</td>
<td>1.6619</td>
<td>0.1767</td>
<td>9.4011</td>
<td>0.0007</td>
<td>99.92%</td>
</tr>
<tr>
<td>Beef extract</td>
<td>8.0000</td>
<td>1.2563</td>
<td>0.1767</td>
<td>7.1068</td>
<td>0.0020</td>
<td>99.79%</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>9.6616</td>
<td>1.6269</td>
<td>0.1767</td>
<td>9.2032</td>
<td>0.0007</td>
<td>99.92%</td>
</tr>
<tr>
<td>K2HPO4</td>
<td>16.421</td>
<td>1.3303</td>
<td>0.1767</td>
<td>7.5254</td>
<td>0.0016</td>
<td>99.83%</td>
</tr>
<tr>
<td>KH2PO4</td>
<td>8.3916</td>
<td>0.3279</td>
<td>0.1767</td>
<td>1.8552</td>
<td>0.1371</td>
<td>80.47%</td>
</tr>
<tr>
<td>MnSO4*4H2O</td>
<td>12.158</td>
<td>-0.5720</td>
<td>0.1767</td>
<td>-3.2558</td>
<td>0.0318</td>
<td>98.02%</td>
</tr>
<tr>
<td>MgSO4*7H2O</td>
<td>5.775</td>
<td>0.2603</td>
<td>0.1767</td>
<td>1.4724</td>
<td>0.2148</td>
<td>78.51%</td>
</tr>
<tr>
<td>Tween-80</td>
<td>6.6333</td>
<td>-0.9563</td>
<td>0.1767</td>
<td>-5.4098</td>
<td>0.0056</td>
<td>99.43%</td>
</tr>
<tr>
<td>NaCl</td>
<td>9.3916</td>
<td>-0.1013</td>
<td>0.1767</td>
<td>-0.5730</td>
<td>0.5973</td>
<td>40.26%</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>10.008</td>
<td>0.9553</td>
<td>0.1767</td>
<td>5.4041</td>
<td>0.0056</td>
<td>99.43%</td>
</tr>
<tr>
<td>Sodium sulfate</td>
<td>10.85</td>
<td>0.8146</td>
<td>0.1767</td>
<td>4.6081</td>
<td>0.0099</td>
<td>99.00%</td>
</tr>
<tr>
<td>Tri-sodium citrate</td>
<td>8.842</td>
<td>-0.2746</td>
<td>0.1767</td>
<td>-1.5535</td>
<td>0.1952</td>
<td>80.47%</td>
</tr>
<tr>
<td>Sodium succinate</td>
<td>10.475</td>
<td>-0.0786</td>
<td>0.1767</td>
<td>-0.4448</td>
<td>0.6794</td>
<td>32.05%</td>
</tr>
<tr>
<td>FeSO4·7H2O</td>
<td>12.4833</td>
<td>-0.5980</td>
<td>0.1767</td>
<td>-3.3828</td>
<td>0.0277</td>
<td>97.22%</td>
</tr>
<tr>
<td>Date juice (as reducing sugar)</td>
<td>16.8603</td>
<td>3.1146</td>
<td>0.1767</td>
<td>17.618</td>
<td>6.1E-05</td>
<td>99.99%</td>
</tr>
</tbody>
</table>

### 3.2. Effect of medium components on lactic acid production

Table 1 represented the independent variables and their respective high and low concentrations used in the optimization study, whereas Table 2 represented the Plackett-Burman experimental design for 20 trials with two levels of concentrations for each variable, which was followed for the optimization of medium components for lactic acid production. The variables X1-X15 represented the medium constituents and D1-D4 represented the dummy variables/assigned variables. Table 4 represents the results of Plackett-Burman experiment with respect to lactic acid production, the effect, standard error, t(0.1), P and confidence level of each component. The components were screened at the confidence level of 95% on the basis of their effects. If the component showed significance at or above 95% confidence level and its effect was negative, it indicated that the component was effective in lactic acid production but the amount required was lower than the indicated low (−) concentration in Plackett-Burman experiment. If the effect was positive, a higher concentration than the indicated high value (+) concentration was required during further optimization studies.

The confidence level of components KH2PO4, MgSO4·7H2O, NaCl, tri-sodium citrate and sodium succinate were below 95% in lactic acid production and hence, were considered insignificant. The rest of the components, peptone, beef extract, yeast extract, K2HPO4, sodium acetate, sodium sulfate, FeSO4·7H2O, MnSO4·4H2O and date juice showed confidence level at or above 95% and were considered to be significant (Table 4).

Peptone, yeast extract and beef extract were selected as a source of nitrogen and were significant for lactic acid production at 99.92%, 99.92% and 99.79% confidence level at 1.0, 0.5 and 1.0 g/l concentration. Lactic acid bacteria are traditionally fastidious microorganisms and have complex nutrient requirements due to their limited ability to biosynthesize B-vitamins and amino acids (Chauhan, 1993;
Fitzpatrick and O’Keeffe, 2001). Moreover, a considerable amount of expensive complex nitrogen source, such as yeast extract must be added to the medium to produce lactic acid in reasonable time. While replacing yeast extract from the medium with ammonium sulfate, considering the cost factor for lactic acid production using date juice, additional requirements of B vitamins has been reported (Nancib et al., 2005). This again resulted in economically unfavorable process considering the cost of lactic acid (Ryu et al., 1999). Thus, considering the cost of organic nitrogen sources tested in the study, peptone was selected as source of nitrogen for further study. Using the same statistical design, peptone was found significant at 1.0 g/l concentration during solid-state fermentation using wheat bran and during selection of media components for lactic acid production by Lactobacillus plantarum NCIM 2084 at 1.0 g/l (Naveena et al., 2005; Krishnan et al., 1998).

Sodium acetate was also found significant at 99.43% confidence level. It enhanced the cell growth and thus influenced the production indirectly (Peters and Snell, 1954). Tween-80 has also been reported to be a significant component for lactic acid production using wheat bran under solid-state fermentation (Naveena et al., 2005) and for production of enzymes. In our study, though it was significant at 99.43% confidence level, it showed negative effect on lactic acid production (Table 4). This could be because the culture had easily available reducing sugars without any need of enzymatic activity. Hence, Tween-80 was not used in further study.

Among the phosphate sources used, only K₂HPO₄ was significant at 99.83% and there was considerable difference in lactic acid production among the two phosphate sources used. Highest production was achieved with K₂HPO₄ and thus it was selected for further studies. Although FeSO₄ · 7H₂O was significant at 97.22% and had a negative effect on lactic acid production, considering the lactic acid production, it was better than other compounds having negative effect and thus its lower concentration was used (for further optimization studies). Same was the case with MnSO₄ · 4H₂O, being 96.82% significant for lactic acid production and since had a negative effect, its lower concentration was used for further optimization. Tri-sodium citrate and sodium succinate were also insignificant; were negative in terms of their effect, and were eliminated. Date juice was significant at 99.99% level and produced 16.86 g/l lactic acid, which was the highest among all the 15 components. Comparison of these components for the lactic acid production in Plackett–Burman design showed that trial number 15 showed maximum lactic acid production as compared to the other trials (Table 4).

In an attempt to study the effect of decreasing pH, fermentation was carried out at flask level incorporating calcium carbonate, to neutralize the lactic acid produced during the fermentation. It was observed that controlling the pH significantly increased the lactic acid production (45.59 g/l) as compared to flask without calcium carbonate (16.5 g/l) (Table 5). It was also observed that in control flask, where pH was not controlled, cell growth was affected by lower pH because of lactic acid production compared to flask with calcium carbonate.

Based on statistical screening methods, media components have been reported to influence lactic acid production by bacteria (Nancib et al., 2005; Ryu et al., 1999) which showed 35% increase in riboflavin production in UV mutant of Eremothecium ashbyii (Pujari and Chandrakumari, 2000) and 35% higher recombinant hirudin production in Saccaromyces cerevisiae (Rao et al., 2000). Using Plackett–Burman experimental design, increase in the yield of a thermotable β amylase and pullulanase by Clostridium thermosulfurogenes SV2 (Reddy et al., 1999), nisin production from whey (Liu et al., 2003) has been reported. Our result on statistical screening of media components using Plackett–Burman design proved useful in selecting significant media components while using date juice as a source of sugar. Use of date juice for lactic acid production with the pH control during the fermentation and scale up for laboratory and pilot scale fermentors may give further ideas on potential of its use for industrial production.

### Table 5
Lactic acid production by Lactobacillus KCPOI with CaCO₂ addition for pH control

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Control</th>
<th>2% CaCO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>Cell growth (CFU/ml × 10⁶)</td>
<td>0.0018</td>
<td>6.89</td>
</tr>
<tr>
<td>Residual sugar (g/l)</td>
<td>52.8</td>
<td>41.5</td>
</tr>
<tr>
<td>Lactic acid (g/l)</td>
<td>97.22</td>
<td>16.9</td>
</tr>
</tbody>
</table>

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*Cell growth was measured by viable count method after appropriately diluting the fermented broth on MRS agar medium and expressed as (colony forming unit) CFU/ml × 10⁶.*

References


