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The Newly Discovered Lactobacillus Plantarum JX183220 uses Cassava (Manihot Esculenta Krantz) Flour

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ABSTRACT
The purpose of the current study was to use Response Surface Methodology to optimize the generation of lactic acid using a new isolate of Lactobacillus plantarum JX183220 and cassava flour (Manihot esculenta Crantz) in semi-solid fermentation. Methodology's Box-Behnken study design was employed. Cassava flour (CF) was employed in a semi-solid fermentation with Lactobacillus plantarum JX183220, a bacteria isolated from goat milk. Initially, preliminary research adjusted a variety of fermentation parameters, including incubation time, inoculum volume, pH, temperature, substrate concentration (cassava flour), and calcium carbonate concentration. The response surface methodology's Box-Behnken design was used to further optimize the substrate concentration, temperature, and pH as potential factors. The fitting of a second-order polynomial regression model, which had a high coefficient of determination, R2, was successful (0.9913). The validation experiment was conducted under the parameters' ideal conditions as determined by the model. According to the preliminary research, Lactobacillus plantarum JX183220 produced the most lactic acid on the fourth day of incubation with 2% inoculum and 0.3% calcium carbonate. Maximum lactic acid production of 18.3679 g/100 g of cassava was achieved by optimization using the Box-Behnken design of RSM at the ideal substrate concentration, temperature, and pH conditions of 36.39°C and 6.43. A validation experiment supported these findings. The best conditions for a newly isolated strain of Lactobacillus plantarum JX183220 to directly convert cassava flour starch to lactic acid were identified. With 15 runs, the RSM's Box Behnken design was determined to be an efficient instrument for maximizing the production of lactic acid. In the future, scientists may use fermentation and sugar-making to make even more lactic acid.

Keywords: lactobacillus plantarum, cassava, flour, box-behnken, fermentation, lactic acid

I. INTRODUCTION

The natural organic acid lactic acid, also known as 2-hydroxypropionic acid or 2-hydroxypropanoic acid, has a long history of application in the food, cosmetic, and pharmaceutical sectors. By 2017, it is anticipated that the global market for lactic acid will grow to 367.3 thousand metric tons. The creation of poly-lactic acid (PLA), a biodegradable, biocompatible, and ecologically benign biopolymer, is the most recent use of lactic acid. Medical sutures, orthopedic implants, medicine delivery systems, and disposable consumer goods all use PLA, which considerably reduces waste disposal issues.
Since chemical synthesis results in a racemic mixture of D- and L-lactic acid, biological fermentation is the preferred approach for producing L-lactic acid.

Both bacterial and fungal species can create lactic acid. Due to mass transfer limitations, there is a limited production rate during fungal fermentation, which results in the development of byproducts including fumaric acid and ethanol. For the wide spectrum of lactic acid production, Lactobacillus species such as L. plantarum, L. delbrueckii, L. casei, L. amylophilus, and L. rhamnosus are the most demanding microorganisms.
Direct fermentation as well as simultaneous saccharification and fermentation are both methods for generating lactic acid. Starch hydrolyzing enzymes are used in simultaneous saccharification and fermentation, which raises the cost of the procedure. Utilizing amylase-producing organisms that can directly convert starch into lactic acid during direct fermentation can significantly lower operating costs. Both Lactobacillus amylovorus and Lactobacillus amylophillus have amylolytic activity. Only a few studies mentioned Lactobacillus plantarum's amylolytic action.

Solid-state fermentation or submerged fermentation is two biological methods for producing lactic acid. With better yields, more concentrated organic acids and enzymes, and the added benefit of potential downstream recovery, solid-state fermentation is preferable to submerged fermentation. However, solid state fermentation takes place when there is little to no free water present. Low moisture levels may cause nutrients to be harder to acquire, which will inhibit microbial growth. Semi-

solid fermentation gives the solid substrate a high water activity as well as carbohydrates, mineral nutrients, and nitrogen sources that make the whole process work better.

Various source materials with high concentrations of lignocelluloses, cellulose, and starch are employed for lactic acid fermentation. Cassava fibrous waste, corn cobs, sugarcane bagasse, molasses, whey, paper sludge, wheat bran, and alfalfa fibers are a few examples of the various substrates employed. These wastes from industry and agriculture contain a lot of carbon and will aid in addressing numerous environmental problems. The sixth-most significant food crop in the world, cassava (Manihot esculenta Cranz), is a starchy crop with 20–30% extractable starch depending on variety and climate. It could be used to make lactic acid because it is a cheap crop that has a lot of carbohydrates.

The typical "one factor at a time" approach to multivariable system optimization is not only time-consuming, but it frequently overlooks the interactions between the parts. An optimization process based on statistical design trials will be used to solve this issue. The goal of response surface methodology (RSM) is to characterize the behavior of a data set such that statistical inferences can be made. It is a collection of mathematical and statistical techniques focused on fitting a polynomial equation to experimental data. The essential variables are screened as part of the initial stage of process optimization, with the goal of keeping them to a maximum of three, four, or five. Following the initial screening, RSM's Box-Behnken design is used to optimize the amounts of these variables.Three-level fractional factorial arrangements, such as the Box-Behnken arrangement, make it possible to efficiently estimate the first- and second-order coefficients of the quadrating mathematical model. Several researchers have used this method to optimize various parameters.

The goal of this study is to use the Box-Behnken design of Response Surface Methodology to find the best way for Lactobacillus plantarum JX183220 to turn starch into lactic acid during semi-solid fermentation with cassava flour.

II. METHOD AND MATERIALS

2.1 Microbes and Substrate

To prevent microbial deterioration, cassava tubers were sun-dried for 4-5 days before being finely ground to the necessary size (1.2 mm). The substrate utilized was cassava flour, which was kept in an airtight container until needed. For this study, Lactobacillus plantarum JX183220 [19] from goat milk was used by the Department of Chemical Engineering and Biotechnology at ANITS in Visakhapatnam, Andhra Pradesh, India.

2.2 Making an inoculum

The modified MRS liquid medium (100 mL, peptone 10.0, beef extract 10.0, yeast extract 5.0, glucose 20.0, Na_2HPO_4, sodium acetate 5.0, tri-ammonium citrate 2.0, $MgSO_4$ 0.2, $MnSO_4$ 0.2, $CaCO_3$ 4.0, Tween 80 0.1 mL, pH 6.8) was used to prepare the inoculum. A loop containing Lactobacillus plantarum JX183220 was transferred from a stock culture to sterile medium and then incubated for 48 hours on an orbital shaker at 37°C and 120 rpm.

2.3 Media Planning

In place of glucose as a carbon source, a modified MRS semi-solid medium including cassava flour (5%) was utilized to produce lactic acid. The cassava flour-infused 100 mL of MRS semi-solid medium was autoclaved. A freshly made 2% inoculum was injected, and it was incubated for 6 days at 37°C.

2.4 Analysis of Initial Optimization

One particular variable was optimized at a time, and its optimum level was then taken into account in the subsequent optimization step. Incubation time, inoculum volume, pH, temperature, substrate concentration, and CaCO3 concentration were the variables that were optimized. The Box-Behnken method of response surface methodology was used to choose the substrate concentration, temperature, and pH for further optimization while keeping the other variables at their ideal levels.

2.5 Lactic Acid Extraction

The fermented substrate was centrifuged at 8000 g for 20 min in a refrigerator. Clear, free-floating supernatants are used to measure lactic acid.

2.6 Techniques for Analysis

Using the calorimetric method of Kimberley and Taylor [20], the amount of lactic acid was calculated, and the yield was given as grams of lactic acid per 100 grams of cassava.

2.7 Experimental Planning

The RSM Box-Behnken design has three levels for variables: low, medium, and high, which are coded as -1, 0, and +1. It is more effective, needs fewer experiments (15 runs), and is easier to understand.

Table 1: Box-Behnken design technique: variable range

Variable factors	Lower level (-1)	Middle level (0)	Upper level (+1)	Step Change ΔX=Difference between levels
Substrate concentration (%)	0.5	2	3.5	1.5
Temperature(°C)	30	35	40	5
pH	5	6	7	1

As a result, this statistical method is employed in the current investigation. For the purpose of producing lactic acid, the concentration of the substrate (cassava flour), temperature, and pH were optimized over the course of 15 runs.

The following equation was used to code the three independent variables. For statistical calculations, these variables are called X_1, X_2, and X_3, respectively.

$$x = (X-X_0)/\Delta X \quad (1)$$

If x is a coded variable, X is a natural variable, X0 is the halfway point (zero level), and ΔX is the step change that signifies the distinction between the progressively higher levels. Table 1 shows the range and levels of the things this study looked at.

III. RESULTS AND CONVERSATION

3.1 Effects of the Incubation Period

Over the course of six days, the amount of lactic acid produced was measured every 24 hours. On the fourth day, the maximum yield of 10.0 g/100 g CF was seen, as indicated in Fig. 1a. Due to the organism's reutilization of products and the significant depletion of nutrients in the fermentation medium after the fourth day, it decreased. The most lactic acid was made on the fifth day of incubation.

3.2 Inoculum Volume Effects

Lactic acid production peaked at 2% inoculum volume (11 g/100 g CF) and progressively declined as inoculum volume increased (Fig. 1b). In semi-solid fermentation, the amount of inoculum changes depending on the make-up of the medium. A low-sugar medium typically requires 2–3% inoculum volume, but higher sugar levels often require 5–10% inoculum volume [23]. Previous studies have also mentioned the use of a 2% (v/v) inoculum for the generation of lactic acid. However, lactic acid generation has also been carried out using the higher inoculum (3% v/v).

3.3 The pH Effect

Utilizing fermentation medium with a pH range of 5.5–8.5 allowed researchers to assess the impact of pH on the generation of lactic acid. As indicated in Fig. 1c, the largest amount of lactic acid was produced at pH 6.5 (9.8 g/100 g CF). The yield was shown to decrease at both higher and lower pH values. The amount of hydrogen ions in the medium influences how quickly microbes grow. The synthesis of metabolic enzymes necessary for the creation of fresh protoplasm is regulated by pH. According to studies utilizing the L. casei strain, lactic acid generation is best within a pH range of 6.0–6.5. However, Ghaly et al. employed L. helveticus to produce lactic acid at pH 5.5.

3.4 Temperature Effect

Different incubation temperatures (25°C, 30°C, 35°C, and 40°C) and their effects on lactic acid generation were researched. From 25°C to 35°C, the production of lactic acid rose, and at 45°C, it reduced (Fig. 1d). Low metabolic rate may be the cause of the decrease in lactic acid output between 25 and 30 degrees Celsius.

3.5 Result of Substrate Concentration

Various substrate concentrations are utilized to produce lactic acid. 1% CF produced the most lactic acid (14.6 g/100 g CF), as shown in Fig. 1(e). The yield of lactic acid decreased above 1% CF, which may be related to the culture medium's increased viscosity. As a result, water activity diminished since the process might have changed from a semi-solid to a solid state. Bacteria typically proliferate at greater water activity levels.

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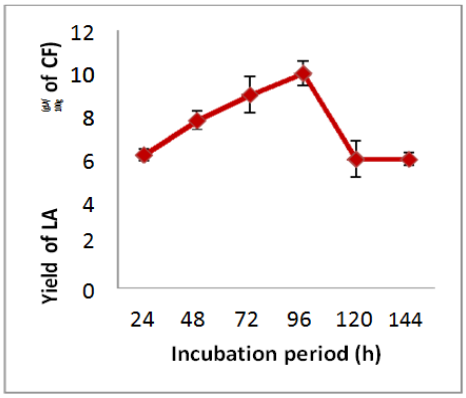


Figure 1a

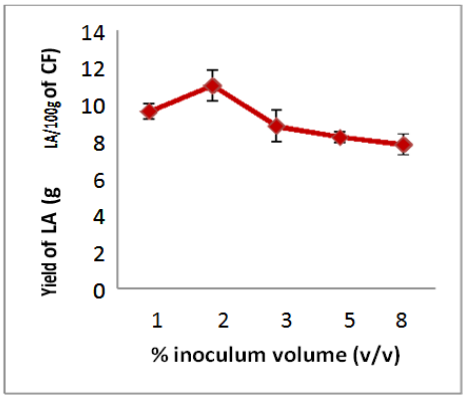
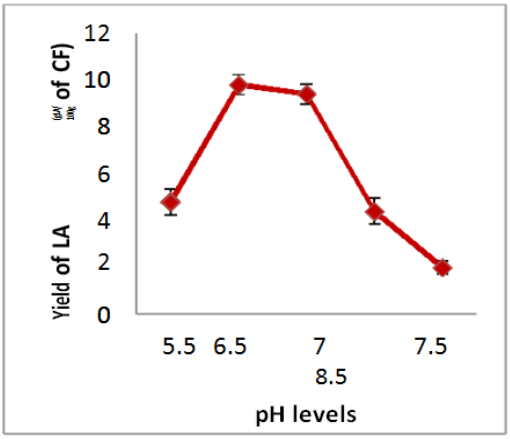


Figure 1b



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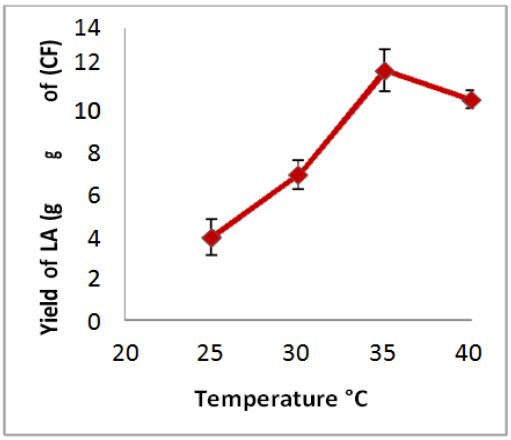

Figure 1d

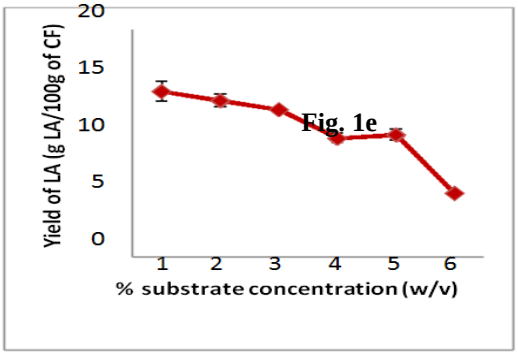

Figure 1e

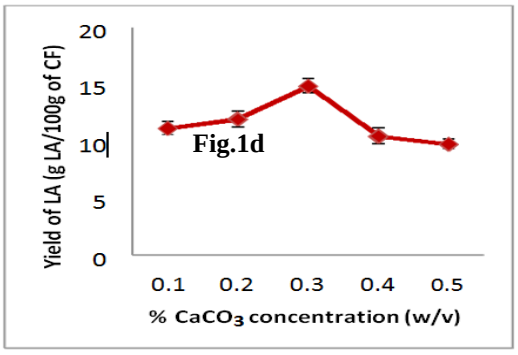

Figure 1f

Figure 1: Effects of (a) the incubation period, (b) the inoculum volume, (c) pH, (d) temperature, (e) cassava flour concentration, and (f) $CaCo_3$ concentration on Lactobacillus plantarum JX183220's generation of lactic acid

3.6 CaCO3 Concentration's Impact

At 0.3% of $CaCO_3$, LA generation was at its maximum (14.9 g/100 g CF) (Fig. 1f). Above 0.3% $CaCO_3$, a drop in LA production may occur as a result of inhibited enzyme activity, which, in turn, promotes the growth of the microorganisms necessary for LA biosynthesis [30]. A minor amount of lactic acid produced by the organism during the growth of biomass was somewhat neutralized by $CaCO_3$.

3.7 Box-Behnken Design, the Process Variables Optimized

With 2% inoculum volume and 0.3% $CaCO_3$, the effects of three potential independent variables (substrate concentration, temperature, and pH) on lactic acid generation by Lactobacillus plantarum JX183220 were studied in a total of 15 trials. The results are shown in Table 2.

Table 2: Shows the observed and predicted responses of Lactobacillus plantarum JX183220 to three variables in both coded and natural units for LA generation.

S. no.	x_1	x_2	x_3	X_1	X_2	X_3	Y = Lactic acid yield, (g/L)	
				Substrate (%w/v)	Temperature (°C)	pH	Experimental	Predicted
1	-1	-1	0	0.5	30	6.0	10.60	10.579
2	-1	1	0	0.5	40	6.0	14.60	15.307
3	1	-1	0	3.5	30	6.0	5.114	4.407
4	1	1	0	3.5	40	6.0	7.80	7.821
5	-1	0	-1	0.5	35	5.0	9.20	9.014
6	-1	0	1	0.5	35	7.0	17.00	16.50
7	1	0	-1	3.5	35	5.0	3.657	4.157
8	1	0	1	3.5	35	7.0	7.514	7.70
9	0	-1	-1	2.0	30	5.0	4.00	4.20
10	0	-1	1	2.0	30	7.0	11.00	11.521
11	0	1	-1	2.0	40	5.0	10.60	10.079
12	0	1	1	2.0	40	7.0	14.00	13.793
13	0	0	0	2.0	35	6.0	17.00	16.670
14	0	0	0	2.0	35	6.0	16.00	16.670
15	0	0	0	2.0	35	6.0	16.80	16.670

The following equation was created in terms of coded variables using the MATLAB 7 built-in function "regstats," and the estimated lactic acid yield values produced by this equation were listed in the last column of Table 2.

$$y = 16.6 - 3.4144x_1 + 2.0357x_2 + 2.7571x_3 - 0.3285x_1x_2 - 0.98575x_1x_3 - 0.9x_2x_3 - 3.8144x1^2 - 3.2571x^2 \quad 2 - 3.4429x_3^2$$

Optimizing the aforementioned equations yielded the following values:

Coded Scale	Natural Scale
$x_{1\ opt}$=-0.51612	$X_{1\ opt}$ = 1.22581 % (substrate conc.)
$x_{2\ opt}$= 0.27802	$X_{2\ opt}$ = 36.3901(Temperature)
$x_{3\ opt}$= 0.43796	$X_{3\ opt}$ = 6.43796 (pH)
	y_{opt} = 18.3679g LA/100 g Cassava

According to the second-order polynomial prediction's R^2 (coefficient of determinant), which is 0.99127, the fitted model could account for about 99.12% of the variation in the lactic acid yield. Since we see the above equation as a hierarchy, the regression equation (2) includes all estimated coefficients, no matter how likely they are to be.

Using MATLAB software and Equation (2), the three-dimensional response surface plots connecting the lactic acid yield with the three process variables were created and are shown in Figure 2.

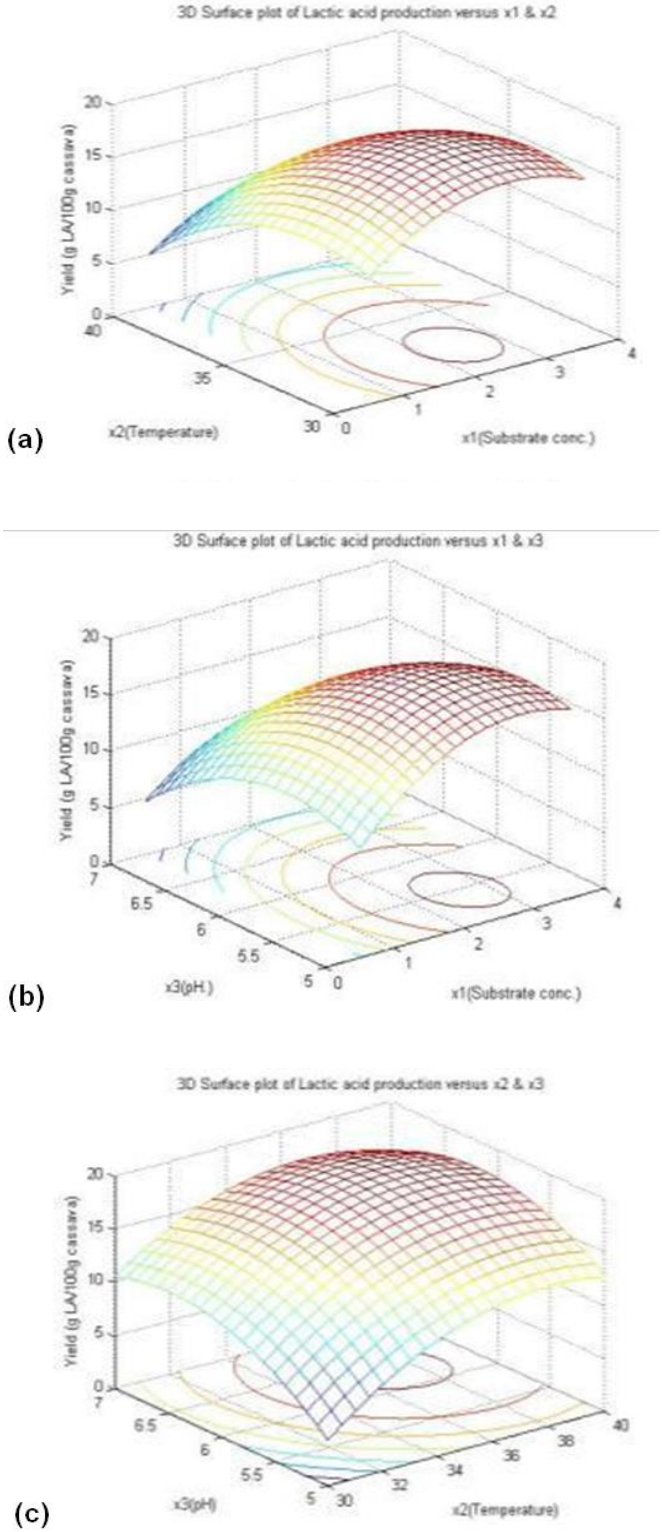

Figure 2: Using the Box-Behnken design, statistical optimization for the synthesis of lactic acid.

(a) Substrate temperature and concentration (b) pH and substrate concentration (c) pH and temperature When substrate concentration and temperature were increased to 1% CF and 36°C, respectively, maximum lactic acid production

(18.36 g/100 g CF) was recorded, and after that, it decreased (Fig. 2a). When temperature is kept constant at its ideal level, the relationship between substrate concentration and pH shows that LA synthesis is best at pH 6.4 with 1% CF (Fig. 2b). A small divergence with the former response was suggested by a temperature and pH interaction (Fig. 2c). In order to get the highest yield of LA (18.36 g/100 g CF), the optimal substrate concentration (1.22%), temperature (36.39 °C), and pH (6.43 °C) levels were determined.

3.7.1 Model Verification

Four runs with the composition (x_1 = 1.22581%, x_2 = 36.3901, and x_3 = 6.43796) were carried out to confirm the aforementioned ideal conditions, and an average result of 18.0343 g LA/100 g cassava was produced. This result proves that the Box-Behnken model is correct because it is very close to the optimal value of 18.3679 g LA per 100 g cassava that was predicted.

IV. CONCLUSION

An economically advantageous solid substrate and carbon source for the formation of lactic acid by an isolated Lactobacillus plantarum could be found in cassava flour, a cheap and conveniently accessible substance. With 15 trials, the Box-Behnken design of RSM showed to be a useful tool for optimizing the synthesis of lactic acid, forecasting a maximum yield of 18.3679 g LA/100 g cassava with the best process variables identified at pH: 6.44, substrate concentration: 1.23%, and temperature: 36.39 °C. Since Lactobacillus plantarum JX183220 can partially break down starches, future research may find that saccharification and fermentation can help it make more lactic acid.

REFERENCES

1. Adnan AFM, & Tan IKP. (2007). Isolation of lactic acid bacteria from Malaysian foods and assessment of the isolates for industrial potential. *Bioresour Technol., 98*. 1380-1385.
2. Sreenath HK, Moldes AB, Koegel RG, & Straub RJ. (2001). Lactic acid production by Simultaneoussaccharificationand fermentation of alfalfa fiber. *J Biosci Bioeng., 92*(6), 518-523.
3. Shen XL, & Xia LM. (2006). Lactic acid production from cellulosic material by synergetic hydrolysis and fermentation. *Appl Biochem Biotechnol., 133*, 251-262.
4. John RP, Nampoothiri KM, & Pandey A. (2006). Saccharification and fermentation of cassava bagasse for L- (+)-lactic acid production using Lactobacilli. *Appl Biochem Biotechnol. 34*(3), 263-272.
5. Naveena BJ, Altaf Md, Bhadrayya K, & Gopal Reddy. (2004). Production of L (+) lactic acid by *lactobacillus amylophilus*GV6 in semi-solid state fermentation using wheat bran. *Food Technol Biotechnol., 42*(3), 147–152.
6. Marques S, Santvos JAL, Gario FM, & Roseiro JC. (2008). Lactic acid production from recycled paper sludge by simultaneous saccharification and fermentation. *Biochem Eng J., 41*, 210-216.
7. Giraud E, Gosselin B, Harin B, Parida L, & Raimbault M. (1993). Purification and characterization of an extracellular amylase activity from *Lactobacillus plantarum* strain A6. *J Appl Bacteriol., 75*, 276-282.
8. Naveena BJ, AltafMd, Bhadrayya K, & Gopal Reddy. (2005). Screening and interaction effects of physical parameters, total N content and buffer on l (+) Lactic Acid production in SSF by *lactobacillus amylophillus GV6* using Taguchi Designs. *Indian J Biotechnol., 4*, 342-346.
9. Hofvendahl K, & Hahn-Hagerdal B. (2000). Factors affecting the fermentative lactic acid production from renewable resources. *Enzyme Microb Technol., 26*, 87-107.
10. Anuradha R, Suresh AK, & Venkatesh KV. (1999). Simultaneous saccharification and fermentation of starch to lactic acid. *Proc Biochem., 35*, 367-375.
11. Altaf Md, Venkateshwar M, Srijana M, & Reddy G. (206). An economic approach for L (+) lactic acid fermentation by *Lactobacillus amylophilus* GV6 using inexpensive carbon and nitrogen sources. *J Appl Microb.*, 372-380.
12. Ramesh RC, Sabita, M, Panda S, & Kar S. (2008). Solid substrate fermentation of cassava fibrous residue for production of α-amylase, lactic acid and ethanol. *J Environ Biol., 29*(1), 111-115.