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Application of Box-Behnken Design for the Optimized Production of Lactic Acid by Newly Isolated *Lactobacillus plantarum* JX183220 Using Cassava (*Manihot esculenta* Crantz) Flour

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Authors' contributions

This work was carried out in collaboration between all authors. Author VS designed the study, supervised and analyzed all the experiments and corrected the first draft of the manuscript. Authors MP, AS and NHV managed literature searches and performed the experiments. Author MP wrote the first draft of the manuscript. Author GHR designed and guided RSM experiments, and corrected the first draft. All authors read and approved the manuscript.

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ABSTRACT

Aim: The present study aimed at optimization of Lactic acid production using new isolate, *Lactobacillus plantarum* JX183220 with cassava flour (*Manihot esculenta* Crantz) in semi-solid fermentation by Response Surface Methodology.
Study Design: Box-Behnken design of Response Surface Methodology was used.
Place: Department of Chemical Engineering and Biotechnology, ANITS, Visakhapatnam.
Materials and Methods: *Lactobacillus plantarum* JX183220 isolated from goat milk was used for the production of Lactic acid using cassava flour (CF) in semi-solid fermentation. Different fermentation parameters such as incubation time, inoculum volume, pH, temperature, substrate concentration (cassava flour), and Calcium carbonate concentration were initially optimized in

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preliminary studies. The substrate concentration, temperature and pH were chosen as potential variables and further optimized using Box-Behnken design of Response Surface Methodology. A second order polynomial regression model was fitted and was found adequate with a high coefficient of determination, R^2 (0.9913). Validation experiment was carried out at optimum conditions of the parameters as determined from the model.

Results: The preliminary experiments revealed that maximum production of lactic acid by *Lactobacillus plantarum* JX183220 was observed on 4th day of incubation with 2% inoculum and 0.3% Calcium carbonate. Optimization using Box -Behnken design of RSM resulted in maximum Lactic acid production of 18.3679 g/100 g of cassava at optimum conditions of substrate concentration, 1.225%; Temperature, 36.39°C and pH 6.43. These results were confirmed by validation experiment.

Conclusion: Optimum parameters for the direct conversion of cassava flour starch to Lactic acid by new isolate, *Lactobacillus plantarum* JX183220 were determined. Box Behnken design of RSM was found to be convenient tool with 15 runs for optimizing lactic acid production. The lactic acid production could be further enhanced by saccharification and fermentation in future studies.

Keywords: Lactobacillus plantarum JX183220; lactic acid; cassava flour; semi-solid fermentation; box-behnken design; response surface methodology.

1. INTRODUCTION

Lactic acid (2-hydroxypropionic acid or 2-hydroxypropanoic acid) is a natural organic acid with a long history of use in food, cosmetic and pharmaceutical industries. The world market for Lactic acid is expected to reach 367.3 thousand metric tons by the year 2017. The most recent application of lactic acid is the production of poly-lactic acid (PLA) which is a biodegradable, biocompatible and environmentally friendly biopolymer. PLA is used in surgical sutures, orthopedic implants, drug delivery systems, disposable consumer products and it significantly alleviates waste disposable problems [1].

For the production of L (+) lactic acid, the most preferable method is biological fermentation since chemical synthesis produces racemic mixture of D- and L- lactic acid. Lactic acid can be produced by both fungal and bacterial species. In fungal fermentation, there is low production rate due to the low reaction rate caused by mass transfer limitation resulting in the formation of by-products such as fumaric acid and ethanol. Lactobacillus species like *Lactobacillus plantarum* [2], *Lactobacillus delbrueckii* [3], *Lactobacillus casei* [4], *Lactobacillus amylophilus* [5] and *Lactobacillus rhamnosus* [6] are the demanding microorganisms for wide range production of lactic acid.

Lactic acid can be produced by direct fermentation [7,8] and also by simultaneous saccharification and fermentation [9,10]. Simultaneous saccharification and fermentation involves the use of starch hydrolyzing enzymes which make the process more expensive. Direct fermentation involves the use of amylase producing organisms which could directly convert the starch into lactic acid, leading to significant reduction in the operating cost. *Lactobacillus amylophillus* [5,8,11] and *Lactobacillus amylovorus* shown amylolytic activity. Few of the studies reported the amylolytic activity in *Lactobacillus plantarum* [12].

Lactic acid can be produced biologically by submerged fermentation or solid state fermentation. Solid state fermentation is more advantageous than the submerged fermentation as it results in higher yields with more concentrated organic acids and enzymes with an added advantage of potential downstream recovery [13]. But solid state fermentation occurs in the absence or near absence of free water. Low moisture content may lead to poor accessibility of nutrients resulting in poor microbial growth. Semi-solid fermentation provides high water activity to the solid substrate and also provides carbohydrates, mineral nutrients, and nitrogen sources which make whole process more economical.

For lactic acid fermentation various raw materials are used which are rich in lignocelluloses, cellulose and starch. Different substrates used are cassava fibrous residue [12], corn cobs [14], sugarcane bagasse [15], molasses [16], whey [17], paper sludge [6], wheat bran [5] and alfalfa fibres [2]. These agricultural and industrial wastes are rich in carbon content and will help to solve many environmental hazards. Cassava

(*Manihot esculenta* Cranz), ranked as the world's sixth important food crop, is a starchy crop having 20-30% extractable starch depending on the varieties and climatic conditions. It is a potential substrate for the production of lactic acid, being a cheap agriculture crop with a rich source of carbohydrates.

The traditional 'one-factor at a time' technique used for optimizing a multivariable system is not only time consuming but also often easily misses the interactions between the components. In order to overcome this problem, optimization based on the statistical design experiments will be carried out. Response surface methodology (RSM) is a collection of mathematical and statistical techniques based on the fit of a polynomial equation to the experimental data, which must describe the behavior of a data set with the objective of making statistical inferences. The first step in process optimization is screening of the important variables, with an aim of limiting them to three, four, or five at maximum. Following the initial screening, the next step is optimizing the levels of these variables by employing Box-Behnken design of RSM. Box-Behnken is three-level fractional factorial arrangement, which allows the efficient estimation of the first- and second-order coefficients of the quadrating mathematical model. Several researchers have applied this technique for optimization of different parameters [16,18].

The present study deals with direct optimized conversion of starch to lactic acid by *Lactobacillus plantarum* JX183220 in semi-solid fermentation using cassava flour by applying Box-Behnken design of Response Surface Methodology.

2. MATERIALS AND METHODS

2.1 Substrate and Microorganism

Cassava tubers were sun-dried for 4-5 days to prevent microbial deterioration and were finely powdered of desired size (1.2 mm). The cassava flour was stored in an air-tight container until required and was used as the substrate. *Lactobacillus plantarum* JX183220 [19] isolated from goat milk in the Department of Chemical Engineering and Biotechnology, ANITS, Visakhapatnam, Andhra Pradesh, India, was used for the present study.

2.2 Inoculum Preparation

The inoculum was prepared in 100 mL of modified MRS liquid medium (g/L: peptone 10.0, beef extract 10.0, yeast extract 5.0, glucose 20.0, Na_2HPO_4 2.0, 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). A loop full of *Lactobacillus plantarum* JX183220 was transferred from stock culture to sterilized medium and incubated at 37°C and 120 rpm for 48 h in an orbital shaker.

2.3 Media Preparation

For lactic acid production, modified MRS semi-solid medium containing Cassava flour (5%) in lieu of glucose as a carbon source was used. The 100 mL MRS semi-solid medium with Cassava flour was autoclaved. Freshly prepared 2% inoculum was inoculated and incubated at 37°C for 6 days.

2.4 Preliminary Optimization Studies

The strategy adopted was to optimize one particular variable at a time and then include its optimum level in the subsequent optimization step. The parameters optimized were: (1) incubation time, (2) inoculum volume, (3) pH, (4) temperature, (5) substrate concentration and (6) $CaCO_3$ concentration. Substrate concentration, temperature and pH were chosen for further optimization by Box-Behnken method of Response surface Methodology, while keeping the others at their chosen optimum levels.

2.5 Extraction of Lactic Acid

The fermented substrate was centrifuged in a refrigerated centrifuge at 8000 g for 20 min. The clear free supernatants are used for the estimation of lactic acid.

2.6 Analytical Techniques

Lactic acid was estimated by calorimetric method of Kimberley and Taylor [20] and the yield is expressed as g LA/100 g cassava.

2.7 Experimental Design

Box-Behnken design of RSM has three levels (low, medium, and high coded as -1, 0, +1) for variables , needs fewer experiments (15 runs), more efficient and easier to interpret [21].

Table 1. Range of process variables for Box-Behnken design

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

Therefore, this statistical technique is used in the present study. A total of 15 runs were used to optimize substrate concentration (Cassava flour), temperature, and pH for the production of lactic acid.

For statistical calculations, the three independent variables are designated as X_1, X_2 and X_3, respectively, and were coded according to the following equation.

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

where, x is coded variable, X is natural variable, X_0 is the middle point (zero level) and ΔX is the step change that represents the difference between the successive levels. The range and levels of the variables investigated in this study were shown in Table 1 above.

3. RESULTS AND DISCUSSION

3.1 Effect of Incubation Time

The amount of lactic acid produced was observed for every 24 h during a period of six days. The maximum yield of 10.0 g/100 g CF was observed on fourth day as shown in Fig. 1a. After fourth day, it was reduced due to the reutilization of products by organism and considerable depletion of nutrients in the fermentation medium. A maximum of yield of lactic acid has been reported on fifth day of incubation [22,5].

3.2 Effect of Inoculum Volume

Lactic acid production was highest at 2% inoculum volume (11 g /100 g CF) and gradually decreases with increase in inoculums volume (Fig.1b). In case of semi-solid fermentation, inoculums level varies according to the medium composition. In general, a low level sugar medium needs 2-3% inoculum volume and higher sugar levels needs 5-10% inoculums volume [23]. The use of 2% (v/v) inoculum for the lactic acid production has been reported in earlier studies also [24,25]. However, the higher inoculum (3%, v/v) has also been used for lactic acid production [26].

3.3 Effect of pH

The effect of pH on lactic acid production was evaluated by using fermentation medium with a pH in the range of 5.5-8.5. The maximum lactic acid production was observed at pH 6.5 (9.8 g/100 g CF) as shown in Fig.1c. At higher and lower pH levels, a decrease in the yield was observed. The hydrogen ion concentration of medium highly influences on the microbial growth. pH limits the synthesis of metabolic enzymes that are responsible for the synthesis of new protoplasm [27]. A pH range of 6.0-6.5 has been reported optimal for lactic acid production using *L. casei* strain [28]. However, pH 5.5 has been used for lactic acid production using *L. helveticus* by Ghaly et al. [29].

3.4 Effect of Temperature

The effect of different incubation temperatures (25°C, 30°C,35°C and 40°C) on production of lactic acid were studied. Lactic acid production increased with increase in temperature from 25°C to 35°C and decreased at 45°C, (Fig.1d). The decrease in lactic acid yield at 25°C and 30°C could be due to low metabolic rate.

3.5 Effect of Substrate Concentration

For the production of lactic acid, various substrate concentrations are used. Fig. 1(e) depicts that 1% CF yielded the maximum amount of lactic acid (14.6 g/100 g CF). Beyond 1% CF level, there was a decrease in the yield of lactic acid which might be due to the increasing viscosity of the culture medium. This led to decreased water activity as the process might have shifted from semi-solid to solid state. Generally bacteria grow at higher water activity [5,7].

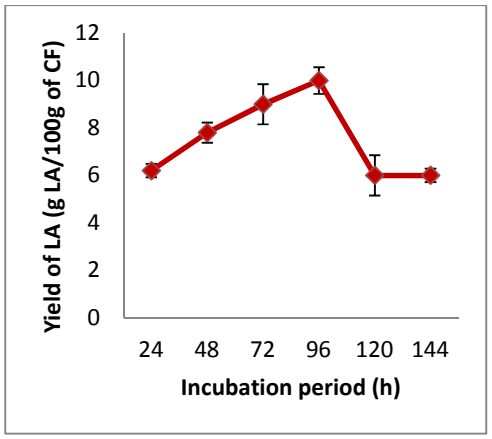

Fig. 1a

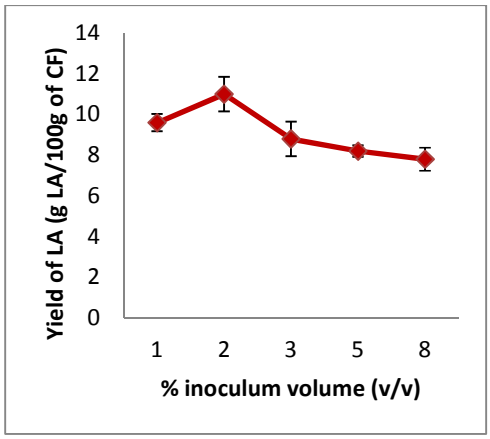

Fig. 1b

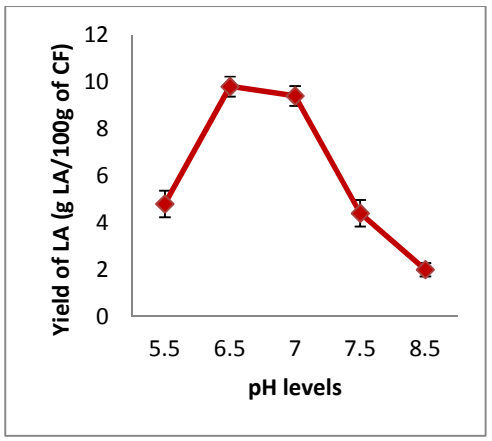

Fig. 1c

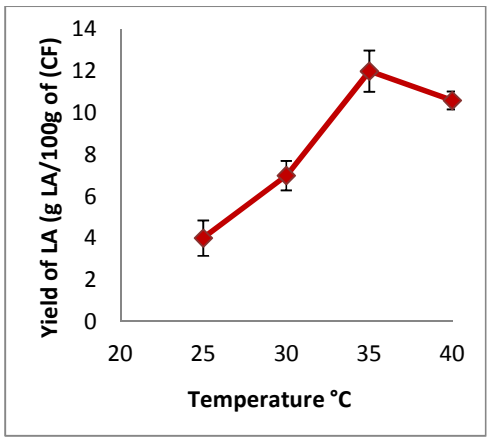

Fig. 1d

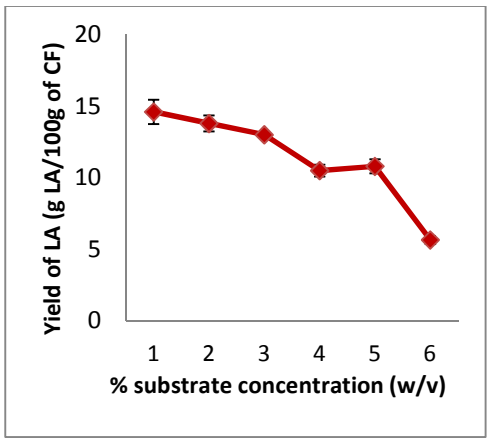

Fig. 1e

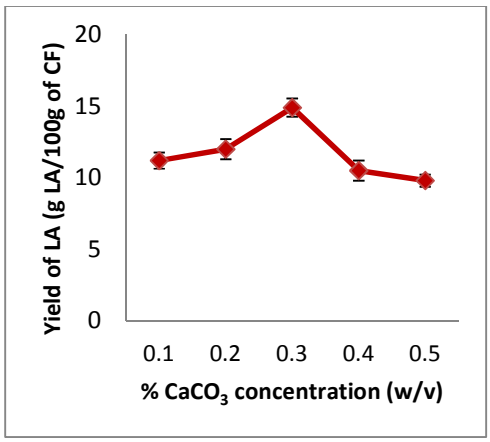

Fig. 1f

Fig. 1. Effect of (a) Incubation time, (b) inoculum volume, (c) pH, (d) Temperature, (c) Cassava flour concentration, (f) $CaCo_3$ concentration on Lactic acid production by *Lactobacillus plantarum* JX183220

3.6 Effect of $CaCO_3$ Concentration

LA production was highest (14.9 g/100 g CF) at 0.3% of $CaCO_3$ (Fig.1f). A decrease in the production of LA above 0.3% $CaCO_3$ might be due to inhibition of enzyme activity and ultimately growth of microorganism responsible for biosynthesis of LA [30]. $CaCO_3$ helped to neutralize a small amount of lactic acid produced by the organism during the development of biomass.

3.7 Optimization of the Process Variables Using Box-Behnken Design

Effect of three potential independent variables (substrate concentration, temperature and pH) for lactic acid production by *Lactobacillus plantarum* JX183220 with 2% inoculums volume and 0.3% $CaCO_3$ were conducted with different combinations in a total of 15 runs and the corresponding results were presented in Table 2.

Table 2. Box-Behnken design matrix of three variables in coded and natural units along with the observed and predicted response of LA production by *Lactobacillus plantarum* JX183220

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

Using MATLAB-7 built-in function 'regstats', the following equation was obtained in terms of coded variables, and the estimated lactic acid yield values by this equation were listed in the last column of Table 2.

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

The optimization of the above equations resulted in 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

The R^2 (coefficient of determinant) of the second-order polynomial prediction is 0.99127, indicating that the variability of approximately 99.12% for lactic acid yield could be explained by the fitted model. As we consider the above equation to be hierarchical, all the estimated coefficients are included in the regression equation (2) irrespective of their probability values.

The three dimensional response surface plots relating the lactic acid yield with the three process variables were generated by MATLAB software using Equation (2) and are presented in Fig. 2.

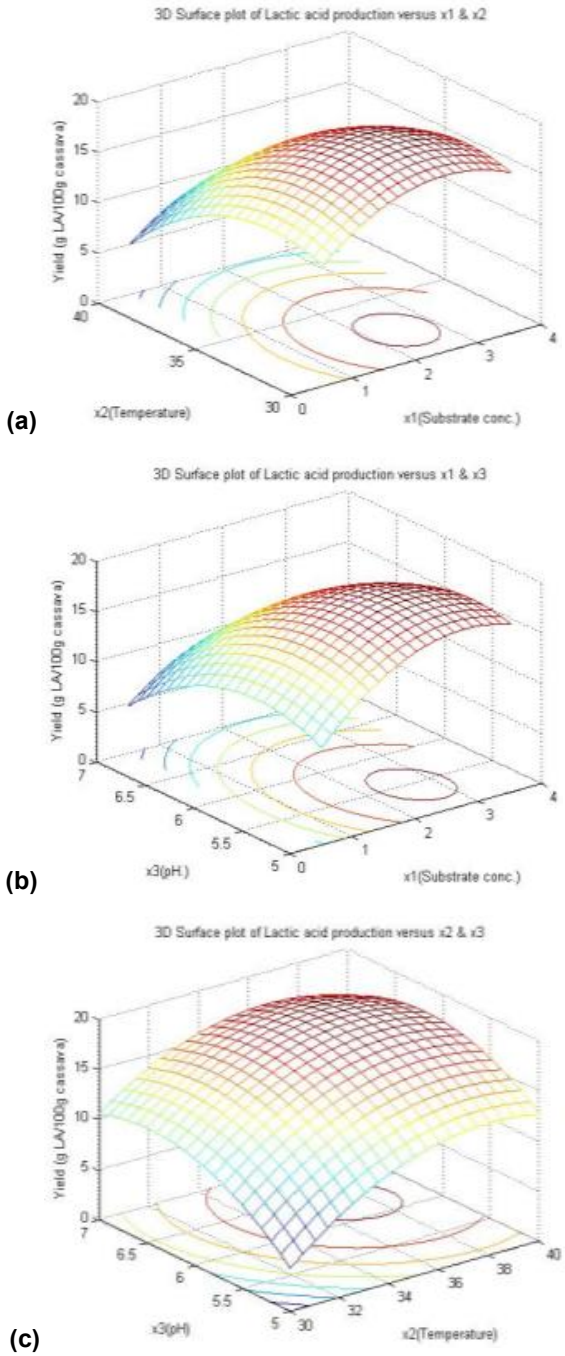

Fig. 2. Statistical optimization for the lactic acid production using Box-Behnken design: (a) Substrate concentration and temperature (b) Substrate concentration and pH (c) Temperature and pH

Maximum lactic acid production (18.36 g/100 g CF) was observed when substrate concentration and temperature was increased upto 1% CF and 36°C, respectively and thereafter it declined (Fig. 2a). The response between substrate concentration and pH when temperature is fixed at its optimum value indicated that pH 6.4 was optimum with 1% CF for LA production (Fig. 2b). An interaction between temperature and pH suggested a little difference with the earlier response (Fig. 2c). Thus, optimum level of substrate concentration (1.22%), temperature

(36.39°C) and pH 6.43 were identified to achieve maximum yield of LA (18.36 g/100 g CF).

3.7.1 Validation of the model

To confirm the above optimum conditions, four runs with the composition (x_1 = 1.22581%, x_2= 36.3901, x_3 = 6.43796) were carried out and an average value of 18.0343 g LA/100 g cassava was obtained. This value is very close to the predicted optimum value 18.3679 g LA/100 g cassava and hence the confirmation experiment validated the optimum conditions predicted by Box-Behnken design.

4. CONCLUSION

Cassava flour, a low cost and easily available material, could provide an economic advantage as solid substrate as well as carbon source for the production of lactic acid by an isolated *Lactobacillus plantarum.* Box-Behnken design of RSM was proved to be a convenient tool (with 15 runs) for optimizing the Lactic acid production, predicting a maximum yield of 18.3679 g LA/100 g cassava with the optimum process variables being found at pH: 6.44, substrate concentration: 1.23%, temperature: 36.39°C. The new isolate, *Lactobacillus plantarum* JX183220 is partially amylolytic and lactic acid production could be increased by Saccharification and fermentation in future studies.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Adnan AFM, Tan IKP. Isolation of lactic acid bacteria from Malaysian foods and assessment of the isolates for industrial potential. Bioresour Technol. 2007;98: 1380-1385.
2. Sreenath HK, Moldes AB, Koegel RG, Straub RJ. Lactic acid production by simultaneous saccharification and fermentation of alfalfa fiber. J Biosci Bioeng. 2001;92(6):518-523.
3. Shen XL, Xia LM. Lactic acid production from cellulosic material by synergetic hydrolysis and fermentation. Appl Biochem Biotechnol. 2006;133:251-262.
4. John RP, Nampoothiri KM, Pandey A. Saccharification and fermentation of cassava bagasse for L- (+)-lactic acid production using *Lactobacilli.* Appl Biochem Biotechnol. 2006a;134(3): 263-272.
5. Naveena BJ, Altaf Md, Bhadrayya K, Gopal Reddy. Production of L (+) Lactic Acid by *Lactobacillus amylophilus*GV6 in Semi-Solid State Fermentation Using Wheat Bran. Food Technol Biotechnol. 2004;42(3)*:*147–152.
6. Marques S, Santvos JAL, Gario FM, Roseiro JC. Lactic acid production from recycled paper sludge by simultaneous saccharification and fermentation. Biochem Eng J. 2008;41:210-216.
7. Giraud E, Gosselin B, Harin B, Parida L, Raimbault M. Purification and characterization of an extracellular amylase activity from *Lactobacillus plantarum* strain A6. J Appl Bacteriol. 1993;75:276-282.
8. Naveena BJ, AltafMd, Bhadrayya K, Gopal Reddy. 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. 2005;4:342-346.
9. Hofvendahl K, Hahn-Hagerdal B. Factors affecting the fermentative lactic acid production from renewable resources. Enzyme Microb Technol. 2000;26:87-107.
10. Anuradha R, Suresh AK, Venkatesh KV. Simultaneous saccharification and fermentation of starch to lactic acid. Proc Biochem.1999;35:367-375.
11. Altaf Md, Venkateshwar M, Srijana M, Reddy G. An economic approach for L (+) lactic acid fermentation by *Lactobacillus amylophilus* GV6 using inexpensive carbon and nitrogen sources. J Appl Microb. 2006;372-380.
12. Ramesh RC, Sabita, M, Panda S, Kar S. Solid substrate fermentation of cassava fibrous residue for production of α-amylase, lactic acid and ethanol. J Environ Biol. 2008;29(1):111-115.
13. Dominguez A, Costas M, Longo MA, Sanroman A. A novel application of solid

state culture: Production of lipases by *Yarrowia lipolytica*. Biotechnol Lett. 2003; 25:1225-1229.
14. Guo W, Jia W, Li Y, Chen S. Performances of *Lactobacillus brevis* for producting Lactic Acid from hydrolysate of Lignocellulosics. Appl Biochem Biotechnol. 2010;161:124-136.
15. Laopaiboon P, Thani A, Leelavatcharamas V, Laopaiboon L. Acid hydrolysis of sugarcane bagasse for lactic acid production. Bioresour Technol. 2010;101: 1036-1043.
16. Coelho LF, de Lima CJB, Rodovalho CM, Bernardo MP, Contiero J. Lactic acid production by new *Lactobacillus plantarum* LMISM6 grown in molasses: optimization of medium composition. Braz J Chem Eng. 2011;28(1):27-36.
17. Ghasemi M, Najafpour G, Rahimnejad M, Beigi PA, Sedighi M, Hashemiyeh M. Effect of Different media on production of lactic acid from whey *by Lactobacillus bulgaricus.* Afr J Biotechnol. 2009;8(1): 081-084.
18. Amenaghawon NA, Nwaru KI, Aisien FA, Ogbeide SE and Okieimen CO. Application of Box-Behnken design for optimization of Citric acid production from corn starch using *Aspergillus niger.* Br. Biotechnol. J. 2013;3(3):236-245.
19. Sridevi V. Isolation of lactic acid bacteria with probiotic potential from goat milk and cow milk. Genbank submission no. JX183220. *Lactobacillus plantarum*, partial sequence of 16s rRNA gene; 2012. Available:http://www.ncbi.nlm.nih.gov/nuccore/JX183220
20. Kimberley AC, Taylor C. A simple colorimetric assay for muramic acid and lactic acid. Appl. Biochem. Biotechnol. 1996;56(1):49-58.
21. Bosque-Sendra JM, Pescarolo S, Cuadros-Rod´riguez L, García-Campana AM, Almansa-López EM. Optimizing analytical methods using sequential response surface methodology. Application to the pararosaniline determination of formaldehyde. Fresenius J Anal Chem. 2001;36:715–718.
22. John RP, Nampoorthi KM, Pandey A. Solid-state fermentation for L-lactic acid production from agro wastes using *Lactobacillus delbrueckii*. Process Biochem. 2006b;41:759-763.
23. Vishnu C, Seenayya G, Reddy G. Direct fermentation of various pure and crude starchy substrates to L (+) lactic acid using *Lactobacillus amylophillus* GV6. World J Microbiol Biotechnol. 2002;18:429-433.
24. Roy D, Goulet J, LeDuy A. Batch fermentation of whey ultrafiltrate by *Lactobacillus helveticus* for lactic acid production. Appl Microbiol Biotechnol. 1986;24:206-213.
25. Gandhi DN, Patel RS, Wadhwa BK, Bansal N, Kaur M, Kumar G. Effect of agro-based by-products on production of lactic acid in whey permeate medium. J Food Sci Techno. 2000;37:292-295.
26. Chiarini L, Mara L, Tabacchioni S. Influence of growth supplements on lactic acid production in whey ultrafiltrate by *Lactobacillus helveticus*. Appl Microbiol Biotechnol. 1992;36:461-464.
27. Panesar PS, John FK, Charles JK, Maria K. Production of l (+) Lactic acid using *Lactobacillus casei* from whey. Braz Arch BiolTechn. 2010;53(1):219-226.
28. Krischke W, Schroder M, Trosch W. Continuous production of L-lactic acid from whey permeate by immobilized *Lactobacillus casei* subsp. *case*i. Appl Microbiol Biotechnol. 1991;34:573-578.
29. Ghaly AE, Tango MSA, Mahmood NS, Avery AC. Batch propagation of *Lactobacillus helveticus* for production of lactic acid from lactose concentrated cheese whey with microaeration and nutrient supplementation. World J Microbiol Biotechnol. 2004;20:65-75.
30. Fu W, Mathews AP. Lactic acid production from lactose by *Lactobacillus plantarum*: Kinetic model and effects of pH, substrate and oxygen. J Biochem Eng. 1999;3: 163-170.

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