Production, optimization and characterization of lactic acid by *Lactobacillus delbrueckii* NCIM 2025 from utilizing agro-industrial byproduct (cane molasses)

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Abstract In the present work *Lactobacillus delbrueckii* was used to utilize agro-industrial byproduct (cane molasses) for lactic acid production under submerged fermentation process. Screening of LAB was done by Fourier transform infra red spectroscopy (FTIR). Effect of different amino acids (DL-Phenylalanine, L-Lysine and DL-Aspartic acid) on the fermentation process was done by high performance liquid chromatography (HPLC). Central composite rotatable design (CCRD) was used to optimize the levels of three parameters viz. tween 80, amino acid and cane molasses concentration during fermentative production of lactic acid. Under optimum condition lactic acid production was enhanced from 55.89 g/L to 84.50 g/L. Further, validation showed 81.50 g/L lactic acid production. Scale up was done on 7.5 L fermentor. Productivity was found to be 3.40 g/L/h which was higher than previous studies with reduced fermentation time from 24 h to 12 h. Further characterization of lactic acid was done by FTIR.

Keywords *Lactobacillus delbrueckii* · cane molasses · lactic acid · FTIR · response surface methodology · CCRD

Introduction

Lactic acid (2-hydroxypropanoic acid, $\text{CH}_3\text{—CH}(\text{OH})\text{—COOH}$) is a natural organic acid with a long history of use in cosmetic and pharmaceutical industries, and also for the production of oxygenated chemicals, plant growth regulators,

and special chemical intermediates (Dumbrepatil et al. 2008; Oshiro et al. 2009; Singhvi et al. 2010; Tashiro et al. 2011). Lactic acid is considered a specialty chemical with various applications both in the food and non-food industries. It has been utilized as a raw material in the production of biodegradable polylactide (PLA) (Tokiwa and Calabia 2006), which has many uses in surgical sutures, orthopedic implants, drug delivery systems, and disposable consumer products (Adnan and Tan 2007). Lactic acid can be produced by either chemical synthesis or microbial fermentation. Chemical synthesis of lactic acid is usually carried out using petrochemical resources, resulting in a racemic mixture of the product, which is a major disadvantage of this approach (Hofvendahl and Hahn-Hagerdal 2000), whereas, the biological fermentation method obtains an optically pure lactic acid by choosing an appropriate strain of lactic acid bacteria (Ryu et al. 2003). Lactic acid is used as a natural preservative in many food products (Altaf et al. 2007). A number of industrial by-products or wastes have been evaluated as substrates for lactic acid production with the aim of decreasing the cost of the process, such as sugarcane (Calabia and Tokiwa 2006) and molasses (Dumbrepatil et al. 2008).

Molasses is a waste product from the sugar manufacturing process, and it usually contains a large amount of sucrose. Molasses and refined sugars, are some of the commonly used carbon sources (Wee et al. 2004) while malt extract, yeast extract and corn steep liquor are the nitrogen sources used for lactic acid production.

Normally molasses consists of about 46 % total sugars, 3.0 % crude protein, 0.0 % fat with 79.5° brix and are used in animal feed as well as for the production of alcohol through fermentation. As it contains high amount of carbohydrates/sugars, it can be used as a cheaper source to produce value added products. Food industrial wastes, high in moisture and rich in carbon source have been considered as an attractive nutrient source for industrial lactic acid production, which is a

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cheaper substrate as a source of sugars to be utilized in a fermentation process subsequently for lactic acid production (Rashid and Altaf 2008). The molasses is a syrupy material left after the removal of sugar from the mother syrup. This viscous material is composed of sucrose, glucose and fructose at a high carbohydrate concentration (Farooq et al. 2012).

In the present work, an attempt was made to enhance lactic acid production utilizing agro industrial by-product (cane molasses) by *Lactobacillus delbrueckii* under submerged fermentation process. Optimization of cultural conditions was done by CCRD and shake flask study was scaled up in the fermentor.

Materials and methods

Bacterial strain and growth medium *Lactobacillus delbrueckii* was procured from National Collection of Industrial Microorganism (NCIM) from National Chemical Laboratory (NCL) Pune, India. The strain used in this work was *Lactobacillus delbrueckii* NCIM 2025, a homofermentative lactic acid bacterium which produces mainly L (+) lactic acid. The stock cultures were maintained at 4 °C in 250 mL MRS broth (DeMan's Sharpe and Rogosa Broth) with sub-culturing after every 10 days throughout the experiment. The medium for cell growth contained (g/L): peptone 10.0, meat extract 10.0, yeast extract 5.0, D-glucose 20.0, Tween 80 1.0, K₂HPO₄ 2.0, sodium acetate 5.0, tri-ammonium citrate 2.0, MgSO₄·7H₂O 0.2, MnSO₄·4H₂O 0.05 in 1000 mL water Bhatt and Srivastava 2008.

Substrate Raw material i.e. cane molasses was procured from National Sugar Institute (NSI), Kanpur, India.

Pretreatment of cane molasses Cane molasses solution (1 L) was boiled with 35 mL of 1 N H₂SO₄ for 30 min, cooled, neutralized with 3 % lime-water (CaO) and was left to stand overnight for clarification. The clear supernatant liquid was diluted to 40 % sugar level and then treated with activated charcoal (1:1) for 2 h in order to reduce its opacity and other interfering compounds.

Inoculum preparation *Lactobacillus delbrueckii* cells from stock cultures were transferred to sterile growth medium (MRS agar plates) and incubated at 37 °C for 24 h. Then the culture was inoculated into MRS broth in screw capped test tubes, incubated for 24 h at 37 °C and was used for fermentation process.

Fermentation The lactic acid fermentation of sugar molasses was carried out by the *Lactobacillus* strain at 37 °C with 0, 6 and 8 % substrate levels. The fermentation media contained (g/L); peptone 10.0, meat extract 10.0, yeast extract 5.0,

Tween-80 1.0, K₂HPO₄ 2.0, Sodium acetate 5.0, tri-ammonium citrate 2.0, MgSO₄·7H₂O 0.2, MnSO₄·4H₂O 0.05, with different percent levels of sugar cane molasses (0, 6, 8 %) and water. (Glucose was replaced with sugar cane molasses). The pH of the final culture medium was adjusted to 7.0±0.5 using 0.1 N HCl/0.1 N NaOH before bacterial inoculation. Production media was prepared in 250 mL conical flask. One mL of the inoculum were added in conical flasks containing 250 mL of production medium and incubated at 150 rpm, 37 °C for 24 h in shaker plus incubator (Sigma, MO, USA) (Tellez-Luis et al. 2003)

Analytical methods

Sugar analysis The total sugar in cane molasses was determined by the phenol-sulphuric method (Dubois et al. 1956) and reducing sugar was determined by the DNS (Dinitrosalicylic) method (Miller et al. 1959).

HPLC analysis HPLC method was used for the quantitative determination of lactic acid (Oh et al. 2005). Samples for lactic acid production were analyzed through HPLC with UV detector at 210 nm using C-18 column. The operating conditions were, mobile phase 5 mM H₂SO₄, flow rate 0.4 mL/min, column temperature 60 °C, injection volume 10 µL. Quantification was based on internal standard method.

Dry cell mass Twenty ml of culture broth was centrifuged at 10,000 rpm for 10 min at 4 °C and cell pellet was obtained. The cell pellet was washed with saline water (NaCl, 0.8 % w/v) and then dried in aluminium weighing dish at 90 °C for 24 h in hot air oven (Perfett India Ltd., Ambala, India).

Sample preparation for FTIR measurement The sample preparation was carried out as suggested by Helm et al. (1991) with some modifications. After the cultivation on agar plates, a platinum loop was used to remove bacterial biomass from the third quadrant of each agar plate, and the biomass was dissolved in 100 µL distilled water. The concentration of the suspension was adjusted so that the intensity of the amide I band (1655 cm⁻¹) in the IR spectrum was between 0.35 and 1.25, which is within the linear range of the DTGS detector. Of each suspension, 35 µL was transferred to an IR transparent optical crystal (ZnSe) in a multisample cuvette (Bruker Optics, Germany). The samples were dried under moderate vacuum (0.1 bar) using anhydrous Silica Gel (Prolabo, France) in a desiccator to form films suitable for FTIR analysis. The FTIR measurement was performed with a Biomodule (Bruker Optics, Germany), specially designed for microorganisms, coupled to an Equinox 55 spectrometer (Bruker Optics, Germany). The spectra were recorded in the region between 4000 and 500 cm⁻¹ with a spectral resolution of 6 cm⁻¹ and an aperture of 5.0 mm (Helm et al. 1991).

Statistical analysis The data obtained from the various experiments were recorded and subjected to statistical analysis as per method of “Analysis of variance” by CCRD. The significance difference between the means was tested against the critical difference at 1 % level of significance by using statistical tool RSM, (MINITAB 15) for data analysis. Statistical optimization of media was done by selecting three factors were chosen at three levels for the optimization process viz. amino acid supplementation, effect of tween 80, cane molasses concentration and two responses i.e. lactic acid and cell biomass were observed. The experimental data obtained from the design were analyzed by the response surface regression procedure using the following second-order quadratic equation:

$$Y_i = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \beta_{ij} x_i x_j$$

Where, Y_i was the predicted response, $x_i x_j$ were independent variables, β_0 was the offset term, β_i was the linear coefficient, β_{ii} was the quadratic coefficient and β_{ij} was the interaction coefficient.

Studies on fermentor Scale-up fermentation of lactic acid production with the optimal medium was carried out in a 7.5 L fermentor (BioFlo/Celligen 115, New Brunswick, USA), containing 3.0 L of media. Agitation speed and culture temperature were controlled at 150 rpm and 37 °C. The fermentor was sterilized in the autoclave (capacity 50 L) at 121 °C for 20 min, cooled and then inoculated with 5 % inoculum (v/v). The pH of the culture broth was maintained at an optimum pH of 7 by automatic addition of acid or base by pH–mV controller (Mettler Toledo, USA). Dissolved oxygen was measured by DO probe (Mettler Toledo, USA). Dissolved oxygen concentration was maintained at 40 % saturation value by cascading the speed of the agitator and air flow rate.

Sampling Sampling was done following the procedure given by Oh et al. (2005). Samples (5–10 mL) were taken from the fermentation medium at different time (every 1–4 h) during the fermentation process. Sample were centrifuged and frozen at –20 °C for further analysis of lactic acid.

Results and discussion

Screening of lactic acid producing microbe (*Lactobacillus delbrueckii*) using FTIR Screening of lactic acid producing microbe was done by FTIR. Fig. 1 represents the FTIR spectrum of *Lactobacillus delbrueckii*. The FTIR spectra of microorganisms are usually divided into five regions. These regions contain information from different cell components: (1) 3000–2800 cm^{-1} : fatty acids in the bacterial cell

membrane (2) 1800–1500 cm^{-1} : amide bands from proteins and peptides (3) 1500–1200 cm^{-1} : mixed region: proteins and fatty acids (4) 1200–900 cm^{-1} : polysaccharides within the cell wall (5) 900–500 cm^{-1} “true” fingerprint region containing bands which cannot be assigned to specific functional groups (Naumann et al. 1991; Curk et al. 1994))

Proximate composition of cane molasses The chemical composition of sugar cane molasses was determined to assess the nutritional potential of the waste material as substrates. The sugar cane molasses contained moisture 23.8 %, ash 11.3 %, dry matter 76.2 %, nitrogen 0.62 %, total sugars 54.8 %, reducing sugars 27.1 % and non-reducing sugars 27.7 %. The results was identical to Akhtar et al. (1997) who also found that the corn steep liquor contained 42–56 % dry matter, 26–45 % protein and 2.5–15 % sugars.

Effect of concentration of cane molasses on lactic acid production Fig. 3a shows that with the increase in the concentration of cane molasses, lactic acid production increased. Lactic acid concentration was 40.0 g/L, when 20.0 g/L cane molasses was used. Kotzamanidis et al. (2002) investigated the effect of yeast extract and molasses, on lactic acid fermentation from beet molasses and found that lactic acid production is stimulated by increase in concentration of yeast extract from 1–5 % and sugar utilization was 93 % with lactic acid production of 90 g/L at 5 % yeast extract. Yeast extract concentration beyond 5 % may be toxic for the cell. According to Yun et al. (2003) cell growth decreases significantly when glucose is used beyond 100 g/L due to inhibition by high substrate concentration. Wee et al. (2004) studied utilization of sugar molasses for economical L (+) lactic acid production by *Enterococcus faecalis* from pH range 5–9 and 0.5–2 % yeast extract w/v and sugar molasses from 15–20 % sugar molasses. They reported lactic acid production of 95 g/L at pH 7.0 with yeast extract concentration 1.5 % and sugar cane molasses concentration of 17 % v/v.

Effect of tween 80 concentration on lactic acid production - Fig. 3a shows that with increase in the concentration of tween 80 (4 g/L) lactic acid production was found to be 45 g/L. According to Feng et al. (2006) tween 80, a non-ionic surfactant has been reported to enhance microbial ability to produce some enzyme. Tween 80 has also been proved beneficial cultivation and fermentation process of *Lactobacillus* (Duggan et al. 1968). In case of most *lactobacillus* strain, unsaturated fatty acid such as tween 80 was essential growth factor. Partenen et al. (2001) reported that the growth of *Lactobacillus casei* was strongly affected by tween 80 in the production L (+) lactic acid from wheat bran by *lactobacillus amylophilus* in solid state fermentation both Naveena et al. (2005) and Nagarjun et al. (2005) pointed out that tween 80 was found to influence the lactic acid productivity.

Fig. 1 Spectrum of lactic acid producing bacteria (*Lactobacillus delbrueckii*) by FTIR

Effect of amino acid supplementation on lactic acid production In the present work, three types of amino acids viz. DL-Aspartic acid, DL-Phenylalanine, L-Lysine and their three combination (i) DL-Aspartic acid+DL-Phenylalanine (ii) DL-Phenyl alanine+L-Lysine (iii) DL-Aspartic acid+L-Lysine were used for the production of lactic acid. In the basal media 50 mg/l concentration of amino acid was used for the production of lactic acid. Combination of two amino acids (1:1) ratio was used in production of lactic acid. In all of these amino acids supplementation trails, the lactic acid production was high in DL-Phenylalanine+L-Lysine. Fig. 2 shows that the HPLC profile of lactic acid production on amino acid supplementation. Different peaks were obtained for citric acid, sucrose, lactic acid, acrylic acid, cis-aconitic acid, and linear dimer with retention time 6.808 min, 7.842 min, 9.86 min, 12.25 min, 14.508 min and 16.617 min, respectively.

Fig. 2 Chromatogram of lactic acid sample: citric acid (1), sucrose (3), lactic acid (5), acrylic acid (6), cis-aconitic acid (7) and linear dimer (8)

Optimization study in shake flask

Interactive effect of tween 80 concentration and cane molasses concentration on lactic acid production Fig. 3a shows that the response surface plot of lactic acid production by *Lactobacillus delbrueckii* NCIM 2025 showing interactive effect of tween 80 and cane molasses. It can be seen that with increased level of cane molasses concentration (30 g/L), the lactic acid production increased exponentially, whereas with the increase in level of tween 80 concentrations (4 g/L) the lactic acid production was (45 g/L) first increased and then decreased. Similar studies were reported by the Beaulieu et al. (1995) showed the effect of molasses and tween 80 on lactic acid production was positive, as molasses was rich in trace elements and vitamins. Yu et al. (2008) reported similar finding for L (+) lactic acid production by *Lactobacillus rhamnosus*.

Fig. 3 Response surface plot of lactic acid production by *Lactobacillus delbrueckii* NCIM 2025 showing interaction between (a) Tween 80 and cane molasses (b) Amino acid and cane molasses (c) Amino acid and tween 80

Interactive effect of cane molasses concentration and amino acid on lactic acid production

As lactic acid bacteria are nutritionally fastidious and require several amino acids and vitamins for growth, it was very important to choose the right nitrogen and carbon sources. Molasses and amino acids predominantly affect lactic acid

production. Hence, a strong interaction between these nutrients for lactic acid fermentation is inevitable as shown in Fig. 3b. Higher concentrations of amino acid (>40 g/L) caused inhibition of lactic acid production. Increase in the concentration of cane molasses (10–30 g/L) lactic acid production was high. According to De Lima et al. (2009) high concentrations of nitrogen can lead to cell death and lactic acid production increases significantly with increase in concentration of cane molasses.

Interactive effect of amino acid and tween 80 on lactic acid production Lactic acid bacteria need a particular amount of nitrogen source for the proper growth. Beyond a given limit of amino acids (above 40 g/L) and tween 80 (above 4 g/L) the growth was negatively impacted shown in Fig. 3c. On using both nitrogen and carbon sources together, it was observed that the LAB growth and production of lactic acid decreases after achieving threshold value. According to Sriphochanart et al. (2011) lactic acid production was increased when amino acid like phenylalanine, lysine and aspartic acid supplementation was done. In case of most *Lactobacillus* strain unsaturated fatty acid such as tween 80 are essential growth factor. Partenen et al. (2001) reported that the growth of *Lactobacillus casei* was strongly affected by tween 80 in the production L (+) lactic acid from wheat bran by *Lactobacillus amylophilus* in solid state fermentation both Naveena et al. (2005) and Nagarjun et al. (2005) pointed out the tween 80 was found to influence the lactic acid productivity.

Statistical optimization of cultural conditions by CCRD The experiments with different combination of cane molasses, tween 80 and amino acid was assayed and calculated after *Lactobacillus delbrueckii* inoculum that was cultivated at 37 °C for 24 h. A total of 20 experiments with different combination of cane molasses, amino acid and tween 80 were performed (Table 1). The ranges and levels of independent variables used in CCRD were obtained from experimental design (Table 2). The result obtained were analyzed using analysis of variance (ANOVA) as appropriate to the experimental design used. The regression equation obtained after analysis of variance gives the production of lactic acid. All the terms regardless of their significance were included in second order polynomial equation:

$$Y = 44.29 - 4.6807A + 2.5173B + 9.3265C - 7.3957A^2 - 5.3062 \\ - 4.0334C^2 - 1.5625AB - 5.0875AC + 0.3700BC \quad (1)$$

The determination coefficient (R^2) of the model was determined to be 0.897 (a value of >0.75 indicates fitness of the model). An R^2 value can be between 0 to 1 and the closer value is to 1.0 the better the model fits the experimental data. Thus the study indicates that 89.7 % of the variation in the response

Table 1 CCRD design for optimization of three variables for production of lactic acid by *Lactobacillus delbrueckii* NCIM 2025

Exp No.	Amino acid (g/L)	Tween 80 (g/L)	Cane molasses (g/L)
1	40.00	3.00	20.00
2	40.00	3.00	20.00
3	20.00	2.00	10.00
4	40.00	3.00	20.00
5	40.00	3.00	03.18
6	73.63	3.00	20.00
7	20.00	2.00	30.00
8	40.00	3.00	20.00
9	40.00	1.32	20.00
10	20.00	4.00	30.00
11	06.36	3.00	20.00
12	40.00	3.00	20.00
13	40.00	3.00	36.81
14	20.00	4.00	10.00
15	40.00	3.00	20.00
16	60.00	2.00	30.00
17	60.00	4.00	10.00
18	40.00	4.68	20.00
19	60.00	2.00	10.00
20	60.00	4.00	30.00

(i.e. lactic acid) was attributed to the independent variables, whereas 10.3 % of the total variance could not be explained by the model. An adjusted R^2 was 0.804, which accounted for the number of predictors in the model. Both of the obtained R^2 values suggested that the model fit the data well. The F statistic was 9.68 and corresponded to a value of $P=0.001$ (the confidence interval was 0.05), which indicated that the model was both adequate and significant (Table 3). The values A, C, A^2 , B^2 , C^2 and AC were significant (Table 4). The larger the magnitude of the t-value and smaller the p-value, the more significant would be the regression coefficient. For the first order effects, judging from the regression coefficient and t-values, it could be concluded that the cane molasses (A) concentration had the most significant effect on the lactic acid

Table 2 Levels of independent variables in experimental plan

Independent variable Ranges and levels	Ranges and levels				
	$-\alpha$	-1	0	1	$+\alpha$
Production medium optimization					
Amino acid	6.36	20	40	60	73.63
Tween 80	1.31	2	3	4	4.68
Cane molasses	3.18	10	20	30	36.18

Table 3 Analysis of variance for the production of lactic acid by *Lactobacillus delbrueckii*

Source	DF	Seq SS	Adj MS	Adj MS	F-value	P-value
Regression	9	3016.07	3016.07	335.119	9.68	0.001*
Linear	3	1573.68	1573.68	524.560	15.16	0.000*
Square	3	1214.70	1214.70	404.900	11.70	0.001*
Interaction	3	227.69	227.69	75.896	2.19	0.152
Residual error	10	3460.2	346.02	34.602	-	-
Lack of fit	5	339.56	339.56	67.913	52.55	0.000*
Pure error	5	6.46	6.46	1.292	-	-
Total	19	3362.09	-	-	-	-

* Significant terms in the model; DF: degree of freedom; Seq SS: sequential sum of squares

Adj MS: adjusted mean square

production, followed by amino acid (C). The quadratic main effect of amino acid ($p<0.001$), tween 80 ($p<0.006$) and cane molasses ($p<0.026$) were the significant factors. Therefore, amino acid, tween 80 and cane molasses act as limiting factors and a little variance in their concentration may alter lactic acid production considerable extent. The p-value is 0.001 which is indicating that it was significant to some extent ($p>0.05$) in this model of lactic acid production (Table 4). However, the amino acid concentration was the significant variable. Interaction between concentration of amino acid and cane molasses were significant ($p<0.034$) (Table 4). Thus, increasing the concentration of cane molasses will increase the lactic acid production (Fig. 3b). Effect of various combinations of amino acid, tween 80 and cane molasses on the cell mass and lactic acid production is shown in Table 5.

Similar study was reported by the Farooq et al. (2012), where the maximum lactic acid production was achieved after 7 days of fermentation in media containing 18 % substrate (cane molasses) level with a mean value of 7.76 ± 0.08 g/

Table 4 Coefficients and t-value for lactic acid production using CCRD

Term	Coefficient	Standard coefficient	t-value	P-value
Constant	44.295	2.399	18.463	0.000*
A: Amino acid	-4.6807	1.592	-2.941	0.015*
B : Tween 80	2.5173	1.592	1.581	0.145
C : Cane molasses	9.3265	1.592	5.859	0.000*
A^2	-7.3957	1.550	-4.773	0.001*
B^2	-5.3062	1.550	-3.424	0.006*
C^2	-4.0034	1.550	-2.603	0.026*
AB	-1.5625	2.080	-0.751	0.470
AC	-5.0875	2.080	-2.446	0.034*
BC	0.3700	2.080	0.178	0.862

* Significant terms in the model

Table 5 Cell mass and lactic acid produced at different CCRD experimental designs for the three variables (amino acid, tween 80 and cane molasses)

Experiment No.	Cell mass (g/L)	Lactic acid (g/L)
1	68.33	45.70
2	64.22	43.25
3	29.40	21.56
4	62.22	43.43
5	56.23	12.15
6	65.45	19.19
7	39.87	40.90
8	59.45	45.67
9	49.89	19.56
10	38.76	47.80
11	27.65	29.80
12	60.23	43.67
13	58.90	55.86
14	32.28	19.86
15	60.29	43.67
16	63.50	25.89
17	62.20	18.95
18	33.22	41.25
19	51.23	19.78
20	56.26	19.42

100 mL (77.6 g/L) at 42 °C. Increase in the concentration of cane molasses and amino acid was followed by significant increase in lactic acid production. Maximum lactic acid production was obtained at 10–40 g/L concentration of cane molasses with amino acid at concentration of 40 g/L.

Scale up in fermentor Shake flask study was then scaled up to lab scale fermentor. The culture was grown in a fermentor to study lactic acid production in batch cultivation. In the fermentor the lactic acid production was high and the fermentation time was reduced. In the shake flask lactic acid production was 55.89 g/L and the fermentation time was 24 h. Scale up in the fermentor the lactic acid production was increased to 84.50 g/L, the fermentation time was decreases to 12 h in comparison to shake flask. Similar study was reported by the Albino et al., (2012), L (+) lactic acid by *Lactobacillus sp. B2* using sugar cane molasses as carbon source. The lactic acid production was 19.5 g/L, production was lower in comparison to current finding. In another study by the Cockl and Stouvenel (2006) reported that the maximum lactic acid production was 35 g/L using a strain of *Lactococcus lactis* subs *lactis* isolated from sugar cane plants. Coelho et al. (2010) reported that the cassava wastewater for the production of L (+) lactic acid by *Lactobacillus rhamnosus* B 103 produces lactic acid of 41.65 g/L after 48 h of fermentation. John et al.

Fig. 4 Spectrum of lactic acid production by FTIR

(2008) reported 40 g/L lactic acid production by the *Lactobacillus delbrueckii* from the starchy wastes.

Characterization of lactic acid using FTIR According to National Institute of Materials and Chemical Research (NIMCR), Japan (2000), FTIR for lactic acid shows two different types of O-H bond - the one in the acid and the simple “alcohol” type in the chain attached to the -COOH group. The O-H bond in the acid group gets absorbed between the range of 2500 and 3300 cm^{-1} and the one in the chain between 3230 and 3550 cm^{-1} . When these two are taken together give immense trough covering the whole range from 2500 to 3550 cm^{-1} and lost in the trough will be absorptions due to the C-H bonds. The presence of strong C=O shows absorption at about 1730 cm^{-1} (Fig. 4).

Conclusions

In the present work, *Lactobacillus delbrueckii* was used to utilize agro-industrial by-product (cane molasses) for lactic acid production under submerged fermentation process. The effect of interactions between the three variables, i.e., amino acid (g/L), tween 80 (g/L) and cane molasses (g/L), on the production of lactic acid produced is shown in Table 5. Under optimum condition lactic acid production was enhanced from 55.89 g/L to 84.50 g/L. Further, validation showed 81.50 g/L lactic acid production. Scale up was done in fermentor. Productivity was found to be 3.40 g/L/h which were higher than in previous studies with reduced fermentation time from 24 to 12 h.

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