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OPTIMIZATION OF LACTIC ACID PRODUCTION FROM CHEAP RAW MATERIAL: SUGARCANE MOLASSES

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Abstract

Biotechnological process is advantageous over chemical one as we can utilize cheap raw materials as carbon source such as agro-industrial byproducts and can produce the pure lactic acid in a very economic way. Sugar cane molasses is available in many countries as byproduct of sucrose production, which can be used as substrate for lactic acid fermentation. Process variables were optimized for the production of lactic acid by *Lactobacillus delbrueckii* using food wastes as substrates. The productivity was found to be affected by fermentation time, temperature and the level of substrate. The maximum lactic acid production was achieved after 7 days of fermentation in media containing 18% substrate level with a mean value of 7.76±0.08 g/100 ml (77.6 g/l) at 42ºC. The maximum recovery of lactic acid with respect to initial total sugar contents of the media (9.91±0.20 g/100 ml) was 78.30%.

Introduction

Lactic acid is one of the functional and valuable compounds utilized in food, pharmaceutical and chemical industries. It is industrially produced either by chemical synthesis or by microbial fermentation. A biological method has advantage that an optically pure lactic acid can be obtained by choosing a strain of lactic acid bacteria, whereas chemical synthesis always results in a racemic mixture of lactic acid (Ryu *et al.*, 2003).

Lactic acid bacteria are traditionally fastidious microorganisms and have complex nutrient requirements (Fitzpatrick & OKeeffe, 2001). Refined sugars (glucose or sucrose) have been more frequently used to produce lactic acid (Hofvendahl & Hahn, 1997). However, these are economically not feasible due to high cost of pure sugars whereas the product (lactic acid) is relatively cheap. The production of lactic acid through fermentation technology in industry is mainly dependent on cost of raw material to be used. Therefore, it is mandatory to have a raw material for industrial production of lactic acid with several characteristics such as low cost, minimum level of contaminants, rapid fermentation rate, high lactic acid production yields, little or no by-product formation and year-round availability (Ryu *et al.*, 2003).

Food industrial wastes, high in moisture and rich in carbon source have been considered as attractive nutrient source for industrial lactic acid production. In Pakistan, thousand tons of agro-based industrial wastes are generated every year (John *et al.*, 2009). The production of sugar cane molasses only has been reported to be 1.3 to 2.1 million tones during 1994 to 2008 (Anon., 2009), which is a cheaper substrate as a source of sugars to be utilized in a fermentation process subsequently for lactic acid production (Rashid & 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 total carbohydrate concentration of 45-60% (Mariam *et al.*, 2009). In Pakistan, sugarcane is grown in the three zones and is an important and the second largest cash crop of Pakistan grown over an area of 963,000 hectares with an average yield of 47 tons/hectare (Ather *et al.*, 2009). Normally molasses contains about 46% total sugars, 3.0% crude protein, 0.0% fat with 79.5° brix (Curtin, 1983) and are used in animal feed as well as for the production of alcohol through fermentation. As it contains high amount of sugars (46%), it must not be considered as a waste material instead researchers should make efforts to produce value added products from this cheaper source of carbohydrates/sugars (Rashid & Altaf, 2008).

In this study, attempts were made for lactic acid production using sugar cane molasses as cheaper available raw substrate through a fermentation process with indigenous bacterial culture (*Lactobacillus delbrueckii*). The fermentation conditions were optimized considering fermentation time, temperature and substrate level as main process factors.

Materials and Methods

Strain and growth medium: The indigenous culture of *Lactobacillus delbrueckii*, a homofermentative lactic acid producer, was utilized in the study. The culture was isolated from yoghurt and was identified by following the procedure given by Harrigan (1998). The stock cultures were maintained at 4ºC 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^{-1}): peptone 10.0, meat extract 10.0, yeast extract 05.0, d-glucose 20.0, tween-80 01.0, K_2HPO_4 02.0, sodium acetate 05.0, tri-ammonium citrate 02.0, $MgSO_4.7H_2O$ 0.2, $MnSO_4.4H_2O$ 0.05 in 1000 mL water.

Inoculum preparation: *Lactobacillus delbrueckii* cells from stock cultures were transferred to sterile growth medium (MRS agar plates) and incubated at 38 ºC for 24 hours. Then the culture was inoculated into MRS broth in screw capped test tubes, incubated for 24 hours at 38 ºC and was used for fermentation process.

Fermentation: The lactic acid fermentation of sugar molasses was carried out by the *Lactobacillus* strain at 34ºC, 38ºC and 42ºC with 0, 6, 12, 18 and 24% substrate levels (Luis *et al.,* 2003). The fermentation media contained (g $100mL^{-1}$); peptone 10.0, meat extract 10.0, yeast extract 05.0, Tween-80 01.0, K_2HPO_4 02.0, Sodium acetate 05.0, tri-ammonium citrate 02.0, $MgSO_4.7H_2O$ 0.2, $MnSO_4.4H_2O$ 0.05, with different percent levels of sugar cane molasses (0, 6, 12, 18, 24) and water 100 mL (Glucose was replaced with sugar cane molasses).

Analysis: Fermentation medium was filtered through Whatman filter paper and the filtrate was used for the estimation of sugars and lactic acid on daily basis up to 8 days of fermentation. Total sugars were estimated using Lane and Eynon method by titration with Fehling's solution (Kirk & Sawyer, 1991). Lactic acid was estimated by high performance liquid chromatography (Akalin *et al.,* 2002) as described below:

Sample preparation: Seven mL of fermented media was added to 40 ml of buffer-acetonitrile mobile phase (0.5 % ($m\ v^{-1}$) $(NH_4)_2HPO_4$ (0.038 M) - 0.4 % (by volume) acetonitrile (0.049 M), at pH=2.24 with H_3PO_4, extracted for 1 hour in orbital shaker and centrifuged at 6000 x *g* for 5 min. The supernatant was collected and filtered once through Whatman #1 filter paper and twice through a 0.45-µm membrane filter, and then used directly for HPLC analysis. Triplicate analyses were performed on all samples.

HPLC analysis: Samples for Lactic acid production were analyzed through HPLC with UV detector (Perkin Elmer-series 200) at 214 nm using RP-18 column (120 x 4.6 mm). The operating conditions were: mobile phase, aqueous 0.5% ($m\ v^{-1}$) $(NH_4)_2HPO_4$ (0.038 M) - 0.2% (by volume) acetonitrile (0.049 M) adjusted to pH=2.24 with H_3PO_4; flow rate 0.3 mL min^{-1}; ambient column temperature. The mobile phase was prepared by dissolving analytical-grade $(NH_4)_2HPO_4$ in distilled deionized water, HPLC-grade acetonitrile, and H_3PO_4. HPLC-grade reagents were used as standards (Sigma Chemical Co., St. Louis, MO). Solvents were filtered through a 0.45-µm membrane filter and degassed under vacuum. Quantification was based on internal standard method.

Statistical analysis: The statistical analyses of the data were carried by following three factor factorial experiment and DMR test by the software Minitab (Steel *et al.,* 1997).

Results

Table 1 indicates the analysis of variance for total sugar utilization and lactic acid production from sugar cane molasses. Different levels (0, 6, 12, 18 and 24) of sugar cane molasses subjected to fermentation at different temperatures (34, 38 and 42ºC) in a specifically designed medium revealed that the fermentation time and substrate levels were highly significant ($p<0.05$) and effective in utilizing total sugars present in molasses during the fermentation process. The effect of temperature was found to be non-significant. Similarly, the first order interactions of all the variables were non-significant except the one between fermentation time and substrate levels on utilization of total sugars in the media during fermentation. The results indicated that the lactic acid production was affected significantly by the selected fermentation time, temperature and substrate levels. However, the first order interactions among time x temperature, temperature x time, and time x temperature x substrate level were found to be non-significant, except the interaction of fermentation time and substrate levels, which was highly significant ($p<0.05$).

Table 1. Mean squares for utilization of total sugars and lactic acid production from sugar cane molasses.

Source of variance	df	Mean squares	
		Utilization of total sugars	Lactic acid production
Fermentation time (D)	8	356.83**	183.34**
Temperature (T)	2	0.04 ^{NS}	0.28**
Substrate level (S)	3	541.05**	205.09**
D x T	16	0.008 ^{NS}	0.03 ^{NS}
D x S	24	23.27**	7.94**
T x S	6	0.002 ^{NS}	0.05 ^{NS}
D x T x S	48	0.003 ^{NS}	0.02 ^{NS}
Error	216	0.055	0.04
Total	**323**		

**Highly significant ($p<0.01$), *Significant ($p<0.05$), ^{NS} Non-significant ($p>0.05$)

Figure 1 shows the utilization of total sugars with the passage of fermentation time. Utilization of total sugars increased as the time of fermentation was increased decreasing the available sugar content in the media. The results indicated that 8.16 g/100 ml total sugar contents were utilized after 8 days of fermentation process with a gradual increase from the initial point. However, the utilization of total sugars was lower at the start of fermentation. This indicates encouraging utility of sugars during fermentation time period (days).

Figure 2 indicates the proportional increased use of available sugars by the *Lactobacillus delbreukii* as the level of dose was increased from 0.00 to 9.91 g $100mL^{-1}$. The highest utilization (8.04 g/100 ml) of total sugars was found in 24% substrate level followed by 18% (6.05 g/100 ml) substrate level. However, utilization of total sugars was not observed in the medium without substrate.

The first order interaction between fermentation time and substrate levels revealed that the highest utilization of total sugars occurred after 8 days of fermentation period whereas, low amount of total sugars was utilized at the start of experiment regardless of substrates levels. However, no total sugars utilization was detected in the media without substrate where no sugar source was added in the media, throughout the fermentation period. The utilization of total sugars ranged from 0.00 to 12.95 g/100

ml (Fig. 3, Table 2). The results further showed that per day utilization of total sugars was the maximum (2.4 g $100mL^{-1}$) at 3rd day of fermentation whereas in subsequent days sugar utilization was continuously decreased (Fig. 4). It was observed that lactic acid production persistently increased up to (5.85 g $100mL^{-1}$) with an increase in fermentation time till the completion of fermentation, although non significant increase was observed in between 7th and 8th day of fermentation (Fig. 5).The lactic acid production from sugar cane molasses was significantly higher at 42 than 34 and 38ºC temperature treatments. The quantity of lactic acid produced was 3.77 g/100 ml, 3.69 g/100 ml and 3.67g/100 ml at 42, 38 and 34ºC temperature, respectively. However, the difference in lactic acid production between 34 and 38ºC temperature was found statistically non-significant (Fig. 6).

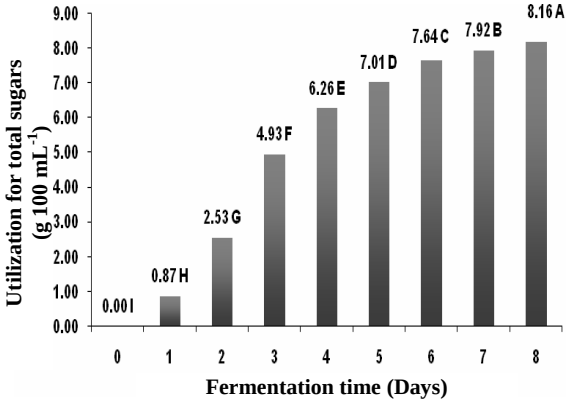

Fig. 1. Effect of fermentation time on utilization of total sugars during lactic acid production from sugar cane molasses.

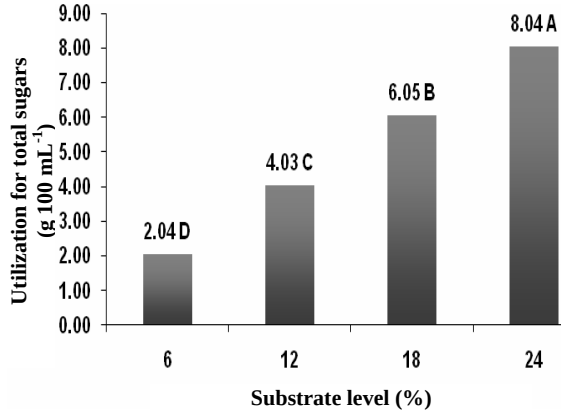

Fig. 2. Effect of substrate level on utilization of total sugars during lactic acid production from sugar cane molasses.

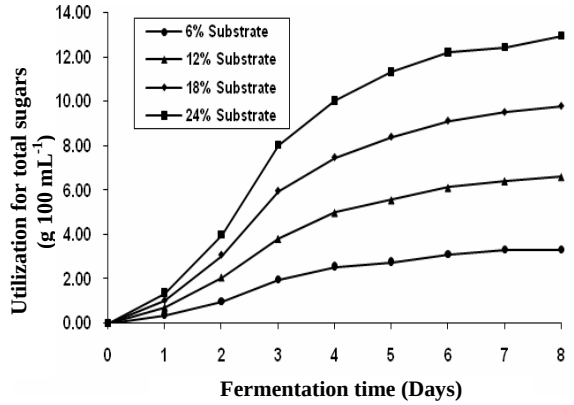

Fig. 3. Effect of fermentation time and substrate level on utilization of total sugars during lactic acid production from sugar cane molasses.

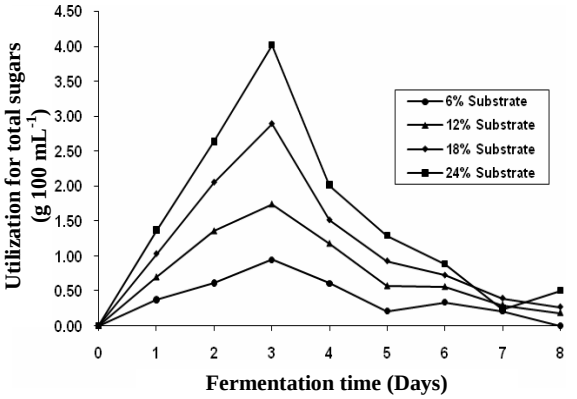

Fig. 4. Effect of fermentation time and substrate level on per day utilization of total sugars during lactic acid production from sugar cane molasses.

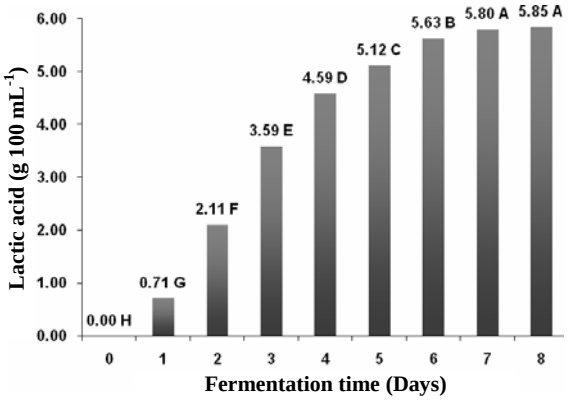

Fig. 5. Effect of fermentation time on lactic acid production from sugar cane molasses.

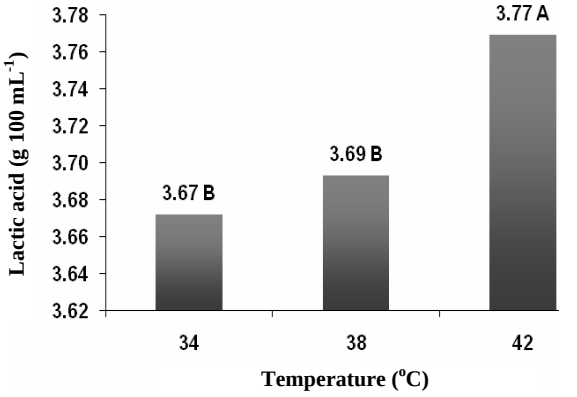

Fig. 6. Effect of temperature on lactic acid production from sugar cane molasses.

Table 2. Effect of fermentation time and substrate level on utilization of total sugars (g/100 ml) during lactic acid production from sugar cane molasses.

Fermentation time (Days)	Substrate level (%)				Mean
	6	12	18	24	
0	0.00 ± 0.00 x	0.00 ± 0.00 x	0.00 ± 0.00 x	0.00 ± 0.00 x	0.00 ± 0.00 I
1	0.38 ± 0.01 w	0.70 ± 0.01 v	1.03 ± 0.02 u	1.37 ± 0.02 t	0.87 ± 0.07 H
2	0.99 ± 0.01 u	2.06 ± 0.02 s	3.08 ± 0.02 q	4.00 ± 0.01 p	2.53 ± 0.21 G
3	1.94 ± 0.01 s	3.80 ± 0.08 p	5.97 ± 0.04 m	8.01 ± 0.06 j	4.93 ± 0.43 F
4	2.55 ± 0.02 r	4.98 ± 0.04 o	7.49 ± 0.07 k	10.03 ± 0.05 e	6.26 ± 0.53 E
5	2.76 ± 0.03 r	5.56 ± 0.09 n	8.41 ± 0.04 i	11.32 ± 0.08 d	7.01 ± 0.60 D
6	3.07 ± 0.02 q	6.12 ± 0.05 m	9.14 ± 0.07 h	12.20 ± 0.08 c	7.64 ± 0.65 C
7	3.30 ± 0.02 q	6.42 ± 0.07 l	9.53 ± 0.14 g	12.44 ± 0.04 b	7.92 ± 0.66 B
8	3.30 ± 0.02 q	6.60 ± 0.05 l	9.80 ± 0.17 f	12.95 ± 0.23 a	8.16 ± 0.69 A
Mean	2.04 ± 0.13 D	4.03 ± 0.26 C	6.05 ± 0.40 B	8.04 ± 0.53 A	

Means carrying same letters are not significantly different

Table 3 Effect of time and substrate on lactic acid production from sugar cane molasses.

Fermentation time (Days)	Substrate level (%)				Mean
	6	12	18	24	
0	0.00 ± 0.00 u	0.00 ± 0.00 u	0.00 ± 0.00 u	0.00 ± 0.00 u	0.00 ± 0.00 H
1	0.31 ± 0.01 t	0.57 ± 0.02 s	0.83 ± 0.02 r	1.14 ± 0.02 q	0.71 ± 0.06 G
2	0.81 ± 0.02 r	1.66 ± 0.03 p	2.58 ± 0.04 n	3.37 ± 0.04 l	2.11 ± 0.18 F
3	1.57 ± 0.03 p	3.069 ± 0.07 m	4.98 ± 0.05 h	4.75 ± 0.13 i	3.59 ± 0.29 E
4	2.06 ± 0.05 o	4.04 ± 0.07 k	6.24 ± 0.11 e	6.01 ± 0.12 f	4.59 ± 0.36 D
5	2.19 ± 0.05 o	4.53 ± 0.06 j	7.10 ± 0.09 c	6.65 ± 0.08 d	5.12 ± 0.40 C
6	2.54 ± 0.07 n	4.94 ± 0.10 hi	7.72 ± 0.10 a	7.34 ± 0.09 b	5.63 ± 0.44 B
7	2.65 ± 0.05 n	5.22 ± 0.07 g	7.76 ± 0.08 a	7.59 ± 0.06 a	5.80 ± 0.45 A
8	2.71 ± 0.06 n	5.41 ± 0.09 g	7.73 ± 0.07 a	7.55 ± 0.09 a	5.85 ± 0.44 A
Mean	1.65 ± 0.11 C	3.27 ± 0.22 B	4.99 ± 0.33 A	4.93 ± 0.30 A	

Means carrying same letters are not significantly different

The lactic acid production with 18% substrate levels was found significantly the highest (4.99 g/100 ml) although 24% substrate yielded 4.93 g/100 ml, which was non-significant with the yield obtained from 18% (Fig. 7). The substrate levels (6 and 12%) proportionally produced maximum lactic acid. However, no lactic acid was produced in the medium lacking substrate due to non-availability of carbon source to the microorganisms.

The interaction of fermentation time and substrate levels (Fig. 8) showed an increasing trend of lactic acid production with increase in fermentation time and substrate levels. Significantly the highest lactic acid production took place after 7 days of fermentation (7.76 ± 0.08 g/100 ml) in the media containing 18% cane molasses substrate as carbon source (Table 3). Rapid increase in lactic acid production was observed at 2nd and 3rd day of fermentation with maximum at 7 days of fermentation whereas, it persisted uptil 8th with a non significant difference with the yield of day 7, corresponding to the utilization of total sugars stated elsewhere (Fig. 9).

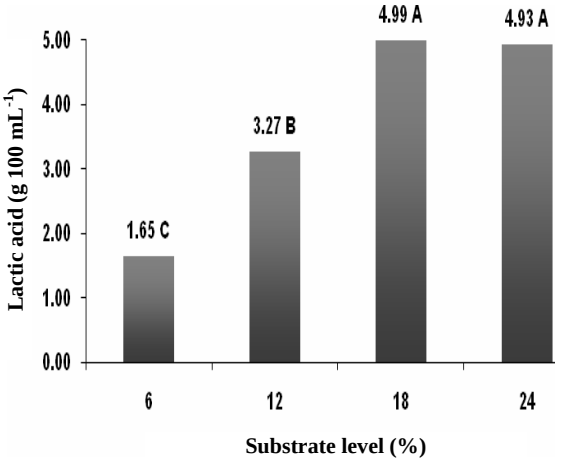

Fig. 7. Effect of substrate level on lactic acid production from sugar cane molasses.

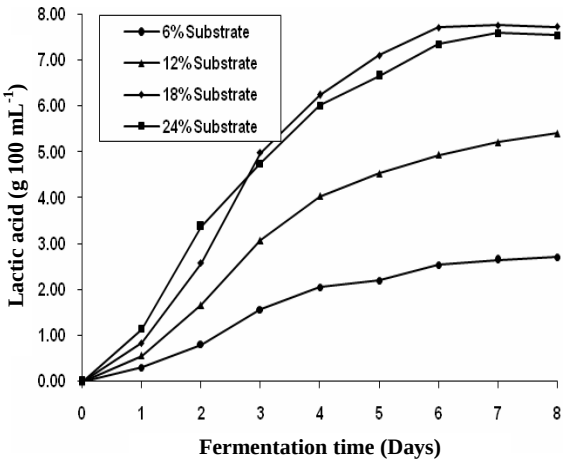

Fig. 8. Effect of fermentation time and substrate level on lactic acid production from sugar cane molasses

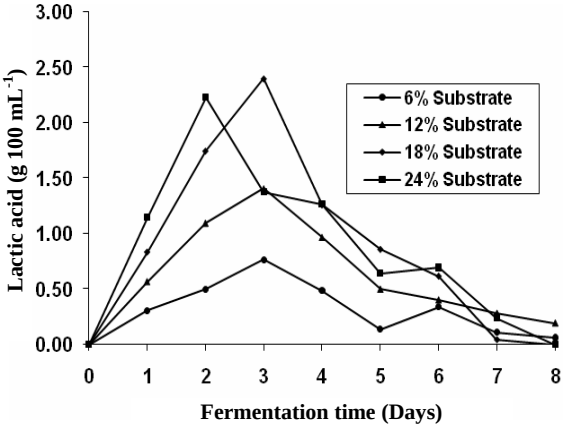

Fig. 9. Effect of fermentation time and substrate level on per day lactic acid production from sugar cane molasses.

Discussion

Microorganisms are not only well known due to their harmful effects such as diseases and food spoilage but also make key contributions to the welfare of humanity (Anon., 1990; Tortora *et al.,* 2010)**.** These are widely used in food industry for production of a large number of fermented food products and at the same time these are also very helpful for conversion of food industrial wastes into value added products (Laufenberg *et al.,* 2003). The ability of microorganisms to convert large and complex molecules into the simplest one depends upon the type of culture and the growth requirements which include both intrinsic and extrinsic environmental conditions (Lucas *et al.,* 2007). The selection of a suitable culture to convert the specific type of substrate into useful products thus plays a vital role in fermentation technology (Krishna, 2005). For the production of useful products at industrial level, attempts are being made by the researchers to identify the cheapest and easily available substrates to reduce the cost of production (John *et al.,* 2006).

Thus overall 12.95 g/100 ml (97.30%) total sugars were utilized during fermentation of sugar cane molasses. The other researcher also found 78 to 89% and 47.2% total sugars conversion to lactic acid under different fermentation conditions (Nancib *et al.,* 2001) while working on the effect of supplementation by different nitrogen sources on the production of lactic acid from date juice by *Lactobacillus casei* subsp. *Rhamnosus*. Similarly, El-Sherbiny *et al.,* (1986); Mintian *et al*., (2005); Richter & Berthold (1998) also observed 80.7, 71 and 89% total sugar utilization during lactic acid production from different substrates used during studies on lactic acid production. In our study, more utilization of total sugars was observed during fermentation of sugar cane molasses in comparison to the findings of the researchers stated therein. The reason might be the selection of culture and substrate (composition) sucrose to avail carbon source of energy in sugar cane molasses. Conversion rate of total sugar to lactic acid basically depends mainly on substrate for growth of the lactic acid bacteria/culture (John *et al*., 2009)

In case of lactic acid production, our results fall within the limits *i.e*., 11.27 to 85.03% lactic acid recoveries after 96 hours (4 days) of fermentation by *Lactobacillus delbrueckii* (Luis *et al.,* 2003). The results are also supported by the findings of other researcher (Kim *et al.,* 2003) who, achieved 91% overall yield of lactic acid at 42°C with initial pH=6.0 of the media during lactic acid production from food wastes through fermentation with *Lactobacillus delbrueckii*.

Several other researchers haw observed different yield production ranging from (51- 97%) the production of lactic acid using various substrates during optimizing the fermentation conditions(El-Sherbiny *et al.,* 1986; Mintian *et al.,* 2005; Richter & Berthold, 1998; Narita *et al.,* 2004; Aksu & Kutsal, 1986; Garde *et al.,* 2002; Bai *et al.,* 2003; Lee *et al.,* 2004). The yield of lactic acid produced in our experiment falls within the range of previous work done. The differences in lactic acid recovery during this study with that of some other researchers might be due to the variation in temperature, substrate levels, types of substrates and the selection of suitable culture for fermentation.

Conclusion

The lactic acid production is significantly influenced by fermentation time, temperature and substrate levels. The effect of these parameters on lactic acid production is linear and after 6 days of fermentation stationary phase is achieved which however depends upon fermentation conditions. The maximum lactic acid was produced after 7 days of fermentation in media containing 18% substrate level with a mean value of 7.76 ± 0.08 g/100 ml (77.6 g/l) at 42ºC. The maximum recovery of lactic acid with respect to initial total sugar contents of the media (9.91 ± 0.20 g/100 ml) was 78.30%. For continuous production of lactic acid under these conditions the media should be reconstituted after 3 days of fermentation as optimum per day acid production is after 72 hours (3 days)

References

Akalin, A.S., S. Gonc and Y. Akbas. 2002. Variation in organic acids content during ripening of pickled white cheese. *J. Dairy Sci*., 85: 1670-1676.

Aksu, Z. and T. Kutsal. 1986. Lactic acid production from molasses utilizing *Lactobacillus delbrueckii* and invertage together. *Biotechnol Lett.*, 8: 157-160.

Anonymous. 1990. Food Protection Committee. Subcommittee on Microbiological Criteria , An evaluation of the role of microbiological criteria for foods and food ingredients, National academy press 2101 constitution avenue, pp. 42-43.

Anonymous. 2009. *Pakistan economic survey 2007-08*. Finance Division, Economic Advisory Wing, Government of Pakistan.

Ather, A., S. Khan, A. Rehman and M. Nazir. 2009. Optimization of the protocols for callus induction, regeneration and acclimatization of sugarcane cv. thatta-10. *Pak. J. Bot*., 41(2): 815-820.

Bai, D.M., Q. Wei, Z.H. Yan, X.M. Zhao, X.G. Li and S.M. Xu. 2003. Fed-batch fermentation of *Lactobacillus lactis* for hyper-production of L-lactic acid. *Biotechnol Lett.*, 25: 1833-1835.

Curtin, L.V. 1983. Molasses-general consideration: In: *Molasses in animal nutrition*. National Feed Ingredients Association, West Des Moines, Iowa.

El-Sherbiny, G.A., S.S. Rizk and G.S. Yousef. 1986. Utilization of beet molasses in the production of lactic acid. *Egyptian J Food Sci*., 14: 91-100.

Fitzpatrick, J.J. and U. OKeeffe. 2001. Influence of whey protein hydrolyzate addition to whey permeate batch fermentations for producing lactic acid. *Proc Biochem*, 37: 183-186.

Garde, A., G. Jonsson, A.S. Schmidt and B.K. Ahring. 2002. Lactic acid production from wheat straw hemicellulose hydrolysate by *Lactobacillus pentosus* and *Lactobacillus brevis*. *Bioresource Technol*., 81: 217-223.

Harrigan, W.F. 1998. *Laboratory methods in food microbiology*. 3rd Ed. Academic Press Limited, London, 532p.

Hofvendahl, K. and B. Hahn-Hagerdal. 1997. L-Lactic acid production from whole wheat flour hydrolyzate using strains of *Lactobacilli* and *Lactococci*. *Enzyme Microbial Technol*, 20: 301-307.

John, R.P., G.S. Anisha, K.M. Nampoothiri and A. Pandey. 2009. Direct lactic acid fermentation: Focus on simultaneous saccharification and lactic acid production. *Biotechnol Advan*, 27: 145-152.

John, R.P., K.M. Nampoothiri and Pandey. 2006. A Solid-state fermentation for L-lactic acid production from agro wastes using *Lactobacillus delbrueckii*. *Process Biochem*, 41: 759-763.

Kim, K., K. Woo, S. Deok, Y. In, K. Eun and Y. Hyon. 2003. Production of lactic acid from food wastes. *Appl Biochem Biotechnol*, 107: 637-648.

Kirk, R.S. and R. Sawyer. 1991. *Composition and analysis of foods*. 9th Ed. Addison Wesley Longman Ltd., Edinburgh Gate, Harlow, England.

Krishna, C. 2005. Solid-State Fermentation Systems-An Overview. *Crit Rev Biotechnol*, 25: 1-30.

Laufenberg, G., B. Kunz and M. Nystroem. 2003. Transformation of vegetable waste into value added products: (A) the upgrading concept; (B) practical implementations. *Bioresource Technol*., 87: 167-198.

Lee, S.M., Y.M. Koo and J. Lin. 2004. Production of lactic acid from paper sludge by simultaneous saccharification and fermentation. *Adv. Biochem. Eng. Biotechnol*., 87: 173-194.

Lucas, A.D., L. Rodriguez, J. Villasenor and F.J. Fernande. 2007. Fermentation of agro-food waste waters by activated sludge. *Water Res*., 41: 1635-1644.

Luis, .S.J.T., A.B. Moldes, J.L. Alonso and M. Vazquez. 2003. Optimization of lactic acid production by *Lactobacillus delbrueckii* through response surface methodology. *J Food Sci*., 68: 1454-1458.

Mariam, I., K. Manzoor, S. Ali and I. Haq. 2009. Enhanced production of ethanol from free and immobilized saccharomyces cerevisiae under stationary culture. *Pak. J. Bot*., 41(2): 821-833.

Mintian, G., M. Koide, R. Gotou, H. Takanashi, M. Hirata and T. Hano. 2005. Development of a continuous electrodialysis fermentation system for production of lactic acid by *Lactobacillus rhamnosus*. *Proc Biochem*, 40: 1033-1036.

Nancib, N., A. Nancib, A. Boudjelal, C. Benslimane, F. Blanchard and J. Boudrant. 2001. The effect of supplementation by different nitrogen sources on the production of lactic acid from date juice by *Lactobacillus casei* subsp. *Rhamnosus*. *Bioresource Technol*, 78: 149-153.

Narita, J., S. Nakahara, H. Fukuda and A. Kondo. 2004. Efficient production of L-(+)-lactic acid from raw starch by *streptococcus bovis* 148. *J. Biosci. Bioengg*., 97: 423-425.

Rashid, M.T. and Z. Altaf. 2008. Potential and environmental concerns of ethanol production from sugarcane molasses in Pakistan. Nature Precedings: hdl:10101/npre.2008.1499.1.

Richter, K., and C. Berthold. 1998. Biotechnological conversion of sugar and starchy crops into lactic acid. *J Agric Engng Res*., 71: 181-191.

Ryu, H.W., J.S. Yun and Y.J. Wee. 2003. Lactic acid. In: *Concise Encyclopedia of Bioresource Technology*. (Ed.): A. Pandey. The Haworth Press, New York, pp 635.

Steel, R.G.D., J.H. Torrie and D. Dickey. 1997. Principles and Procedures of Statistics: A Biometrical Approach. 3rd Ed McGraw Hill Book Co, NY, USA.

Tortora, G.J., Funke B.R. and C.L. Case. 2010. Microbiology: an introduction. 10th Ed. Pearson Education, Inc., publishing as Pearson Benjamin Cummings, 1301, Sansome St., San Francisco. pp 1.

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