Asian Journal of Chemistry Vol. 15, Nos. 3 & 4 (2003), 1822–1824

NOTE

Optimization of Parameter for Submerged Lactic Acid Fermentation by *Lactobacillus bulgaricus* BS-18

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The optimization of parameters, *viz.*, incubation period, temperature, pH and concentration of sucrose substrate have been studied for *Lactobacillus bulgaricus* BS-18. It has been found that submerged lactic acid fermentation attains its best activity when a 10.5% sucrose solution is allowed to ferment for 5 days at 48°C temperature by maintaining the pH value of the fermentation medium at 6.2.

Key Words: Lactic acid, Fermentation, *Lactobacillus bulgaricus*.

The optimization of culture conditions for various lactic acid bacteria have been studied by different workers[1–5], Liu and Chen[6] reported significant yield of lactic acid from 11–23% sucrose at 32–33°C temperature and 6.0 pH. Tiwari and Pandey[7] reported maximum production of lactic acid by *L. bulgaricus* when 20% solution of molasses was allowed to ferment for 6 days at 47°C and 5.8–6.0 pH. Singh *et al.*[8] (1988) found that *L. delbrueckii* attains its best activity of producing lactic acid when 5% solution of sugar at 6.2 pH is allowed to ferment at 46°C for 6 days. In the recent past Singh *et al.*[9–15] studied lactic acid fermentation by different strains of lactic acid bacteria and found their best activity when sugar substrate concentration was taken in between 10–15%; pH 5.8–6.2, tempeature 42–48°C and incubation period 4–6 days.

In the present communication we have confined our study to optimize the parameters for maximum production of lactic acid by submerged fermentation using the bacterial strain of *L. bulgaricus* BS-18.

Medium: The composition of the production medium for each fermentor flask containing 100 mL production medium is as below:

Sucrose: 10% malt-extract: 0.375%; $(NH_4)_2HPO_4$: 0.25%; $CaCO_3$: 10.185%; distilled water: to make 100 mL; pH: 6.2 (adjusted by adding requisite amount of phosphate buffer solution); temperature : 48°C.

Sterilization: The growth and production media were sterilized in an autoclave maintained at 15 lbs steam pressure for 30 min.

Strain: *Lactobacillus bulgaricus* BS-18 was used in the present study. The strain was procured from NCL, Pune, India.

Assay methods: Evaluation of lactic acid formed and sucrose left unfermented was made colorimetrically[16].

Age of the inoculum: 48 h old.

Quantum of the inoculum: 0.5 mL bacterial suspension of *L. bulgaricus* BS-18.

TABLE-1
EFFECT OF CONCENTRATION OF SUCROSE SUBSTRATE, pH, TEMPERATURE AND INCUBATION PERIOD ON SUBMERGED LACTIC ACID FERMENTATION BY *LACTOBACILLUS BULAGRICUS* BS-18

% of sucrose	pH	Temp. in (°C)	Incubation period (days)	Corresponding yield of lactic acid* in g/100 mL				Corresponding amount of sucrose* left unfermented in g/100 mL			
2.5	5.8	30	2	1.356	4.410	4.487	2.989	0.173	4.091	3.958	5.512
5.5	5.9	35	4	3.358	5.413	4.925	3.992	1.182	3.083	3.641	4.030
10.5†	6.0	40	5†	6.960‡	6.701	5.945	6.958‡	1.302	1.778	2.587	1.300
15.5	6.1	43	6	10.178	6.805	6.930	6.311	2.169	1.389	1.389	1.128
20.5	6.2†	48†	9	13.291	6.964‡	6.966‡	6.118	3.492	1.272	1.284	1.122
25.5	6.3	50	12	16.683	6.952	5.782	5.843	4.487	1.275	2.710	1.117
30.5	6.5	51	14	20.296	6.352	—	—	5.973	2.118	—	
35.0	6.6	52	16	—	6.118	—	—	—	2.352	—	
40.0	6.7	54	18	—	5.002	—	—	—	3.440	—	
45.0	6.8	55	20	—	—	—	—	—	—	—	

*Each value represents mean of three trials; Experimental deviation (±) 0.5 to 2.5%.

†Optimum values of sucrose solution, pH, temp. and incubation period;

‡Optimum yield of lactic acid.

Temperature (°C): 30, 35, 40, 43, 48, 50, 51, 52, 54 and 55.

pH: 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.5, 6.6, 6.7, 6.8.

Incubation period: 4, 5 and 6 days.

Concentrations of sucrose solution: 2.5, 5.5, 10.5, 15.5, 20.5, 25.5, 30.5, 35, 40 and 45%.

The data recorded in Table-1 shows that a 10.5% sucrose solution serves as an optimum substrate solution for the fermentative production of lactic acid. It is obvious that higher concentration of sucrose interferes with the lactic acid producing activity of *L. bulgaricus* BS-18, and inhibits the yield of lactic acid.

The results recorded in the table show that *L. bulgaricus* BS-18 attains its best activity when the pH of the medium is maintained at 6.2. In much acidic medium, *L. bulgaricus* BS-18 failed to give significant yield of lactic acid. However, at pH 6.3 and onwards the yield of lactic acid has been found discouraging.

It is obvious from the table that *L. bulgaricus* BS-18 shows its best activity at the temperature of 48°C. Lower temperature of the expeiment at 30 and 35°C caused insignificant yield of lactic acid. Higher temperatures, *i.e.*, 50°C and onwards also deactivated the enzymatic system of lactic acid fermentation process and the yield of lactic acid was very much insignificant.

It is also obvious from the results that an incubation period of 5 days is most favourable for lactic acid production by *L. bulgaricus* BS-18. No increase in the yield of lactic acid has been observed even after incubation for 6 and 9 days.

REFERENCES

1. E. Johannsen, *J. Appl. Bact.*, **35**, 415 (1972).
2. H. Diar, *J. Gen. Microbiol.*, **73**, 233 (1972).
3. K. Vladimir, D. Josef and J. Strejoeck, *Chem. Absir.*, **65**, 4616b (1966).
4. K.P. Tiwari and N. Mishra, *Proc. Nat. Acad. Sci. India*, **48**, 170 (1978).
5. S.P. Singh, N. Rathor, G. Samdani, R.P. Sinha and A.K. Sinha, *Mendel*, **3** (1986).
6. P.W. Liu and S.T. Chen, *Chem. Abstr.*, **50**, 2117 (1956).
7. K.P. Tiwari and A. Pandey, *Proc. Nat. Acad. Sci. India*, **49**, 37 (1979).
8. S.P. Singh, N. Rathor, A.K. Sinha and L.K. Sinha, *Mendel*, **4**, 341 (1988).
9. S.P. Singh, Md. Shamin, K.P. Kamal and K.B. Lal, *Indian J. Agric. Chem.*, **30**, 73 (1997).
10. S.P. Singh, S. Kumar, U.N. Verma and B. Singh, *Orient. J. Chem.*, **14**, 359 (1998).
11. S.P. Singh, S. Kumar, B. Singh and B.K. Singh, *Asian J. Chem.*, **10**, 377 (1998).
12. S.P. Singh, S. Kumar and B.P. Pandey, *Asian J. Chem.*, **10**, 645 (1998).
13. S.P. Singh, S. Kumar and D.C. Mandal, *Vijnana Parishad Anusandhan Patrika*, **43**, 73 (2000).
14. S.P. Singh, V. Kumar and S.S. Chandra, *Indian J. Agric. Chem.*, **34**, 80 (2001).
15. S.P. Singh and V. Kumar, *J. Chemtracks*, **3**, 68 (2001).
16. M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, *Anal. Chem.*, **28**, 350 (1956).

(*Received*: 30 October 2000; *Accepted:*) 12 May 2003 AJC-3113