http://dx.doi.org/10.22037/afb.v4i2.13514

Research Article

APPLIED FOOD BIOTECHNOLOGY, 2017, 4 (2):85-92
Journal homepage: www.journals.sbmu.ac.ir/afb

pISSN: 2345-5357
eISSN: 2423-4214

Enhanced Sorbitol Production under Submerged Fermentation using *Lactobacillus plantarum*

Khan Nadiya Jan[1], Abhishek Dutt Tripathi[2], Shubhendra Singh[2]*, Diksha Surya[2], Surendra Prasad Singh[2]

1- SantLongowal Institute of Engineering and Technology, Punjab, India.

2- Center of Food Science and Technology, BHU, Varanasi, U.P, India.

Abstract

Background and Objective: Sorbitol is a non-toxic and slightly hygroscopic compound with different applications. *Zymomonas mobiles* produces sorbitol from sucrose or mixtures of glucose and fructose (formation is coupled with the dehydrogenation of glucose to glucono-δ-lactone). Recombinant *Zymomonas mobilis* may produce sorbitol and gluconic acid from glucose and fructose using different divalent metal ions with reduced the ethanol yield and significantly increased yield of sorbitol. Current study envisaged to alter the media components, physical process parameters and supplementation of amino acids for enhanced sorbitol production.

Materials and Methods: Several process variables were evaluated on sorbitol production including carbon sources (glucose, fructose, maltose, sucrose), carbon concentrations (5, 10, 20 and 25 g l^{-1}), nitrogen sources (peptone, tryptone, yeast extract, beef extract and organic nitrogen mix), temperatures (25, 29, 33, 37, 41°C), pH (6, 6.5, 7, 7.5, 8), agitation rate (50, 100, 150, 200 rpm) and amino acids (cysteine, cystine, tryptophan) in batch cultivation of *Lactobacillus plantarum* NCIM 2912. Shake flask cultivation performed under optimum conditions like temperature 37°C, pH 7.0 and agitation rate of 150 rpm, resulted in enhanced sorbitol production. Comparative study of sorbitol production in solid state fermentation and submerged fermentation was also evaluated.

Results and Conclusion: Batch cultivation under submerged conditions further performed in 7.5-l lab scale bioreactor (working volume 3.0-l) under optimized conditions resulted in maximum cell biomass of 8.95±0.03 g l^{-1} and a sorbitol content of 9.78±0.04 g l^{-1} after 42.0 h of fermentation. Scale up study on bioreactor resulted in maximum sorbitol yield ($Y_{p/x}$) and productivity of 1.11 g g^{-1} and 0.50 g l^{-1} h^{-1} under submerged fermentation, respectively.

Conflict of interest: The authors declare no conflict of interest.

Article Information

Article history:

Received	14 Aug 2016
Revised	14 Oct 2016
Accepted	26 Feb 2017

Keywords:
- Characterization
- HPLC
- *Lactobacillus plantarum* NCIM 2912
- Media components
- Physical parameters
- Sorbitol

***Corresponding author:**
Shubhendra Singh,
Center of Food Science and Technology, BHU, Varanasi, U.P, India.

Tel: +91-7275162549
Fax: +542-2368993
E-mail:
shubhendra_bhu@rediffmail.com

1. Introduction

Sorbitol ($C_6H_{14}O_6$), also referred to as D-glucitol, is naturally found in many fruits as berries (except white grapes), cherries, plums, pears, and apples [1]. It offers a sweet alternative for diabetes, and body-weight special diets due to its low caloric value (2.4 calories per gram versus 4.0 for sugar) of sorbitol. It is a non-metabolized alcoholic sugar that belongs to the family of low-calorie sugars, and can replace sucrose or lactose in food products with a nearly same sweetness and taste. Besides this sorbitol has a stabilizing effect on food by serving as fat replacer [2].

World sorbitol production has been estimated to be more than 5 million ton per year, traditionally from glucose catalytic hydrogenation using nickel as the catalyser, at high pressures and temperatures [3]. Sorbitol is a non-toxic and slightly hygroscopic compound being employed in large scale as texturizer, humectant, stabilizer and conditioner in several industries pharmaceutical, cosmetic, confectionary, etc [4-6]. It is produced as an important precursor for the production of ascorbic acid. Sorbitol has been shown to display an in vivoanti-cariogenic effect as it is not fermented by *Streptococcus* (*S.*) *mutants*, which are the most potent cariogenic bacterium [7]. Sorbitol functions in textile applications basically as a dispensing agent, humectant bodying agent and sequestering agent. FDA has approved the use of a "does not promote tooth decay" health claim in labeling for sugar-free foods that contain sorbitol or other polyols. Sorbitol can withstand high temperatures and does not participate in Maillard (browning) reactions. It functions well in many food products such aschewing gums, candies, frozen desserts, cookies, cakes, icings and fillings as well as oral care

products, including toothpaste and mouthwash. European Union has provided a Nutritional Labeling Directive stating that all polyols, including sorbitol, have a caloric value of 2.4 calories per gram which is less than glucose. Sorbitol has also a potential in vivo prebiotic effect, specifically to increase the lactobacilli number and butyrate production in the intestine [8] Sorbitol capable of causing hyperglycemia because it is converted to fructose in the liver [9].

In 1984, the capability of the ethanol-producing bacterium *Zymomonas* (*Z.*) *mobilis* to produce sorbitol from sucrose or mixtures of glucose and fructose was disclosed and reported that sorbitol formation is coupled with the hydrogenation of glucose to form glucono-δ-lactone [10]. Most of the work is done on different Zymomonas strains for evaluating their capacity of sorbitol production by varying the cultural conditions and by permeabilization of cells. A recombinant *Z. mobilis* strain was constructed for the production of sorbitol and gluconic acid from glucose and fructose was made using different divalent metal ions which drastically reduced the ethanol yield and significantly increased the yield of target product [11]. Kinetics of lactobionic acid and sorbitol production, from lactose/fructose substrates, using the enzymatic glucose–fructose oxidoreductase/glucono-δ-lactonase system was studied. In mentioned study higher initial concentrations of substrate led to a larger action of the enzymatic complex but did not cause any relevant increase on the rate of product formation [12]. Besides *Z. mobilis*, only a few other organisms have been reported to produce sorbitol. Sorbitol production by *Lactobasillus* (*L.*) *casei* and *L. plantarum*was also remarkable [2]. In another study whey permeate was used as substrate for sorbitol production by *L. casei* and conversion rate of 9.4% of lactose into sorbitol was obtained using an optimized fed-batch system. Further it was demonstrated that the mixed polyol production in *L. casei* can be avoided by mutating the mltD gene [13]. A strain from *Candida* (*C.*) *famata* was also found to reduce sorbose to sorbitol and iditol at a ratio of 3:2, respectively [14].

Current Study envisaged to alter the media components, physical process parameters and supplementation of amino acids for enhanced sorbitol production. This research work was carried out to evaluate different process parameters like temperature, pH, agitation speed, nitrogen source, carbon source for enhanced sorbitol production under submerged fermentation by *L. plantarum* sp. NCIM 2912 and also to investigate the possibility of sorbitol production in Solid state fermentation. Scale up of shake flask cultivation was done in 7.5-l bioreactor. Growth kinetics in batch mode under optimized physical conditions was also studied.

2. Materials and Methods

2.1. Screening for selection of potent sorbitol producing strains

The screening was done in submerged and solid state fermentation as mentioned below in materials and methods. Level of parameters is also mentioned in SSF and SMF separately. Main focus of the present study was to select a potent strain with highest sorbitol producing capability. Among wide range of microbes four strains selected for this study were *Alcaligens sp.* NCIM No. 5085, *Pseudomonas* (*P.*) *aeruginosa* NCIM No. 2948, *L. plantarum* NCIM No. 2912, *L. casei* NCIM No. 2025. The strains like *Alcaligens sp*., *P. aeruginosa*, and *Bacillus sp.* produced small amount of sorbitol. However *L. plantarum*, and *L. casei* showed the capability to produce sorbitol in large quantity and hence were selected for further study. All strains used under present study were procured from NCL (National Chemical Laboratory, pune, India) and Bacillus sp. was obtained from School of Biochemical Engineering, IT, BHU, Varanasi, India.

2.2. Media components for bacterial growth

L. casei NCIM 2502 and *L. planetarium* NCIM 2912 were used for fermentative sorbitol production. The *L. casei* was maintained on nutrient agar at 5°C and was sub-cultured monthly. However *L. plantarum*was maintained on media containing (g l^{-1}): 10 proteose peptone, 5 yeast extract, 10 beef extract, 20 dextrose, 1 tween 80, 2 ammonium citrate, 5 sodium acetate, 0.1 magnesium sulphate, 0.05 manganese sulphate, 2 dipotassium phosphate and 2.5 agar. The media was then incubated at 37°C for 72 h and was sub-cultured monthly.

2.3. Shake flask cultivation condition

2.3.1. Solid state fermentation

Solid state fermentation was carried out using different substrates including rice bran, wheat bran, orange peel powder. 15 g of each substrate was taken and mixed with 20 ml of the solution containing 0.20 g K_2HPO_4, 0.1 g Tween 80, 0.04 g $MgSO_4$, 0.006 g $MnCl_2$ and 0.2 g ammonium citrate in 100 ml flasks. All the chemicals were of analytical reagent grade and procured from Merck, USA. The media was then incubated in an incubator plus shaker with an agitation speed of 150 rpm and temperature 37°C for 72 h.

2.3.2. Submerged fermentation

Submerged fermentation experiments carried out in cotton plugged 250 ml erlenmeyer flasks containing 100 ml of production medium (g l^{-1}): 10 Tryptone, 8 beef extract, 4 yeast extract, 2 K_2HPO_4, 1 tween 80, 0.07 $MnCl_2$, 0.41 $MgSO_4$-$7H_2O$, 2 ammonium citrate, 5 sodium acetate and 20 glucose. Media was sterilized at 121°C for

15 min. All the chemicals were of analytical reagent grade and procured from Merck, USA. The pH of final culture medium was adjusted to 7.0 ± 0.2 using 0.1 N HCl per 0.1 N NaOH solution before bacterial inoculation.

For production of sorbitol, 200 ml of media (containing 20 g l^{-1} glucose) was taken in a 500 ml flask and was inoculated with 10 ml of inoculum. Different experimental trials were performed by keeping the flasks under shaking condition for 72 h at varying agitation speed, temperature, pH, carbon source, carbon concentration and nitrogen source in incubator plus shaker (Sigma, USA). Also the effect of amino acid supplementation was observed under submerged conditions.

2.3.4. Scale up in 7.5-l Bioreactor

Shake flask study was then scaled up to a lab scale bioreactor. The culture was grown in a 7.5-l Bench top bioreactor (BioFlo/Celligen 115, New Brunswick, USA) to study sorbitol production in batch cultivation using the selected comparatively greater sorbitol producing organism *L. plantarum* NCIM (2912). Working volume of bioreactor was kept at 3.0-l.

2.4. Analytical methods

2.4.1. Dry cell mass

20 ml culture broth obtained from different trials was centrifuged at 680 ×g for 15 min at 4°C and cell pellet was obtained. The cell pellet was washed with distilled water and then dried in petri-plate at 80°C for 24 h.

2.4.2. Glucose (reducing sugar concentration)

Reducing sugar concentration in the samples was determined by Di-nitro salicylic acid test.

2.4.3. Sample preparation for HPLC Analysis

20 ml of fermented media, which was prepared in submerged fermentation (200 ml) was taken from conical flask containing glucose as carbon source. The media was then centrifuged at 8000 ×g or 15 min at 4°C. Supernatant was then passed through 0.22 µ filter.

2.4.5. Sorbitol estimation

Quantitative analysis of sorbitol was done by collecting 2 ml of media containing sorbitol obtained at different time intervals and injected into Aminex HPX87C column (300 mm ×7.8 mm, at temperature 80°C). Distilled and deionized water was used as eluent with flow rate 0.6 ml min^{-1}. Acetonitrile: water in the ratio 90:10 were used as mobile phase. Analysis was done by measuring the retention time [15].

2.4.6. Fermentation optimization

Initial step for fermentation optimization was the selection of best carbon source. One factor at one time approach was used. Four carbon sources including glucose, maltose, sucrose and fructose were selected. Among all these glucose at concentration 20 g l^{-1} showed best result for growth and sorbitol production (Table 1). Process for sorbitol production was optimized by number of factors involving carbon sources (glucose, fructose, maltose, sucrose), carbon concentration (5, 10, 20, 25 g l^{-1}), nitrogen source (peptone, tryptone, yeast extract, beef extract and organic nitrogen mixture), temperatures (25, 29, 33, 37, 41°C), pH (6, 6.5, 7, 7.5, 8), agitation speed (50, 100, 150 and 200 rpm) and amino acid (cysteine, cystine, tryptophan). All the experiments were done in triplicates.

2.4.7. Bioreactor

Seed culture was prepared in a 500 ml Erlenmeyer flask containing 200 ml media. Batch cultivation was carried out at optimum temperature of 37°C in a 7.5-l bioreactor (BioFlo/Celligen 115, New Brunswick, USA) containing 3.0-l of media. The reactor was sterilized in an autoclave at 121°C for 20 min, cooled and inoculated with 50 ml l^{-1} inoculum. pH of the culture broth was maintained at optimum pH (7.0) by automatic addition of acid or base by pH-mV controller (MettlerTolledo USA). Dissolved oxygen was measured by DO probe (MettlerTolledo, USA).

Table 1. Effect of different carbon source on biomass, sorbitol production of *L. plantarum* sp. NCIM 2912

Carbon source	Biomass (g l^{-1})	Sorbitol (g l^{-1})	Substrate left (g g^{-1})	Specific growth rate (h^{-1})
Glucose	2.57	1.99	4.9	0.014
Sucrose	1.05	0.35	7.3	0.002
Maltose	1.56	0.88	6.4	0.005
Fructose	2.07	1.59	5.8	0.010

3. Results and Discussion

Several microbial strains like *Bacillus sp*, Alcaligens, Pseudomonas, *L. casei* and *L. plantarum* were selected for checking the cell mass and sorbitol production. Among these strains *L. plantarum* and *L. casei* showed the best results for sorbitol production at temperature of about 37°C, pH 7 and agitation of about 150 rpm (Fig 1).These results were in correlation with the results of Cazetta et al. in which sorbitol production were 300 g l^{-1} in similar conditions [16].

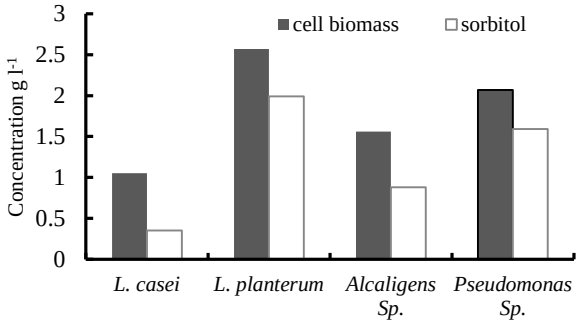


Fig 1. Effect of different strains on sorbitol and biomass production by *L. plantarum* sp. NCIM 2912

3.1. Solid state fermentation

In the present study SSF was carried out and low cost raw materials such as rice bran (15% v v^{-1}) wheat bran (15%) and orange peel (15%) were selected to study the cell mass and amount of sorbitol produced. Among these strains, *L. plantarum* showed the cell mass of 0.01 g ml^{-1} and sorbitol production of 0.0053 g ml^{-1} in rice bran at temperature of about 37°C, and there was no cell mass and sorbitol in wheat bran and orange peel powder by *L. casei.* Other microbial strains were unable to utilize solid substrate for sorbitol production. Rice bran contains approximately a total carbohydrate of 82% w w^{-1} and the main composition is hemicelluloses [17]. However, the sorbitol produced by *L. plantarum* in SSF was very low therefore submerged fermentation was practiced.

3.2. Submerged fermentation

In submerged fermentation sorbitol was produced in greater amount (9.78±0.04 g l^{-1}) compared to SSF (5.3 g ml^{-1}). Therefore SMF process was further optimized by varying cultural conditions including media components and physical parameters to elevate the amount of sorbitol produced. The results found here are in agreement with the results obtained by Mishra et al., for ethanol production in SSF and SMF using *Saccharomyces cerevisiae* and *C. albicans* utilizing agro-industrial waste [18].

3.3. Fermentation optimization

Initial step for fermentation optimization was the selection of best carbon source. One factor at one time approach was used. Four carbon sources including glucose, maltose, sucrose and fructose were selected. Among all these, glucose at concentration of 20 g l^{-1} showed best result for growth and sorbitol production (Table 1 & 2).

Nitrogen source was also varied and included peptone, tryptone, beef extract, yeast extract, and organic nitrogen mix (consisting of beef extract, yeast extract, and tryptone). Organic nitrogen mix proved to be the best nitrogen source at 20 g l^{-1} (Table 3). Previously Borsari et al concluded that the maximum sorbitol production (4.66 g l^{-1}) taking pantothenic acid, NaCl and Yeast extract at levels 0.01, 0.2, 4 g l^{-1}, respectively [19].

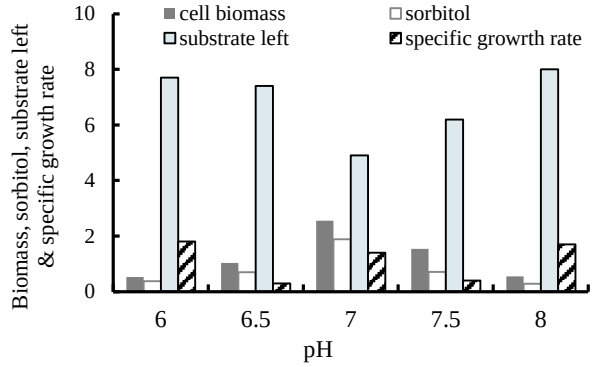
Table 2. Effect of different carbon concentration on biomass, sorbitol production of *L. plantarum* sp. NCIM 2912

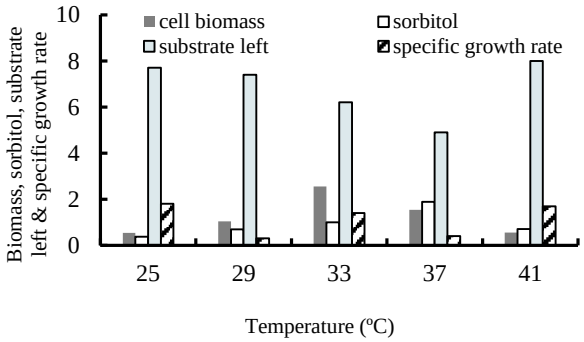
Carbon source	Biomass (g l^{-1})	Sorbitol (g l^{-1})	Substrate left (g g^{-1})	Specific growth rate (h^{-1})
5	0.57	0.15	7.55	0.014
10	1.05	0.35	7.25	0.002
15	1.56	0.88	6.30	0.005
20	2.57	1.99	5.05	0.014
25	2.07	1.59	5.7	0.010

Table 3. Effect of different nitrogen source on biomass, sorbitol production of *L. plantarum* sp. NCIM 2912

Nitrogen source	Biomass (g l^{-1})	Sorbitol (g l^{-1})	Substrate left (g g^{-1})	Specific growth rate (h^{-1})
Tryptone	1.55	0.67	6.35	0.005
Yeast extract	1.05	0.57	7.20	0.002
Beef extract	0.56	0.25	7.65	0.017
Peptone Organic	0.55	0.24	7.80	0.014
Nitrogen mix	2.04	1.02	5.48	0.010

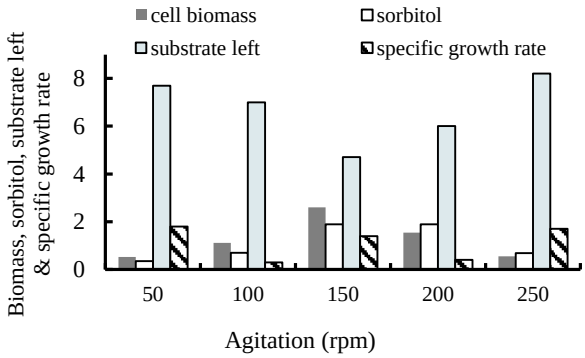
For further optimization effect of different physical parameters on sorbitol content was evaluated which showed that increase in pH, agitation and temperature up to 7.00, 150 rpm and 37˚C enhanced sorbitol content. However, further increase in these parameters resulted in a decrease in sorbitol content (Figures 2A, 2B, 2C). Finally, different amino acids (cysteine, cystine and tryptophan) were added to the media to study their role in improving sorbitol production. Among these cysteine increased the sorbitol production to greater extent (Table 4). Amino acid supplementation is done because of its positive responses, including reduced lag time and increased cell viability [20].





A. Effect of pH

B. Effect of temperature



C. Effect of agitation

Fig 2. Effect of agitation on production of sorbitol and biomass (g l^{-1}), substrate left (g g^{-1}) and specific growth rate (100^{-1}) (h^{-1}) of *L. plantarum* sp. NCIM 2912

3.4. Scale up in 7.5-l bioreactor

Batch cultivation study was carried out to understand the kinetics of sorbitol production under optimized condition of media components i.e., temperature of 37˚C, pH 7.0, agitation of 150 rpm and also with cysteine supplementation of 0.5 g l^{-1}. Fig 3 represents the sorbitol production under optimized conditions by *L. plantarum* utilizing glucose and organic nitrogen mix as carbon and nitrogen sources at initial concentration of 20.0 g l^{-1} and 2.0 g l^{-1}, respectively. pH was kept at 7.0±0.2 throughout the production process. Agitation speed was kept at 150 rpm at 37°C. Fig 3 clearly revealed that after a lag phase of 12.0 h biomass increased to 8.95 g l^{-1} at 42.0 h. Maximum sorbitol production was found to be 9.78 gl^{-1} after 42.0 h of fermentative production. Sorbitol yield ($Y_{p/x}$) in terms of cell biomass produced was found to be 1.11 g g^{-1} of biomass, total sugar concentration decreased to 0.72 g l^{-1} at the end of production phase in comparison to initial concentration of 20.0 g l^{-1}. HPLC analysis reveal-ed the sorbitol production which is in correlation with previous finding which showed similar peaks at same retention time i.e. 2.4 min for sorbitol in pharmaceutical formation (Fig. 4) [15].

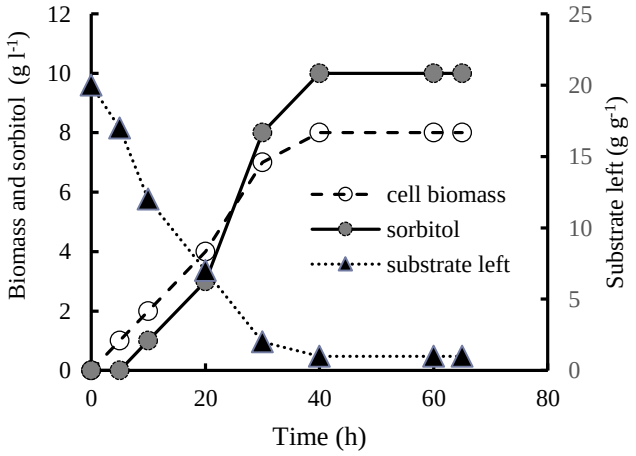


Fig 3. Biomass and sorbitol production in scale up 7.5-l bioreactor by *L. plantarum* sp. NCIM 2912

4. Conclusion

L. plantarum NCIM gave maximum sorbitol yield under optimized condition comprising; temperature 37°C, pH 7.0 and agitation speed of 150 rpm utilizing glucose at concentration 20 g l^{-1} as substrate. Batch cultivation perfor-med in 7.5-l lab scale bioreactor under optimized condition resulted in sorbitol yield ($Y_{p/x}$) in terms of cell biomass as 50%, yield in terms of substrate consumed ($Y_{p/s}$) was 1.12 g sorbitol g l^{-1} glucose consumed and productivity of 0.50 g $l^{-1}h^{-1}$, respectively. Current findings suggest that very low sorbitol production occurred in SSF compared to SMF.

Table 4. Effect of amino acid supplementation on biomass, sorbitol production of *L. plantarum* sp. NCIM 2912

	Cell Biomass ($g\ l^{-1}$)	Sorbitol ($g\ l^{-1}$)	Substrate left ($g\ g^{-1}$)	Specific growth rate ($g\ l^{-1}\ h^{-1}$)
Cysteine	4.04	5.19	3.50	0.018
Cystine	3.54	4.37	4.45	0.020
Tryptophan	3.05	2.92	4.70	0.017

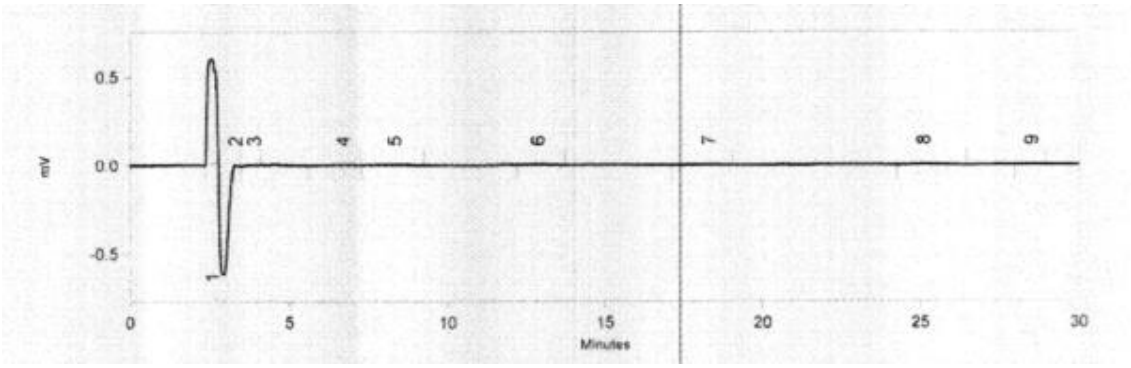


Fig 4. HPLC profile of sorbitol produced by *L. plantarum* sp. NCIM 2912

5. Acknowledgements

We are grateful to National Institute of Pharmaceutical Education and Research for its skillful help in HPLC analyses.

6. Conflict of Interest

There is no conflict of interest between authors and all the authors mutually agree to submit the manuscript in the Journal.

References

1. Wrolstad RE, Shallenberger RS, Free sugars and sorbitol in fruits-a complication from the literature. J Assoc Off Anal Chem 1981; 64(1) 91-103.
2. Ladero V, Ramos A, Wiersma A, Goffin, P, Schank A, Kleerebezem M, Hugenholtz J, Smid EJ, Hols P. High level production of the low-calorie sugar sorbitol by *Lactobacillus plantarum* through metabolic engineering, Appl Environ Microbiol. 2007; 63: 2117-2123. doi: 10.1128/AEM.02304-06
3. Vogel R, Ullmann's Encyclopedia of Industrial Chemistry, 6th ed, Vol 34, Wiley-VCH, Weinheim, Germany. 2003; 52: 492-498.
4. Albert R, Strätz A, Vollheim G. Die katalytische herstellung von zuckeralkoholen und deren verwendung (In German). Chem. Ing. Tech. 1980, 52, 582-587
5. Collins PM, Ferrier RJ. Monosaccharides: Their chemistry and their roles in natural products. Great Britain: John Wiley Sons; 1995: 587.
6. Silveira MM, Jonas R. The biotechnological production of sorbitol. Appl Microbiol Biotechnol. 2002; 59: 400-408. doi 10.1007/s00253-002-1046-0
7. Hayes C. The effect of non-cariogenic sweeteners on the prevention of dental caries: A review of the evidence. J Dent Educ. 2001; 65: 1106-1109.
8. Sarmiento-Rubiano LA, Zuniga M, Perez-Martinez G. Yebra MJ. Dietary supplementation with sorbitol results in selective enrichment of lactobacilli in rat intestine. Res Microbiol. 2007: 158: 694-701. doi: org/10.1016/j.resmic.-2007.07.007
9. Miller SA, Frattali VP. Saccharin. In: Kretchmer, N; Hollenbeck, C.B, eds. Sugars and sweeteners. Boca Raton, FL: CRC Press, 1991; 245-255
10. Leigh DA, Scopes RK, Rogers PL. A proposed pathway for sorbitol production by *Zymomonas mobilis.* Appl Microbiol Biotechnol. 1984; 20: 413-415. doi: 10.1007/BF00261944
11. Liu C, Dong H, Zhong J, Ryu DDY, Bao J. Sorbitol production using recombinant *Zymomonas mobilis* strain, J. Biotechnol. 2010; 148: 105-112. doi: 10.1016/j.jbiotec.-2010.04.008
12. Borges da Silva EA, Pedruzzi I, Rodrigues AE. Adsorption (2011) 17: 145. doi: 10.1007/s10450-010-9304-4.
13. Boeck RD, Sarmeinto-Rubiano LA, Nadal I, Monedero V, Perez-Martinez, G, Yebra JM. Sorbitol production from lactose by engineered *Lactobacillus casei* deficient in sorbitol transport system and mannitol-1-phosphate dehydrogenase. Appl Microbiol Biotechnol. 2010; 85: 1915-1922. doi: /10.1016/j.femsle.2005.06.010
14. Nissen L, Perez-Martinez G, Yebra MJ. Sorbitol synthesis by an engineered *Lactobacillus casei* strain expressing a sorbitol-6- phosphate dehydrogenase gene within the lactose operon. FEMS Microbiol Lett. 2005; 24 (9): 177-183. doi: 10.1016/j.femsle.2005.06.010
15. Sasahara, H, Izumori, K. Reduction of L-sorbose by halotolerant yeast, Candida famata 234 B. *Seibutsu-kogaku*, 1994; 72:299-304.

16. Simonzadeh N, RonsenB. An isocratic HPLC method for the determination of sorbitol and glycerol in pharmaceutical formulations. J Chromatogr Sci. 2012; 50(7): 644-647. doi: 10.1093/chromsci/bms044.

17. Cazetta ML, Celligio MAPC, Buzato JB, Scarmino IS, Silva da RSF. Optimization study for sorbitol production by *Zymomonas mobilis* in sugar cane molasses. Process Biochem. 2005; 40: 747-751. doi: 10.1016/j.procbio.2004-01.041

18. Claye SS, Idouraine A,Weber CW. Extraction of insoluble fiber from five sources. Food Chem. 1996; 57: 305-310. doi: 10.1016/0308-8146(95)00250-2

19. Mishra J, Kumar D, Samanta S, Vishwakarma M, Comparative study of ethanol production from various agro residues by using *Saccharomyces cerevisiae* and *Candida albicans*. J Yeast Fungal Res. 2012; 3(2): 12-17. doi:10.5897-JYFR11.025

20. Borsari R, Buzato JB. Evaluation of supplementation of sucrose medium on the synthesis of *Zymomonas mobilis* bioproducts. Acta Biol Sci; 2010; 32(3): 229-233. doi: 10.4025-/actascibiolsci.v32i3.1519

http://dx.doi.org/10.22037/afb.v4i2.13514

Research Article

APPLIED FOOD BIOTECHNOLOGY, 2017, 4 (2): 85-92
Journal homepage: www.journals.sbmu.ac.ir/afb

pISSN: 2345-5357
eISSN: 2423-4214

افزایش تولید سوربیتول با استفاده از *لاکتوباسیلوس پلانتاروم* در شرایط تخمیر غوطه ور

خان نادیا جان [۱]، آب هیشک دات تریپاتی[۲]، شابهندرا سینگ*[۲]، دیکشا سرایا[۲]، سارندرا پراساد سینگ[۲]

۱- سن لانگووال انستیتو مهندسی و تکنولوژی،پنجاب، هند.

۲- مرکز علوم تغذیه و تکنولوژی، BHU(Banaras Hindu University)، واراناسی، U.P، هند.

چکیده

سابقه و هدف: سوربیتول ترکیبی غیرسمی و کمی نم‌گیر با کاربردهای کوناکون است. *زایموموناس موبایلز* سوربیتول را از سوکروز یا مخلوط از گلوکز و فروکتوز تولید می‌کند (با جدا کردن هیدروژن از گلوکز و تشکیل گلوکونو-گاما- لاکتون)، *زایموموناس موبایلز* نوترکیب می‌تواند با استفاده از یون‌های فلزی گوناگون با دو بار مثبت ، از گلوکز و فروکتوز، سوربیتول و گلوکونیک اسید تولید کند،که با کاهش راندمان اتانول و افزایش قابل توجه راندمان سوربیتول همراه باشد. مطالعه حاضر در نظر دارد به منظور افزایش تولید سوربیتول، ترکیبات محیط کشت، پارامترهای فیزیکی فرایند و افزودن مکمل‌های آمینو اسیدها را تغییر دهد.

مواد و روش‌ها: در کشت غیرپیوسته *لاکتوباسیلوس پلانتاروم* NCIM 2912، متغیرهای گوناگون فرایند، شامل منابع کربن (گلوکز، فروکتوز، مالتوز، سوکروز)، غلظت‌های کربن ($g\ l^{-1}$ ۵، ۱۰، ۲۰ و ۲۵)، منابع ازت (پپتون، تریپتون، عصاره مخمرو مخلوط ازت‌های آلی)، درجه حرارت‌های (°C۲۵، ۲۹، ۳۳، ۳۷ و ۴۱)، pH (۶، ۶/۵، ۷، ۷/۵ و ۸)، سرعت همزدن (۵۰، ۱۰۰، ۱۵۰ و ۲۰۰ دور در دقیقه) و آمینو اسیدها (سیستئین، سیستین، تریپتوفان) بررسی شد. فلاسک محیط کشت تحت شرایط بهینه، مانند درجه حرارت °C ۳۷، pH، ۷/۰ و سرعت همزدن ۱۵۰ دور در دقیقه تکان داده شد و نتیجه‌اش افزایش تولید سوربیتول بود. تولید سوربیتول در تخمیر در حالت جامد و تخمیر غوطه‌ور نیز مقایسه و مورد بررسی قرار گرفت.

یافته‌ها و نتیجه گیری: کشت غیرپیوسته در شرایط غوطه‌ور در بیوراکتوری آزمایشگاهی ۷/۵ لیتری (حجم کاری ۳ لیتر) و در شرایط بهینه شده انجام و بیشینه توده سلولی $g\ g^{-1}$ ۸/۹۵±۰/۰۳ و میزان سوربیتول $g\ l^{-1}$ ۹/۷۸±۰/۰۴ پس از ۴۲/۰ ساعت تخمیر به دست آمد. با افزایش مقیاس بیوراکتور، بیشینه راندمان تولید سوربیتول ($Y_{p/x}$) و بهره‌وری به ترتیب $g\ g^{-1}$ ۱/۱۱ و $g\ l^{-1}$ ۰/۵۰در شرایط تخمیر غوطه‌ور به دست آمد.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ تعارض منافعی وجود ندارد.

تاریخچه مقاله

دریافت ۱۴ اوت ۲۰۱۶
داوری ۱۴ اکتبر ۲۰۱۶
پذیرش ۲۶ فوریه ۲۰۱۷

واژگان کلیدی

- ویژگی ها
- HPLC
- *لاکتوباسیلوس پلانتاروم* NCIM 2912
- ترکیبات محیط کشت
- پارامترهای فیزیکی
- سوربیتول

***نویسنده مسئول**

شابهندرا سینگ، مرکز علوم تغذیه و تکنولوژی، BHU (Banaras Hindu University)، واراناسی، U.P، هند.

تلفن:
دورنگار:
پست الکترونیک:
shubhendra_bhu@rediffmail.com