World Applied Sciences Journal 17 (9): 1083-1086, 2012
ISSN 1818-4952

Fermentative Lactic Acid from Deproteinized Whey Using *Lactobacillus bulgaricus* in Batch Culture

[1]*Sakineh Fakhravar,* [2]*Ghasem Najafpour,* [1]*Saeed Zeinali Heris,*
[3]*Mohammad Izadi and* [4]*Afsaneh Fakhravar*

[1]School of Chemical Engineering, Ferdowsi University of Mashhad, Iran
[2]Biotechnology Research Lab., School of Chemical Engineering,
Noushirvani University of Technology, Babol, Iran
[3]Chemical Engineering Department of Amirkabir University of Technology, Tehran, Iran
[4]Department of Chemistry, Faculty of Basic Science, Mazandaran University, Babolsar, Iran

Abstract: Lactic acid is an organic acid which has a wide range of application in food, pharmaceutical, leather and textile industries. Lactic acid is produced via chemical synthesis and also fermentative processes. In microbial fermentation, lactic acid is produced by lactic acid bacteria. The aim of present research was to investigate the effect of lactose concentration in production of lactic acid from cheese whey using *Lactobacillus bulgaricus* (ATCC 8001, PTCC 1332) in a batch culture. Fermentation media was inoculated, stirred at 180 rpm and incubated at 32°C. Substrate consumption and lactic acid production were determined. Four defined lactose concentrations were used in fermentative lactic acid production. Maximum lactic acid production of 24.57 $g.l^{-1}$ was achieved with initial lactose concentration of 40 $g.l^{-1}$. As the concentrations of lactose in the fermentation media was reduced from 40 to 10 $g.l^{-1}$, the yield of lactic acid production from 0.69 to 0.81 g lactic acid per g lactose was improved.

Key words: Lactose · Lactic acid · Whey · Fermentation · *Lactobacillus bulgaricus*

INTRODUCTION

Lactic acid (α-hydroxy propionic acid) ($C_3H_6O_3$) [1] is a natural organic acid which has number of applications in pharmaceutical, food and chemical industries as an acidulant, preservative and substrate for the production of some other organic acids [2]. Nowadays, production of lactic acid has increased due to demand for its use as a raw material for biodegradable polymers (PLA) and renewable plastics [3, 4]. Lactic acid manufactured by chemical synthesis or microbial fermentation. In recent years, the amount of lactic acid obtained via biotechnological methods has significantly increased [5, 6]. There are two optical isomers of lactic acid; L (+) lactic acid, D (-) lactic acid and racemic (DL) lactic acid. D (-) lactic acid is harmful to human metabolism [5]. Microbial fermentation lead to optically pure isomers L(+) or D(-) lactic acid from renewable sources, while chemical synthesis always produces racemic (DL) lactic acid from petrochemical resources [1, 6].

Approximately 90% of lactic acid was produced by lactic acid bacteria (LAB) via fermentation of carbohydrates. The genus *Lactobacillus* belongs to a large group of LAB which is gram-positive and safe organisms. The genus *Lactobacillus* is a heterogeneous group of lactic acid bacteria with important implications in food fermentation [7]. *Lactobacilli* have been used for decades in food preservation as starters for dairy products, fermented vegetables, fish and etc.

Fermentation comes from a variety of sugar known as substrates. Several carbohydrate substances such as corn and potato starch, molasses and whey are used for lactic acid production [1]. Hydrolyzed sugars from starch and molasses are abundant substrate for industrial use. The choice of substrate depends upon its availability, treatment required prior to fermentation and processing costs. Pure sugar is the best substrate for lactic acid production but the process for purification of sugar is most probably an expensive process. Therefore, reasonably

Corresponding Author: Dr. Ghasem Najafpour, Biotechnology Research Lab., School of Chemical Engineering, Noushirvani University of Technology, Babol, Iran.

priced material such as whey permeate, starchy raw, molasses and beet are used as suitable substrates [1].

Production of lactic acid from pretreated whey by *Lactobacillus bulgaricus* is considered as downstream waste material in diary process. Whey is considered as wastewater obtained as byproduct of cheese production process after the separation fat and casein from milk. The mentioned whey contains high value of chemical oxygen demand (COD), in the range of 50000-70000 $g.l^{-1}$ [8]. Therefore, disposal of the whey is a major pollution problem for the dairy industry and threatening our safe environment. To overcome the existing problem, utilization of whey in a desired fermented product is highly appreciated in most of industrial processes [9, 10]. Whey is a rich source of lactose, nitrogenous substances including vitamins and other essential nutrients for the growth of certain bacteria. Therefore, whey is a potent and suitable raw material for the production of wide variety of bio-based products processed via biotechnological methods. Whey are also used as substrate for production of organic acid, ethanol, single cell protein and methane [11].

The purpose of this present research (production of lactic acid from ultra filtered (UF) whey in batch process) was investigated in a wide range of lactose concentrations. In this regard, lactose concentration was adjusted by dilution of the whey and the prepared lactic acid was compared in four media contained different lactose concentrations.

MATERIALS AND METHODS

Microorganism: *Lactobacillus Bulgaricus* (ATCC 8001, PTCC 1332) was used for the present work. This strain was obtained from Iranian Research Organization for Science and Technology (IROST). The strain was cultured in media which containing yeast extract (1 $g.l^{-1}$) and K_2HPO_4 (0.2 $g.l^{-1}$).

Whey Preparation: Deproteinased whey was obtained by heating [12]. Whey was filtrated in order to separate the coagulated proteins. Then lactose in presence of dilute acid, hydrolyzed to galactose and glucose (1ml HCl in 100 ml whey). After 24 h, the whey was neutralized with 1M NaOH solution. The pH of pretreated whey was adjusted to 7. Deproteinated and hydrolyzed whey was prepared as suitable media for formentation.

Culture and Fermentation Conditions: The microorganism was grown in anaerobically medium culture which contained 1 $g.l^{-1}$ yeast extract. MRS sterilized at 121°C for 20 min [13]. After adding whey to MRS, culture medium incubated at 32°C and 180 rpm, for 24h. Stock culture for batch experiment was prepared by combination of 95% volume of whey and 5 % volume of seed culture. Batch fermentation was performed at 32 °C and 180 rpm in a flask with 200 ml working volume.

Analytical Method

Bacterial Growth: Bacterial growth was measured by optical density using spectrophotometer (Unico 2100, USA) at a wavelength of 620 nm. The optical density data was converted to cell dry weight using the corresponding calibration curve which was previously obtained and results were expressed in $g.l^{-1}$.

Lactose Concentration: Lactose concentration was determined via reducing reagent 3, 5-dinytrisalicylic acid (DNS) method. The sample was drawn in every 12 h. The DNS reagent solution (1 ml) added to (1 ml) sample and the sample was heated for 15 min, then cooled for 7-8 min. Finally, by addition of 8ml of distillated water, the absorbance was recorder at a wavelength of 540 nm by means of a spectrophotometer (Unico 2100, USA). The lactose concentration was determined by the use of standard calibration curve.

pH: The pH meter, HANA 211 (Romania) glass-electrode was employed for measuring pH values in the aqueous phase.

Lactic Acid: Lactic acid was measured by means of HPLC (column: shim-pack CLC-ODS:SPD 6A at 210 nm) [14]. Samples were quantified according to analytical external standards with HPLC grade. Calibration curve was carried out with different concentration of pure lactic acid.

RESULTS AND DISCUSSION

In this study, lactic acid production was performed in a batch fermentation. The substrate was ultra filtrated whey inoculated with *Lactobacillus bulgaricus* (ATCC 8001, PTCC 1332). In this experiment, effect of lactose concentration on lactic acid production was investigated. Dilution was performed with distillated water in four different concentration of lactose (Table 1).

Table 1: Lactose concentration in four different medium by dilution of whey

Medium Number	Dilution ratio (Whey: Distillated water)	Lactose concentration ($g.l^{-1}$)
1	----	40
2	3:1	30
3	1:1	20
4	1:3	10

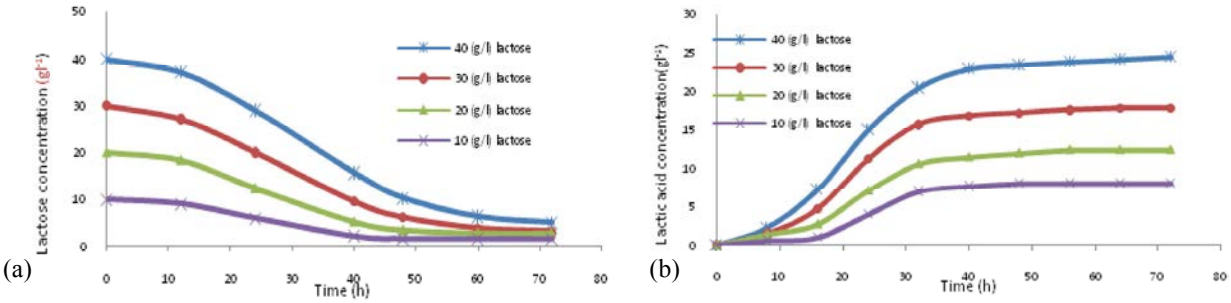

Fig. 1: (a) Lactose consumption and (b) lactic acid production for, four different lactose concentrations medium

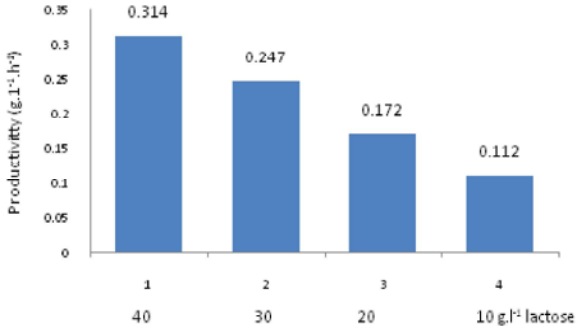

Fig. 2: Comparison between lactic acid productivity for four different lactose concentration medium

Lactose consumption and lactic acid production with 10, 20, 30 and 40 $g.l^{-1}$ lactose concentration profiles are shown in Figures 1.a and 1.b, respectively. In the sample initially contained 40 $g.l^{-1}$ lactose, at 72 h incubation, the lactose concentration was reduced to 3.7 $g.l^{-1}$; while lactic acid production has reached to 24.57 $g.l^{-1}$. About 91% conversion of lactose was obtained.

For medium contained 30 $g.l^{-1}$ lactose, at 60 h incubation, 17.83 $g.l^{-1}$ lactic acid produced. The final lactose concentration was 3.12 $g.l^{-1}$; that corresponds to 89% of lactose conversion. In a medium contained 20 $g.l^{-1}$ lactose, produced 12.4 $g.l^{-1}$ lactic acid at 48 h incubation and lactose concentration was decreased to 2.78 $g.l^{-1}$. It means that, about 86% of lactose was converted to lactic acid and other biological products. Finally for 10 $g.l^{-1}$ lactose concentration, lactic acid production and final lactose were 8.1 and 1.56 $g.l^{-1}$, respectively. The lactose conversion has reached to 84% at 38 h incubation time. Results showed that incubation time, lactic acid production and optical density were decreased as the initial concentration of lactose was decreased. The growth of microorganism is strongly depended on lactose concentration. Thus, in low lactose concentration, organism may reached to the end stage of fermentation that means to death phase earlier than medium contained high lactose concentration. The medium with 40 $g.l^{-1}$ had more productivity than media contained low lactose because of sufficient substrate are present for the bacterial growth and lactic acid production (Figure 2).

CONCLUSION

Batch fermentation of lactic acid from the treated whey permeate by *Lactobacillus bulgaricus* (ATCC 8001, PTCC 1332) was successfully carried out with several initial lactose concentrations. Consequently, whey could be attractive, alternative and cheap substrate replacing costly sugars for lactic acid fermentation. The lactic acid production and optical density of microorganism significantly increased with an increasing initial lactose concentration from 10 to 40 $g.l^{-1}$. Medium with 40 $g.l^{-1}$ lactose, had the maximum concentration of lactic acid (24.57 $g.l^{-1}$). The rate of lactose consumption and yield of productivity of lactic acid were 91% and 0.61 $g.g^{-1}$ lactose, respectively. The maximum rate of productivity was 0.314 $g.l^{-1}h^{-1}$. Therefore, high lactose concentration was a suitable medium with desired nutrients for the growth of *Lactobacillus* strain. High concentration of sugar in culture may inhibit the growth and cause adverse effect on lactic acid production. Therefore, minimization of supplementary nutrients for lactic acid production may be

desired. In other words, factories should focus on how to maximize the efficiency with desired medium condition. Lactose concentration is one of them that is reasonably necessary for bacterial growth and should be studied for the optimum lactose concentration with high lactic acid productivities without substrate inhibition.

ACKNOWLEDGMENTS

The authors would like to thank Gela Company for supplying whey and also special thanks to Biotechnology Research Lab. in Babol Noushirvani University of technology.

REFERENCES

1. Reddy, G., M. Altaf, B.J. Naveena, M. Venkateshwar and E. VijayKumar, 2008. Amylolytic bacterial lactic acid fermentation, Biotechnol. Advances, 26: 22-34.
2. Li, Z., L.U. Han, Y. Ji, X. Wang and T. Tan, 2010. Fermentative production of lactic acid from hydrolysate of wheat bran by *Lactobacillus rhamnosus*., Biochem. Eng., 49: 138-142.
3. Schepers, A.W., J. Thibault and C.H. Lacroix, 2006. Continuous lactic acid production in whey permeate/yeast extract medium with immobilized *Lactobacillus helveticus* in a two-stage process: Model and experiments, Enzyme Microbiol Technol., 38: 324-337.
4. Nancib, N., A. Nancib, A. Boudjelal and C. Benslimane, 2001. F. Blanchard, J. Boudrant, The effect of supplementation by different nitrogen sources on the production of lactic acid from date juice by *Lactobacillus casei* subsp. Rhamnosus, Bioresour. Technol., 78: 149-153.
5. Romani, A., R. Yanez, G. Garrote and J.L. Alonso, 2008. SSF production of lactic acid from Cellulosic biosludges. Bioresour. Technol., 99: 4247-4254.
6. Bonomo, M.G.A., T. Ricciardi, E. Zotta and G. Parente, 2008. Molecular and technological characterization of lactic acid bacteria from traditional fermented sausages of Basilicata region. Meat Sci., 80: 1238-1248.
7. Giraffa, G., N. Chanishvili and Y. Widyastuti, 2010. Importance of lactobacilli in food and feed biotechnology, Research Microbiol., 161: 480-487.
8. Gonzalez, M.I., S. Alvarez, F. Rieraa and R. Alvarez, 2007. Economic evaluation of an integrated process for lactic acid production from ultrafiltered whey, Food Eng., 80: 553-561.
9. Panesar, P.S., J.F. Kennedy, D.N. Gandhi and K. Bunko, 2007. Bioutilisation of whey for lactic acid production, Food Chem., 105: 1-14.
10. Roukas, T. and p. Kotzekidou, 1998. Lactic acid production from deproteinized whey by mixed cultures of free and coimmobilized *Lactobacillus casei* and *Lmtococcus zactis* cells using fedbatch culture, Enzyme Microbiol. Technol., 22: 199-204.
11. Siso, M., 1996. The biotechnological utilization of cheese whey: a review, Bioresour Technol., 57: 1-11.
12. Alonso, S., M. Herrer, M. Rendueles and M. Diaz, 2010. Residual yoghurt whey for lactic acid, production, Biomass and Bioenergy, 34: 931-938.
13. Nancib, A., N. Nancib, D. Meziane-Cherif, A. Boubendir, M. Fick and J. Boudrant, 2005. Joint effect of nitrogen sources and B vitamin supplementationof date juice on lactic acid productionby *Lactobacillus casei* subsp. rhamnosus, Bioresource Technology, 96: 63-67.
14. Ghasemi, M., G. Najafpour, M. Rahimnejad, P. Aeineh Beigi, M. Sedighi and B. Hashemiyeh, 2009. Effect of different media on production of lactic acid from whey by *Lactobacillus bulgaricus*. African Journal of Biotechnology, 8(1): 081-084.