Polish Journal of Chemical Technology, 18, 1, 58—63, 10.1515/pjct-2016-0010

A study on the effect of parameters on lactic acid production from whey

Hamidreza Ghafouri Taleghani[1], Ghasem D. Najafpour[1*], Ali Asghar Ghoreyshi[2]

[1]*University of Mazandaran, Faculty of Chemical Engineering, Babolsar, Iran*
[2]*Babol Noshirvani University of Technology, Biotechnology Research Lab, Faculty of Chemical Engineering, Babol, Iran*
[*]*Corresponding author: e-mail: najafpour8@nit.ac.ir*

In batch fermentation of whey, selection of suitable species at desired conditions such as substrate, product concentrations, temperature and inoculum size were investigated. Four *Lactobacillus* species and one *Lactococcus* species were screened for lactic acid production. Among them *L. bulgaricus* ATCC 11842 were selected for further studies. The optimal growth of the selected organism for variable size of inocula was examined. The results indicated that inoculum size had insignificant effect on the cell and lactic acid concentration. The effect of temperature was also studied at 32, 37, 42 and 47°C. Results showed that the concentration of cell dry weight increased with increment of temperature from 32 to 42°C. The maximum cell and lactic acid concentration was obtained at 42°C. The effect of initial substrate concentration on lactic acid production was also examined. The optimum initial lactose concentration was found to be 90 g/l.

Keywords: lactose, lactic acid, fermentation, *Lactobacillus bulgaricus,* whey.

INTRODUCTION

Lactic acid is the most widely occurring hydroxy carboxylic acid found in most living organisms[1]. Lactic acid is an important chemical used in variety of food-related industries; however its main potential use is an intermediate agent for manufacturing of the biodegradable polymer and poly lactic acid[2, 3].

There are two main routes for producing lactic acid; synthetic route through hydrolysis of lactonitrile and microbial route through fermentation of carbohydrates using lactic acid bacteria (LAB)[4]. Production of lactic acid by fermentation has received much attention in past decade as evidenced by a number of patents and scientific articles which has been published in recent years[5, 6].

Conversion of dairy waste streams into lactic acid is a process with several beneficial facts[7]. On one hand cheese waste poses an environmental threat and on the other hand it represents an abundant renewable raw material for production of several value-added products such as lactic acid[8].

There are several vital factors affecting the fermentative production of lactic acid[9]. The choice of substrate, LAB species and operating conditions are a few influential parameters which should be considered for lactic acid production.

Substrate for the lactic acid fermentation process can be chosen from a variety of carbon sources, such as glucose, xylose, sucrose and lactose[10–13]. The choice of substrate depends upon its availability, cost and pretreatment required for fermentation[14].

There are several species of bacteria that are capable to produce lactic acid from lactose such as *L. bulgaricus*[8], *L. plantarum*[15] and *L. casei*[16, 17].

Media temperature is one of the important factors that affects the growth of microorganism[18] Dong-Hoon</author><author>Lim, Wan-Taek</author><author>Lee, Mo-Kwon</author><author>Kim, Mi-Sun</author></authors></contributors><titles><title>Effect of temperature on continuous fermentative lactic acid (LA. Most species have a characteristic range of temperature, in which they can grow and propagate, but they do not grow at the same rate over the whole range of temperatures[19]. Microbial growth is governed by the rate of chemical reaction catalyzed by enzymes within the cell[20].

The lactic acid bacteria requires substrates with high nitrogen content and it has a particular demand for vitamins B[21]. The nitrogen sources required for the fermentation medium are supplied in the form of yeast extract, cotton oil, soy flour, tryptone and peptone[22].

In the current study, lactose hydrolysate from cheese whey was used as substrate for growth of LAB. This substrate supplied sufficient nutrients for some organisms. Four *Lactobacillus* species and one *Lactococcus* species were screened for lactic acid production; among them the desired species was selected for further studies. The influence of temperature, inoculum size, and initial substrate concentration on the cell growth and lactic acid production was investigated.

MATERIAL AND METHODS

Microorganism and medium

The *L. delbrueckii* ATCC 9649, *L. casei* ATCC 39392, *L. bulgaricus* ATCC 11842 and *Lactococcus lactis* ATCC 11454 were obtained from Iranian Research Organization Science and Technology (IROST). Man-Rogosa-Sharpe (MRS) medium was used for cultivation of bacteria. The MRS medium consists of yeast extract, 5 g; meat extract, 5 g; peptone, 10 g; K_2HPO_4, 2 g; diammonium citrate, 5 g; glucose, 20 g; sodium acetate, 2 g; $MgSO_4 \cdot 7H_2O$, 0.58 g; $MnSO_4 \cdot 4H_2O$, 0.2 g, in one liter medium. The media were sterilized at 121°C for 15 minutes before inoculation.

Substrate preparation

Sweet cheese whey was obtained from a dairy plant (Kalle, Mazandaran, Iran). The whey was first filtrated in order to separate the coagulated proteins. Then lactose in the presence of dilute acid was hydrolyzed to galactose and glucose (1 ml HCl in 100 ml whey). After 24 h, the whey was neutralized with 1M NaOH solution. The pH of pretreated whey was adjusted to 6.5. A 0.3% (w/v) yeast extract was added to the whey in all fermentation

experiments except when the effect of nitrogen source was examined. Deprotonated and hydrolyzed whey was prepared as suitable medium for fermentation. The initial lactic acid concentration in the cheese whey was less than 2 g/l.

Analytical methods

Substrates and products were analyzed by HPLC (Shimadzu-Japan) equipped with a Shim-pack CLC-ODS column. The column, maintained at 75°C, was eluted with 4 mM H_2SO_4 at a flow rate of 0.4 ml/min for 20 min. The retention time of lactic acid under these conditions was 18 min. The samples were centrifuged at 5000 rpm for 5 minutes and then filtered through 0.2 μm paper filter (Whatman). To obtain the desired peak height, 10 μl of clear sample solution was injected to HPLC.

Growth rates were monitored by measuring optical density (the absorbance at 620 nm) using a Unico 2100 spectrophotometer. The dry cell weight was measured using a pre-developed calibration curve.

Fermentation

The bacteria were cultured in 100 ml MRS medium in 250-ml Erlenmeyer flasks. The medium temperature was adjusted according to the proposed temperature for the optimum growth of the respective organism, i.e., 30°C for the *Lactococci* strains, 32°C for *L. casei* and 37°C for *L. delbrueckii.* For each strain, several parallel fermentations were carried out with different conditions to obtain the highest cell growth. The fermentations were carried out in vessels with 500 ml volume and controlled at the optimal growth temperature for the respective organism. The vessels were sealed with a rubber cap with ports to allow insertion of a sampling device. Stirring at approximately 180 rpm was applied using an incubator shaker.

RESULTS AND DISCUSSION

Microorganism

The growth of several LAB and their lactic acid production ability was investigated in some batch fermentation experiments. Four microorganisms, *L. delbrueckii*, *L. casei*, *L. delbrueckii* and *Lactococcus lactis* were used for the fermentative production of lactic acid from whey. These bacteria are very important for the dairy industry because of their use in milk fermentation to convert substrate to lactic acid for product preservation. Therefore, it is important to determine the performance of each species. Each experimental test was repeated in various conditions and the best results were reported.

Figure 1 shows the cell concentration in batch fermentation process using whey as carbon source. Results indicated that all strains were able to ferment lactose except *L. delbrueckii*. Figure 1 also shows that *L. bulgaricus* and *L. Casei* had the highest cell concentrations.

The exponential phase, defined as the time with maximal lactic acid production, was around 36 h and ended at about 48 h in all fermentations. There was a strong relationship between the cell concentration and substrate consumption.

Figure 2 demonstrates the utilization of lactose of whey as substrate. As it was expected, *L. bulgaricus* can utilize

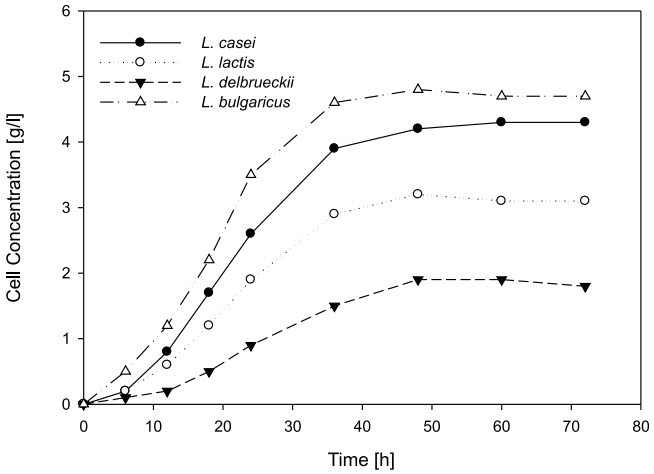

Figure 1. Cell growth profiles for the selected microorganisms

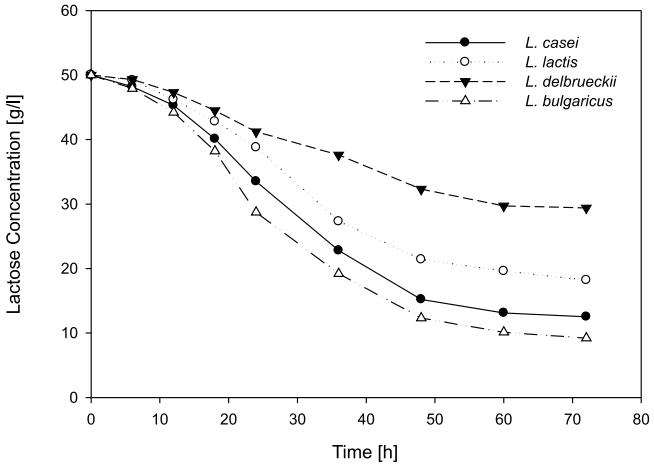

Figure 2. Utilization of lactose as substrate by the selected microorganisms

up to 80% of lactose. However, only 35% of lactose was consumed even after 72 hours by *L. delbrueckii.* The fermentations were considered completed when substrate was depleted or the lactic acid production was ceased.

The lactic acid production using different strains is illustrated in Figure 3. All of the four organisms produced lactic acid. *L. bulgaricus* exhibited the highest lactic acid concentration and *L. delbrueckii* produced the lowest amount of lactic acid. The fermentation with *L. delbrueckii* was stopped after 72 hours of incubation since small amount of lactic acid was produced. The maximum lactic acid concentration of 23.3 and 21.5 g/l

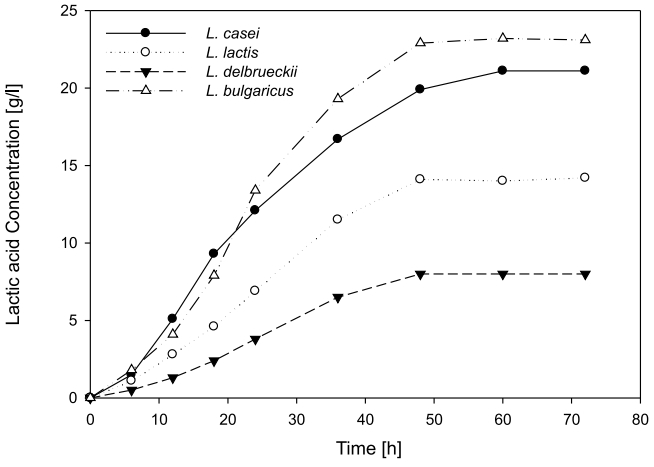

Figure 3. Lactic acid production by the selected organisms

was obtained with *L. bulgaricus* and *L. Casei*, respectively. The results proved that *L. bulgaricus* is the most potent strain for lactic acid production. Therefore, further investigations on lactic acid production were performed with this strain. The yield of lactic acid based on total sugar was 70–80% for three strains whereas the yield for *L. delbrueckii* was much lower than other species.

Growth kinetics

Kinetic models are being widely used for better understanding of the microorganism growth. Several growth-related kinetic models were tested in this study. However, among them logistic model was capable of predicting the growth curve of *Lactobacilli* bacteria and was well fitted with experimental data. Logistic model is a suitable model for prediction of lag, exponential and stationary phases of growth curve. The specific growth rate predicted by this model is presented by the following equation:

$$\frac{dx}{dt} = \mu_{max}\, x\left(1 - \frac{x}{x_{max}}\right) \tag{1}$$

Where μ_{max} is the maximum specific growth rate (h^{-1}) and x_{max} is the maximum cell dry weight concentration (g/l). This equation is known as the Riccati equation, which can be easily integrated to give the logistic equation:

$$x = \frac{x_0 \exp(\mu_{max}\, t)}{1 - \left(\frac{x_0}{x_{max}}\right)(1 - \exp(\mu_{max}\, t))} \tag{2}$$

Determination of growth kinetic parameters

Growth kinetics is typically categorized to either structured and unstructured models or segregated and non-segregated models[20]. In this study, the governing model was selected to be unstructured and non-segregated in logistic category. Data obtained in batch fermentation were used to determine the cell growth kinetic parameters. The kinetic parameters were calculated by non-linear least square analysis. MATHLAB software was applied to predict the logistic growth kinetic parameters for all cultures. The defined kinetic parameters are summarized in Table 1.

The high coefficient of determination (R^2) shows the high accuracy and capability of the model to interpret the experimental data. According to data presented in Table 3, *L. bulgaricus* has the highest specific growth rate of 0.18 h^{-1}.

Table 1. Kinetic parameters of logistic model for different bacteria at different temperatures

Bacteria	X_0 [g/l]	x_{max} [g/l]	μ_{max} [1/h]	R^2
L. bulgaricus	0.15	4.75	0.18	0.998
L. casei	0.11	4.27	0.17	0.997
L. lactic	0.08	3.12	0.15	0.996
L. delbrueckii	0.05	1.88	0.13	0.996

Effect of inoculum size

In industrial lactic acid fermentation process, the inoculum size range is usually between 3 to 10% (v/v) of the fermentation broth volume[23].

The assumption that inoculum size has insignificant effect on microbial growth once growth is initiated; however suitable size of inoculum would eliminate the probable occurrence of lag phase. Furthermore, the effect of inoculum size on *L. bulgaricus* has not been reported in any previous study. In this work, the growth of *L. bulgaricus* using inoculum sizes ranging between 1–15% was examined. The results indicated that inoculum size had no significant effect on the cell concentration as the size of inoculums exceeded certain percentages. The highest cell concentration of 4.6 g/l was obtained with inoculum size of 10% (see Fig. 4). In addition, inoculum size could have an effect on the duration of the lag phase. It was expected that duration of the lag phase decrease with an increase in inoculum size but when inoculum size was increased to above 5%, the lag phase decreased. A long lag phase is undesirable because it is time consuming and the medium is used to maintain a viable culture prior to the growth. Therefore, 5% inoculum was performed better than 10% because the lag phase for 10% inoculum was a little longer than 5%. Effect of inoculum size on lactic acid concentration is given in Table 2.

Table 2. Effect of inoculum size on lactic acid concentration

Inoculum size [%]	LA [g/l]	Yield [%]
1	12.3	43.1
5	23.1	66.3
10	24.6	72.7
15	20.5	62.5

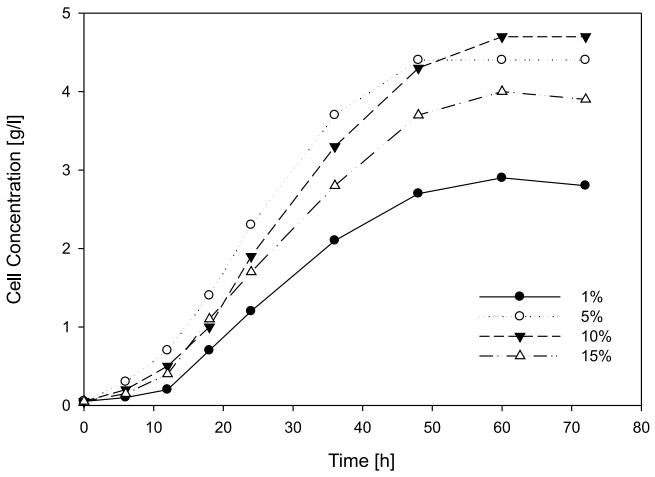

Figure 4. Effect of inoculum size on *L. bulgaricus* growth during lactic acid fermentation. Experimental conditions: initial lactose concentration: 50 g/l; pH: uncontrolled, temperature: 40°C and yeast extract: 0.3%

However, based on lactic acid yield data presented in the table, the maximum concentration of lactic acid of 24.6 g/l was obtained at inoculum size of 10%.

Effect of temperature

Temperature is one of the most important factors that influences the growth of microorganism but the effect of temperature on lactic acid production has only been studied in few reports[9]. *L. bulgaricus* is mesophilic bacterium which grows in temperature range of 30 to 50°C[24].

The effect of temperature on lactic acid fermentation was investigated at 32, 37, 42 and 47°C using whey with 50 g/l of lactose concentration, 0.3% of yeast extract and 5% of inoculum. The effect of temperature on bacterial growth is depicted in Figure 5.

The results indicated that the lag phase of bacterial growth at 32°C was longer than other temperatures, as

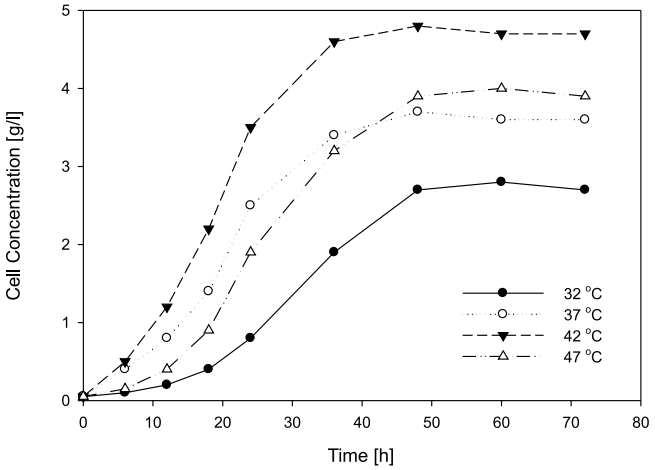

Figure 5. The effect of temperature on bacterial growth. Experimental conditions: initial lactose concentration: 50 g/l; pH: uncontrolled; inoculum: 5% and yeast extract: 0.3%

the bacteria needed to be adapted to the environment. The maximum concentration of cell dry weight increased with increase of temperature from 32 to 42°C. The maximum concentration of dry cell weight obtained at 42°C was 4.7 g/l. The exponential phase of the bacterial growth for 32, 37, 42 and 47°C was started at about 12, 4, 4 and 6 h, respectively.

Figure 6 shows the effect of temperature on lactose utilization during the fermentation process. It appears that the consumption of lactose at 42°C was faster than other temperatures. However, after 72 hours of incubation, the carbohydrate concentration profile shows the substrate is not fully utilized.

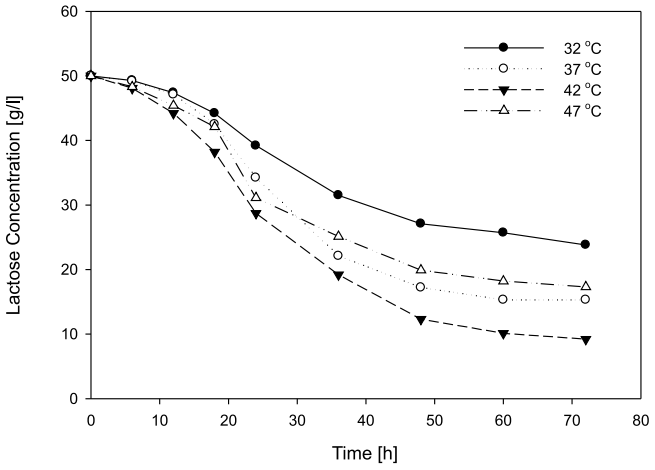

Figure 6. Effect of temperature on lactose consumption. Experimental conditions: initial lactose concentration: 50 g/l; pH: uncontrolled; inoculum: 5% and yeast extract: 0.3%

The effect of temperature on lactic acid production is given in Figure 7. The maximum concentration of lactic acid obtained at the temperature of 42°C was 24.3 g/l. It can be seen that when the temperature was increased to 42°C, the lactic acid production increased. Nevertheless, when the temperature was further increased to 47°C, the lactic acid concentration decreased to 17.5 g/l.

The results indicated that the suitable temperature for the production of lactic acid by *L. bulgaricus* was 42°C. According to Hofvendahl et al.[25], optimal growth

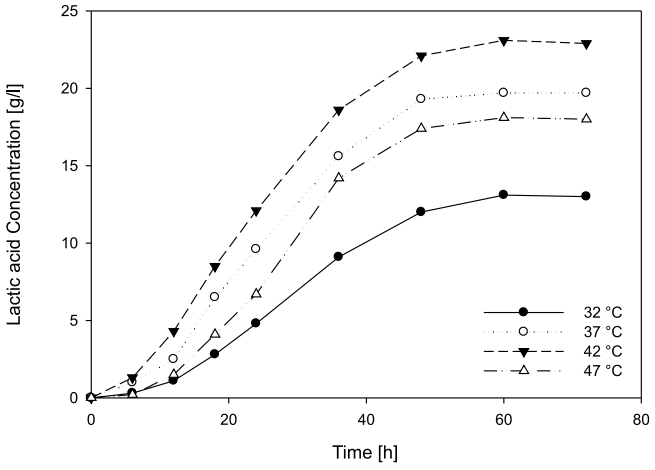

Figure 7. Effect of temperature on lactic acid production. Experimental conditions: initial lactose concentration: 50 g/l; pH: uncontrolled; inoculum: 5% and yeast extract: 0.3%

temperature for the *L. bulgaricus* was 45°C. Audet et al.[26] and Samuel et al.[27] reported that temperature of 42°C is the optimal growth temperature for *L. bulgaricus* While Mozzi et al.[28] reported that 37°C is the desired growth temperature for *L. bulgaricus*. These differences may be due to the diversity of substrate, product and operating conditions.

Effect of initial lactose concentration

The effects of initial lactose concentration on cell growth, lactic acid production and lactose utilization were investigated. Figure 8 shows the growth profile of *L. bulgaricus* in batch fermentation process using lactose of whey as carbon source. The range of lactose concentration was 30 to 120 g/l. The results indicate that the cell dry weight concentration was related to substrate concentration. High substrate and product concentrations may cause growth inhibition, as the microorganisms may be intoxicated in an undesired condition. With initial lactose concentration of 90 g/l, maximum dry cell weight of 5.0 g/l was obtained. When the substrate concentration was increased to 120 g/l, the concentration of biomass decreased to 3.2 g/l; this proved that substrate inhibition existed.

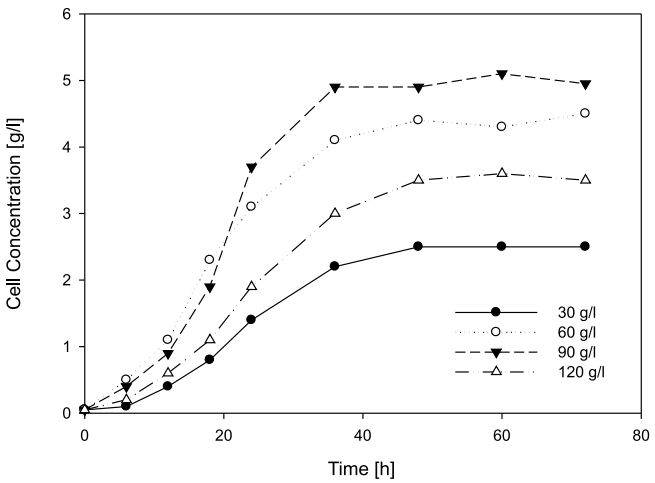

Figure 8. Effect of initial substrate concentration on cell concentration. Experimental conditions: temperature: 42°C; pH: uncontrolled; inoculum: 5% and yeast extract: 0.3%

The effect of initial lactose concentration on product formation is shown in Figure 9. The highest concentration of lactic acid which obtained at 90 g/l lactose was 32.1 g/l. When the concentration of lactose was increased, the lactic acid production decreased; that was due to inhibitions caused by the high substrate concentration. At high substrate concentration of 120 g/l, the concentration of lactic acid dropped to 14.9 g/l that was due to existing inhibition in batch process.

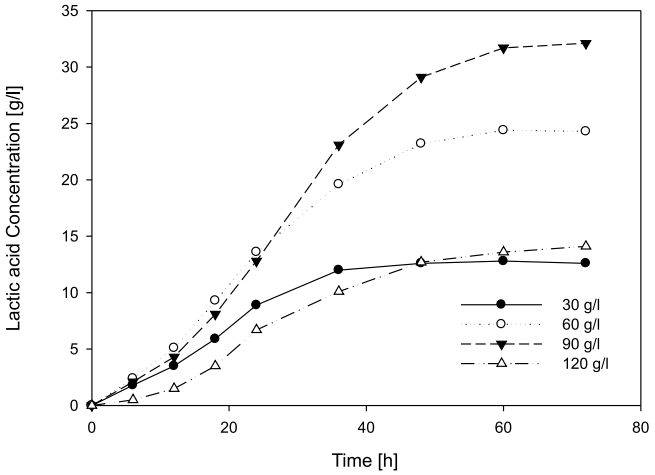

Figure 9. Effect of initial substrate concentration on lactic acid production. Experimental conditions: temperature: 42°C; pH: uncontrolled; inoculum: 5% and yeast extract: 0.3%

In comparison to similar works conducted by other investigators, the reported concentration of lactic acid at controlled pH was slightly higher than the values obtained in present work[29, 30]. When pH was uncontrolled, the pH dropped to values lowers than 3. This condition was unfavorable for the growth activity of the fermentative lactic acid bacteria and the microorganisms were unable to fully utilize the substrate.

Figure 10 illustrates the utilization of various concentrations of lactose as substrate. It appears that the consumption of lactose decreased when the initial lactose concentration was increased. In the course of fermentation, with initial lactose concentration of 30 g/l, 87% of the substrate was consumed. At initial lactose concentration of 120 g/l, consumption of lactose dropped to 50%.

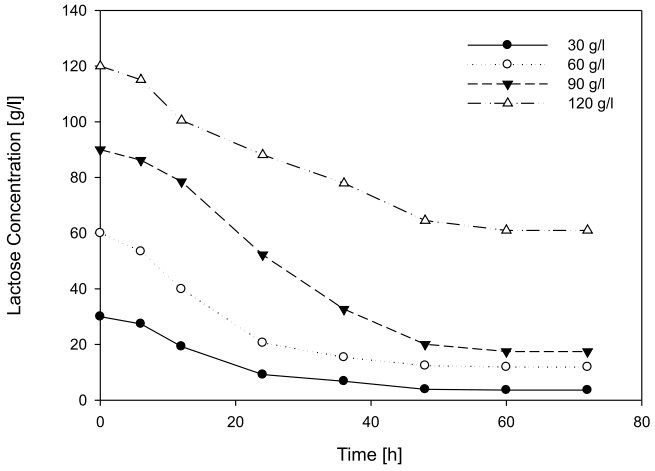

Figure 10. Effect of initial substrate concentration on lactose utilization. Experimental conditions: temperature: 42°C; pH: uncontrolled; inoculum: 5% and yeast extract: 0.3%

Effect of nitrogen sources

The effect of nitrogen source on lactic acid production by *L. bugaricus* was investigated using whey as substrate, at the temperature of 42°C and inoculum size of 5%. The results are summarized in Table 3. The results showed that lactic acid production increased with increasing the concentration of supplements, specially yeast extract (Y.E.). The highest production rate was found with addition of 1% of yeast extract. However, the addition of yeast extract during large scale fermentation is unlikely due to the extra cost imposed to the fermentation process.

Table 3. Effect of nitrogen source, type and concentration on lactic acid production

Nitrogen source [%]	Cell [g/l]	LA [g/l]	Yield [%]
M.E. 0.3	2.5	11.2	38.8
Peptone 0.3	2.8	13.4	45.9
Y.E. 0.1	3.2	16.1	49.2
Y.E. 0.3	4.7	24.3	71.1
Y.E . 1	5.2	29.5	79.5
Y.E. 0.1 + peptone 0.1	3.5	17.9	51.3
Y.E. 0.3 + peptone 0.3	4.9	26.2	73.4

The yeast extract exhibited the highest yield of lactic acid, followed by meat extract (M.E.) and peptone. Therefore, yeast extract is the best nitrogen source for lactic acid production using *L. bulgaricus.* The use of yeast extract as nitrogen source not only increased the bacterial growth but also reduced the time required for the completion of fermentation. This could be due to the nutritional value of yeast extract which contains substances such as amino acid, peptides, vitamins, and several organic acids including pyruvic and glyseric acid which are needed for the *L. bulgaricus* growth.

The maximum obtained cell concentration was 5.2 g/l which was achieved by 1% yeast extract. The results also indicated that yeast extract alone at high concentration gave higher lactic acid production than yeast extract and peptone at low percentages. Furthermore, addition of peptone to yeast extract had little effect on the production of lactic acid.

CONCLUSIONS

The cheese whey waste from dairy industry was used as carbon source for lactic acid production. The different bacteria were tested for their growth behavior and lactic acid production ability. *L. bulgaricus* had high performance and then it was selected for further studies. The effects of some parameters such as temperature, inoculum size, substrate concentration and nitrogen source were studied. The optimal values of tested variables for maximal lactic acid production were found to be: inoculum size of 5%, temperature of 42°C, yeast extract of 1% and initial lactose concentration of 90 g/l.

ACKNOWLEDGMENT

The authors are thankful to Biotechnology Research Center, Noshirvani University of Technology, Babol, Iran, for the facilities provided to accomplish the present research.

LITERATURE CITED

1. Najafpour, G., Hashemiyeh, B., Asadi, M. & Ghasemi, M. (2008). Biological treatment of dairy wastewater in an upflow anaerobic sludge-fixed film bioreactor. *Am. Eurasian J. Agric. Environ. Sci.* 4(2), 251–257.

2. Wee, Y., Kim, J. & Ryu, H. (2006). Biotechnological production of lactic acid and its recent applications. *Food Technol. Biotechnol.* 44(2), 163.

3. Tango, M. & Ghaly, A. (2002). A continuous lactic acid production system using an immobilized packed bed of *Lactobacillus helveticus*. *Appl. Microbiol. Biotechnol.* 58(6), 712–720, DOI: 10.1007/s00253-002-0970-3.

4. Vodnar, D.C., Venus, J., Schneider, R. & Socaciu, C. (2010). Lactic acid production by *Lactobacillus paracasei* 168 in discontinuous fermentation using lucerne green juice as nutrient substitute. *Chem. Eng. Technol.* 33(3), 468–474. DOI: 10.1002/ceat.200900463.

5. John, R.P., Nampoothiri, K.M. & Pandey, A. (2007). Fermentative production of lactic acid from biomass: an overview on process developments and future perspectives. *Appl. Microbiol. Biotechnol.* 74(3), 524–534. DOI:10.1007/s00253-006-0779-6.

6. Abdel-Rahman, M.A., Tashiro, Y. & Sonomoto, K. (2013). Recent advances in lactic acid production by microbial fermentation processes. *Biotechnol. Adv.* 31(6), 877-902. DOI: 10.1016/j.biotechadv.2013.04.0027.

7. Panesar P.S., Kennedy J.F., Gandhi D.N. & Bunko K. (2007). Bioutilisation of whey for lactic acid production. *Food Chem.* 105(1), 1–14. DOI: 10.1016/j.foodchem.2007.03.035.

8. Fakhravar, S., Najafpour, G., Heris, S.Z., Izadi, M. & Fakhravar, A. (2012). Fermentative Lactic Acid from Deproteinized Whey Using *Lactobacillus bulgaricus* in Batch Culture. *World Appl. Sci. J.* 17(9), 1083–1086.

9. Hofvendahl, K. & Hahn–Hägerdal, B. (2000). Factors affecting the fermentative lactic acid production from renewable resources. *Enzyme Microb. Technol.* 26(2), 87–107. DOI: 10.1016/S0141-0229(99)00155-6.

10. Guo, Y., Yan, Q., Jiang, Z., Teng, C. & Wang, X. (2010). Efficient production of lactic acid from sucrose and corncob hydrolysate by a newly isolated Rhizopus oryzae GY18. *J. Ind. Microbiol. Biotechnol.* 37(11), 1137–1143. DOI: 10.1007/s10295-010-0761-2.

11. Pagana, I., Morawicki, R. & Hager, T.J. (2014). Lactic acid production using waste generated from sweet potato processing. *Int. J. Food Sci. Tech.* 49(2), 641–649. DOI: 10.1111/ijfs.12347.

12. Saito, K., Hasa, Y. & Abe, H. (2012). Production of lactic acid from xylose and wheat straw by *Rhizopus oryzae*. *J. Biosci. Bioeng.* 114(2), 166–169. DOI: 10.1016/j.jbiosc.2012.03.007.

13. Tay, A. & Yang, S.T. (2002). Production of L (+) lactic acid from glucose and starch by immobilized cells of *Rhizopus oryzae* in a rotating fibrous bed bioreactor. *Biotechnol. Bioeng.* 80(1), 1–12. DOI: 10.1002/bit.10340.

14. Yen, H.,W. & Kang, J.L. (2010). Lactic acid production directly from starch in a starch-controlled fed-batch operation using *Lactobacillus amylophilus*. *Bioprocess Biosystems Eng.* 33(9), 1017–1023. DOI: 10.1007/s00449-010-0426-6.

15. Fu, W. & Mathews, A.P. (1999). Lactic acid production from lactose by *Lactobacillus plantarum*: kinetic model and effects of pH, substrate, and oxygen. *Biochem. Eng. J.* 3(3), 163–170. DOI: 10.1016/S1369-703X(99)00014-5.

16. Khiralla, G., Rasmy, N., El-Malky, W. & Ibrahim, M. (2009). The role of fermented soymilk with potential probiotic properties in the treatment of diarrhea in young rats. *Pak. J. Biotech.* 6(1/2), 89–100.

17. Korbekandi, H., Abedi, D., Jalali, M., Fazeli, M.R. & Heidari, M. (2007). Optimization of *Lactobacillus casei* growth and lactic acid production in batch culture. *J. Biotechnol.* 131(2 Suppl.), 182–183. DOI: 10.1016/j.jbiotec.2007.07.923.

18. Kim, D.H., Lim, W.T., Lee, M.K. & Kim, M.S. (2012). Effect of temperature on continuous fermentative lactic acid (LA) production and bacterial community, and development of LA-producing UASB reactor. *Bioresour. Technol.* 119, 355–361. DOI: 10.1016/j.biortech.2012.05.027.

19. Tango, M. & Ghaly, A. (1999). Effect of temperature on lactic acid production from cheese whey using *Lactobacillus helveticus* under batch conditions. *Biomass Bioenergy.* 16(1), 61–78. DOI: 10.1016/S0961-9534(98)00062-2.

20. Najafpour, G. (2006). *Biochemical engineering and biotechnology*. Elsevier.

21. Liu, B., Yang, M., Qi, B., Chen, X., Su, Z. & Wan, Y. (2010). Optimizing l-(+)-lactic acid production by thermophile Lactobacillus plantarum As.1.3 using alternative nitrogen sources with response surface method. *Biochem. Eng. J.* 52(2–3), 212–219. DOI: 10.1016/j.bej.2010.08.013.

22. Hujanen, M. & Linko, Y.Y. (1996). Effect of temperature and various nitrogen sources on L (+)-lactic acid production by *Lactobacillus casei*. *Appl. Microbiol. Biotechnol.* 45(3), 307–313. DOI: 10.1007/s00253005068823.

23. Clark, D.S. & Blanch, H.W. (1997). *Biochemical engineering*. New York: CRC Press.

24. Taylor, J. (2001). *Microorganisms and Biotechnology*. United Kingdom: Nelson Thornes.

25. Hofvendahl, K. & Hagerdal, B.H. (1997). L. Lactic Acid Production from Whole Wheat Flour Hydrolysate Using Strain of *Lactobacilli* and *Lactococci*. *Enzyme Microbiol. Technol.* 20(3), 303–307. DOI: 10.1016/S0141-0229(99)00155-6.

26. Audet, P., Paquin, C. & Lacroix, C. (1989). Sugar utilization and acid production by free and entrapped cells of *Streptococcus salivarius* subsp. *thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Lactococcus lactis* subsp. *lactis* in a whey permeate medium. *Appl. Environ. Microbiol.* 55(1), 185–189.

27. Samuel, W.A., Lee, Y. & Anthony, W. (1980). Lactic acid fermentation of crude sorghum extract. *Biotechnol. Bioeng.* 22(4), 757–777. DOI: 10.1002/bit.260220404.

28. Mozzi, F., Oliver, G., de Giori, G.S. & de Valdez, G.F. (1995). Influence of temperature on the production of exopolysaccharides by thermophilic lactic acid bacteria. *Milchwissenschaft* 50(2), 80–82.

29. Büyükkileci, A.O. & Harsa, S. (2004). Batch production of L (+) lactic acid from whey by *Lactobacillus casei* (NRRL B–441). *J. Chem. Technol. Biotechnol.* 79(9), 1036–1040. DOI: 10.1002/jctb.1094.

30. Chiarini, L., Mara, L. & Tabacchioni, S. (1992). Influence of growth supplements on lactic acid production in whey ultrafiltrate by *Lactobacillus helveticus*. *Appl. Microbiol. Biotechnol.* 36(4), 461–464.