Effect of Na-alginate and bead diameter on lactic acid production from pineapple waste using immobilized *Lactobacillus delbrueckii* ATCC 9646

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ABSTRACT

Batch fermentation of glucose to lactic acid was performed using immobilized *Lactobacillus delbrueckii subsp. Delbrueckii ATCC 9646* under anaerobic condition for 72 hours. Liquid pineapple waste medium was used as the fermentation medium and the effects of Na-alginate concentration and bead diameter on cell growth, glucose consumption and lactic acid production were investigated. The results indicate that Na-alginate and bead diameter have a significant effect on the lactic acid production. The maximum concentrations of lactic acid produced at 56 hours of fermentation were 29.39 gL^{-1} at 2.0% Na-alginate concentration and 30.27 gL^{-1} for 1.0mm bead diameter with initial glucose concentration of 31.3 g/L. Thus, it is observed that the conversion yields for lactic acid production are 93.8% and 96.7% at 2.0% Na-alginate and 1.0mm of bead diameter respectively.

1. INTRODUCTION

Application of immobilized living cells is a new and rapidly growing area in biotechnology. Cell immobilization can improve production rates of lactic acid while reducing medium requirements and inhibitions. Entrapment in calcium alginate bead is the most widely used procedure for immobilization [1]. Prasad and Mishra (1995) immobilized *Saccharomyces cerevisiae* in calcium alginate matrix, Mohamed et al. (2000) entrapped cells of *Bacillus amyloliquefaciens* in calcium alginate and Gough et al. (1998) immobilized *Kluyveromyces marxianus* in a calcium alginate matrix. The mild condition for immobilization and its simplicity are some of the reasons calcium alginate was chosen as the immobilization matrix [2-4]. In this study, *Lactobacillus delbrueckii* strain has been entrapped in the bead matrix through which substrates and products diffuse in and out easily. Stability of the beads is important to maintain high conversion of substrate to product. The concentration of sodium alginate and bead diameter were found to have a pronounced effect on the stability of the bead, which will effect lactic acid production [5].

Several authors have studied the effect of Na-alginate concentration on lactic acid production by immobilized organisms. Bead diameter is another factor that affects the lactic acid fermentation using immobilized *Lactobacillus delbrueckii.* Goksungur and Guvenc (1999) in his early work with beet molasses used various Ca-alginate bead sizes ranking from 1.3 to 3.2 mm diameter. It was reported that the highest lactic acid production was obtained with cell entrapped in the 1.3 to 1.7mm Ca-alginate bead [6]. Abdel-Naby et al. (1992) reported maximum lactic acid was produced with cell entrapped in 2.0mm Ca-alginate bead.

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When the bead diameter is increased beyond 3.0mm, the production of lactic acid is increased.

Goksungur and Guvenc (1999) reported that maximum lactic acid production, 5.93% was obtained with beads prepared at 2.0% w/v of Na-alginate concentration. Abdel-Naby et al. (1992) investigated lactic acid production using Ca-alginate immobilized beads and found that maximum lactic acid produced with beads containing 3.0% Ca-alginate concentration and obtained lower yields with beads made of 4.0 and 6.0% Ca-alginate due to diffusion problems [7]. Although there has been much work on lactic acid, none of them have used pineapple waste as the substrate for fermentation using the immobilization technique.

Since, the pineapple canning industry is one of the many food industries producing large quantities of solid and liquid wastes and due to the stringent environmental regulations regarding to waste disposal a special interest has developed in using the pineapple waste, which is rich in nutrients such as glucose. Thus the ability to utilize these liquid effluent into useful by products such as lactic acid will help reduce or eliminate sources of pollution [1].

2. MATERIAL AND METHODS

2.1 Immobilization cell

L. delbrueckii cells grown in 25ml MRS broth were mixed with an equal volume (1:1 v/v) of Na-alginate solution and were stirred for 5 minutes. The mixed solution obtained was then placed in a syringe and allowed to drop into a sterile 0.2M $CaCl_2$ solution that was stirred continuously. Alginate drops solidified upon contact with $CaCl_2$, forming beads and thus entrapping bacteria cells. The beads were allowed to harden for 30 minutes at 37°C and then washed with sterile saline solution to remove excess calcium ions and untrapped cells.

2.2 Fermentation conditions

2.2.1 Effect of Na-alginate concentration and bead diameter

The submerged fermentations were carried out in 250 ml Erlenmeyer flasks containing 100 ml of pineapple waste with 31.3 gL^{-1} of glucose concentration. Flushing the flasks with nitrogen and sealing them with tight fitting rubber stoppers maintained anaerobic conditions. The fermentation flasks were placed in a incubator shaker with an agitation rate of 150 rpm.

i) The effect of Na-alginate concentration on fermentation was conducted at various concentrations ranging from 1.0%, 2.0%, 4.0%, 6.0% and 8.0% for 72 hours. Initial pH of the fermentation medium was 6.5; with 1.0 mm bead diameter at 37°C.

ii) The effect of bead diameter was studied for various bead diameter 1.0, 3.0 and 5.0mm. These flasks were incubated at 37°C and 5 g bead.

3. RESULTS AND DISCUSSION

Lactic acid bacteria were immobilized in Ca-alginate beads prepared from different concentration of Na-alginate (1.0%, 2.0%, 4.0%, 6.0% and 8.0%) and their fermentation efficiency ware investigated in liquid pineapple waste containing 31.3 gL^{-1} of glucose initially. Fig. 1 shows the growth pattern for the five concentrations of sodium alginate. The lag phase of bacterial growth for 1, 4, 6 and 8% Na-alginate concentration are longer; 24 hr compared to the 2% Na-alginate concentration, which is only 8 hr. Increasing the Na-alginate concentration above 2% only prolong the lag phase and the bacteria does not exhibit improved growth.

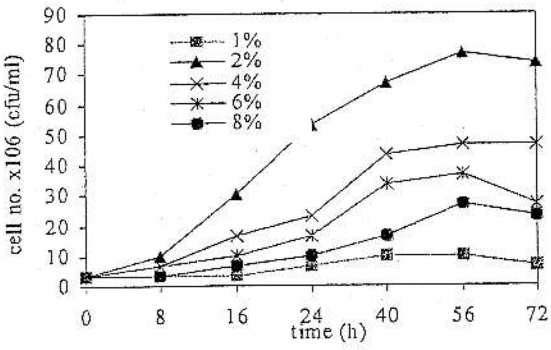

Fig.1. Effect of sodium alginate concentration on cell concentration

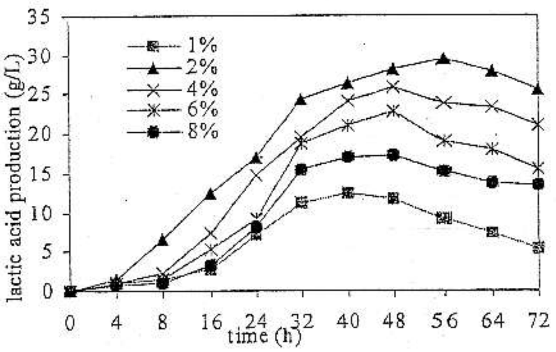

Fig. 2. Effect of sodium alginate concentration on lactic acid production

This longer lag phase could be due to the bacteria requiring to adapt with their environment. The exponential growth can be seen in all the flasks accept for the flask containing 1.0% of Na-alginate concentration. The 2.0% Na-alginate conc. produces more cell number compared to other samples. The exponential phase begins after 8 hours and the cell grows gradually until 56 hr where the death phase begins. Thus, the presence of only 2.0% Na-alginate concentration in the calcium alginate beads creates the optimum condition for *L. delbrueckii.*

The effect of Na-alginate concentration on the lactic acid production is depicted in Fig.2. The highest lactic acid production is obtained for the 2.0% of Na-alginate concentration with a yield of 29.39 gL^{-1}. Increasing the Na-alginate concentration above 2.0%, decrease the lactic acid production due to the lower diffusion efficiency of the beads. However when only 1.0% of Na-alginate concentration is used, the beads were disrupted in the medium at the end of fermentation.

Fig.3 showed the growth pattern for three different sizes of bead diameter. The 1.0mm bead produced more cell number (73.3 x 10^6 $cfuml^{-1}$) compared to the 3.0 mm (50.0 x 10^6 $cfuml^{-1}$) and 5 0mm (26.7 x 10^6 $cfuml^{-1}$) beads. The lag phase of bacterial growth for 3.0mm and 5.0mm are longer than 1mm bead diameter. The 1mm bead diameter went into exponential phase growth at the 8^{th} hr until 24^{th} hr before the stationary phase started. The high cell growth promotes lactic acid production, which started at about the same time. Different patterns were observed for the 3.0mm and 5.0mm beads, where the exponential growth started only after from 16^{th} hr. The number of cells produced for bigger beads were less compared to the 1mm bead. Thus, when the bead diameter is increased to 3.0mm, the bacteria grew even more slowly producing less lactic acid.

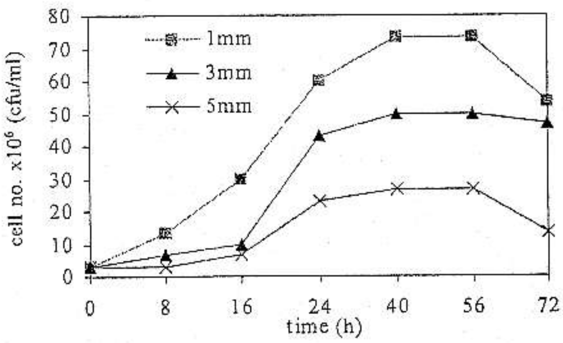

Fig.3. Effect of bead diameter on cell concentration

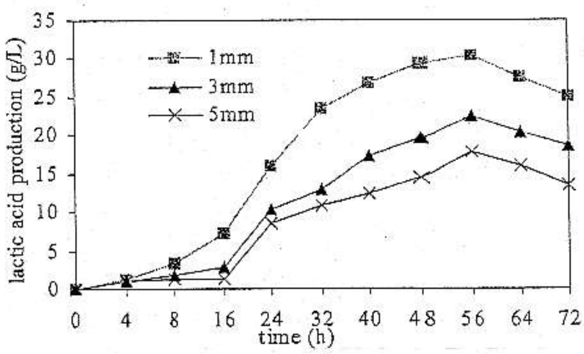

Fig.4. Effect of bead diameter on lactic acid production

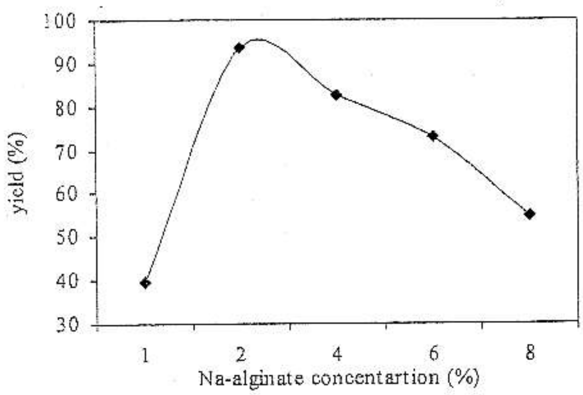

Fig.5.Effect of Na-alginate on lactic acid yield

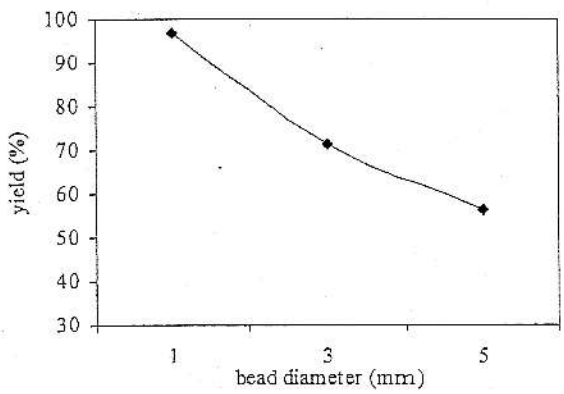

Fig.6.Effect of bead diameter on lactic acid yield

A similar trend is also observed for the production of lactic acid in Fig.4. Maximum lactic acid concentration is attained for the 1mm bead diameter with a yield of 30.27 gL^{-1}. A further increase in the bead diameter to 5mm results in a decrease of lactic acid production to 17.65 gL^{-1}. Abdel-Naby et al. (1992) had studied the effect of bead diameter for lactic acid production and found the optimum lactic acid yield was obtained using a 2mm bead diameter. Lactic acid production increased as bead diameter continues to decrease.

Fig. 5 shows the pattern of lactic acid yield during the fermentation process at various Na-alginate concentrations. The results show the highest yield of lactic acid was obtained when 2.0% of Na-alginate concentration was used in lactic acid fermentation process. Increasing Na-alginate concentration beyond this value do not result in any increase of lactic acid yield. These results seem to be in agreement with those obtained by Goksungur and Guvenc [6] where optimum Na-alginate concentration is 2.0%. Too low Na-alginate concentration results in very soft beads whilst increased Na-alginate to above 2.0% hardens the beads, thus causing diffusion problems to occur. At high Na-alginate concentration, the bacteria do not get enough nutrients (food) as the substrate has difficulty in diffusing through the beads. However when only 1.0% Na-alginate concentration is used, the beads which are too soft as mentioned earlier are easily broken because their mechanical strength are lower and the bacteria leaks out from the beads [8].

Effect of bead diameter on lactic acid yield is clearly revealed in Fig.6. The optimum bead diameter for the fermentation of lactic acid for cell entrapped in Ca-alginate is 1.0mm with a yield of 30.27 gL^{-1} and 96.7%. Increasing bead diameter beyond this value did not improve lactic acid production. Smaller bead diameter yields more lactic acid production, due to an increase in the surface volume ratio [9]. A further increase in bead diameter to 5.0mm results in a decrease of lactic acid production to 17.65 gL^{-1} or 50.7%.

REFERENCES

[1] I. Ani, W. Suzana and H.B. Mat, *Water and Environmental Management Series*, (2003).
[2] B. Prasad and I.M. Mishra, *J. of Bioresource Technology*, **53**, (1995).
[3] S.Gough, N. Barron, V.I. Lozinsky and A.L. Zulbov, *J. of Bioprocess Engineering*, **19**, *(1998)*.
[4] A. Mohamed, M. Reyad and F. abdel-Fattah, J. of Biochemical Engineering, 5, (2000).
[5] J. Rajagopalan., S.T. Pillutla, V. Sonal, *J. of Fermentation and Bioengineering*, 73,4 (1992).
[6] Y. Goksungur and U. Guvenc, *J. of Chem. Technol. and Biotechnol.*, **74**, (1999).
[7] M. Abdel-Naby, K. Mok, and C. Lee, *UNIDO Proceedings*, (1992).
[8] P. Boyaval and J. Goulet, *J. Enzyme Microbiol Technol.*, **10**, (1988).
[9] D. Guoqiang, R. Kaul and B. Mattiasson, *Appl. Microbiol. Biotechnol.*, **36**, (1991).