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Determination of Critical Point of pO_2 Level in the Production of Lactic Acid by *Lactobacillus rhamnosus*

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Abstract: The study was conducted to determine the critical point of pO_2 level in the production of lactic acid by *Lactobacillus rhamnosus*. The fermentation process was successfully carried out in laboratory scale fermenter/bioreactor using different pO_2 level (the main parameter that significantly affects the growth of *L. rhamnosus* and lactic acid production) together with two other parameters; the agitation rate and pH. From the result, it was observed that the best production of lactic acid with the concentration of 16.85 g L^{-1} or 1.68% production yield has been obtained at the operating parameters of 5% pO_2 level, agitation speed of 100 rpm and sample pH 6. The critical point of pO_2 was found to be between 5 and 10%.

Key words: Lactic acid, Lactobacillus, fermentation, critical pO_2, laboratory scale fermenter

INTRODUCTION

Lactic acid is an organic acid and it is being used in many kind of industries such as pharmaceutical, chemical, cosmetic and food industries (Wang *et al*., 1995). Traditionally, it has been employed in food industry as preservative, pH regulator and taste enhancing additive (Hujanen *et al*., 2001). However, its most important applications are as a preservative and acidulant in foods, prosthetic device which controls the delivery of drugs in pharmaceutical agents, precursor for production of polymers like polylactic acid and as moisture agents in cosmetic (Mirdamadi *et al*., 2002). Recently, effort is being enforced in its new applications; the degradable plastics made from polylactic acid which demand great amount of lactic acid. This will greatly expand the market of lactic acid so that the more economical process of lactic acid production must be developed (Nancib *et al*., 2001).

Lactic acid bacteria which are the facultative anaerobe or microaerophilic are poorly grown in the presence of oxygen. It grows best when the concentration of oxygen is lower than it is in the air (SigLeton, 1995). Different from the normal bacteria which are normally grown well in the presence of oxygen; these bacteria still can keep growing even under the anaerobic condition. But, for all organisms, including the obligate aerobes, high concentration of oxygen may caused toxicity for them (Teuber, 1993). Thus, it is very important to control the partial pressure of the oxygen (pO_2) in keeping them to grow significantly.

Apart of it, there are many other fermentation parameters that also significantly affect the growth and metabolic production of lactic acid such as temperature, pH, agitation speed, etc. In the present study, the effect of pO_2 together with the agitation speed and pH was study in order to determine the critical value of pO_2 in enhancing the growth of the bacteria. Nevertheless, the growth kinetic study of the microbial cultures can be used to estimate the cost effective production of lactic acid in large scale (Yankov *et al*., 2004).

MATERIALS AND METHODS

Inoculum Preparation and Bioreactor Experimental Design: *Lactobacillus rhamnosus*, a homo fermentative producer of lactic acid was used in this study. The inoculums was prepared by inoculating a single colony of bacteria into 10 mL broth media and incubated at 37°C for 24 h. One milliliter of inoculum was transferred into bijou bottle containing 9 mL media. Cultures were incubated for 10 h at 37°C before being transferred into 250 mL inoculum flask containing 100 mL media.

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Table 1: Operational condition in bioreactor fermentation

	F1	F2	F3
Parameters	RPM	pH	pO_2 (%)
Run 1	100	6	5
Run 2	100	6	10
Run 3	100	6	15
Run 4	100	6	20

Fermentation processes was carried out based on a sing Le factor design where the effect of pH was initially determined. It was then followed by the determination of the effect of pO_2 and agitation on the lactic acid production (Table 1).

Fermentation: A 2 L Stirred Tank Bioreactor (STBR), B-Braun fermenter, with batch mode of operation, was operated at various values of dissolved oxygen's partial pressure (pO_2) at fixed stirrer speed of 100 rpm. The culture temperature was controlled at 37°C. The medium composition are as follows: Peptone, 10.0 g L^{-1}; Yeast extract, 4.0 g L^{-1}; glucose, 9.8 g L^{-1}; Lactose, 20.0 g L^{-1}; Tween 80, 1.0 mL; Potassium phosphate (K_2HPO_4), 2.0 g L^{-1}; Sodium acetate, 5.0 g L^{-1}; Ammonium sulfate $(NH_3)_2SO_4$), 2.0 g L^{-1}; Magnesium sulfate ($MgSO_4$), 0.2 g L^{-1} and Manganese sulfate ($MnSO_4$), 0.05 g L^{-1}. Culture media were sterilized at 121°C for 20 min. The culture pH was adjusted to pH 6 by adding 2 M NaOH. The partial pressure of oxygen (pO_2) was adjusted by cascade airflow with the maximum supply of air at about 3 L per minute. The pO_2, rpm airflow rate and pH levels were monitored during the 48 h of fermentation process.

Sampling and analytical methods: Three milliliter sample was withdrawn at every 2 h interval into a bijou bottle for the measurement of the optical density (OD_{660nm}), total cell number, cell dry weight, glucose and lactate concentration. For glucose and lactate analysis, 1 mL sample was withdrawn into a 1.5 mL centrifuge tube and centrifuged at 3000 rpm for 10 min before undergoing analysis using YSI biochemistry analyzer. Optical density analysis (OD), Total Cell Number (TCN) and Cell Dried Weight (CDW) were analyzed as described previously (Karim *et al*., 2005).

RESULT AND DISCUSSION

As shown in Fig. 1, Run 1 had slightly higher cell concentration compared to the other three Runs. Growth rate during the exponential phase for all Runs were seen to be similar where the cells concentration was rapidly increased from 2 h until 6 h. The highest cell concentration was obtained at Run 1 and Run 2 with the concentration of 23 and 22 mg L^{-1} cells, respectively. This

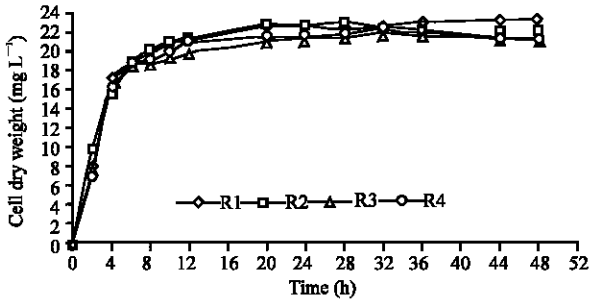

Fig. 1: The cell concentration between four Runs

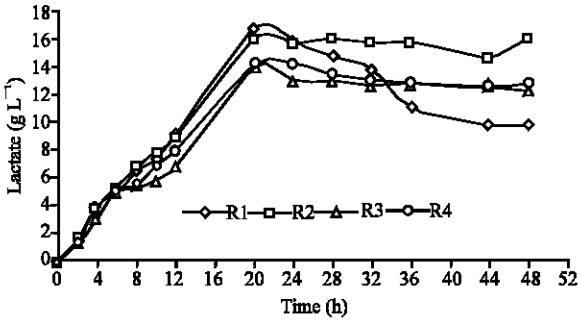

Fig. 2: Lactate production at four Runs

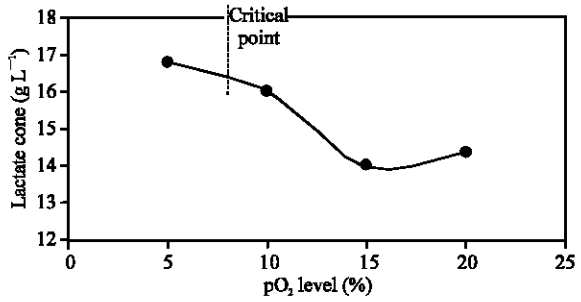

Fig. 3: Critical point of pO_2 level for *Lactobacillus rhamnossus*

indicated that *Lactobacillus rhamnosus* cells grow faster in the environment with acidic condition (pH 6), agitation rate of 100 rpm and pO_2 level between 5 and 10%. The cells then kept growing slowly till reached the stationary phase at 8 h. In Run 3 and 4 where the operation conditions were set with pO_2 level at 15 and 20%, respectively, adaptation of the cells in this environment was a little bit slow but due to the suitable pH condition (acidic), the cells had also grown well. This finding was inline with the finding of Roehr (1996) who had found that *Lactobacillus spp* bacteria were well grown in the acidic condition with pH 6 and low pO_2 level between 5 and 10%.

From Fig. 2, it is observed that, after 20 h of fermentation, Run 1 had produced the highest concentration of lactic acid (16.8 g L^{-1}) followed by Run

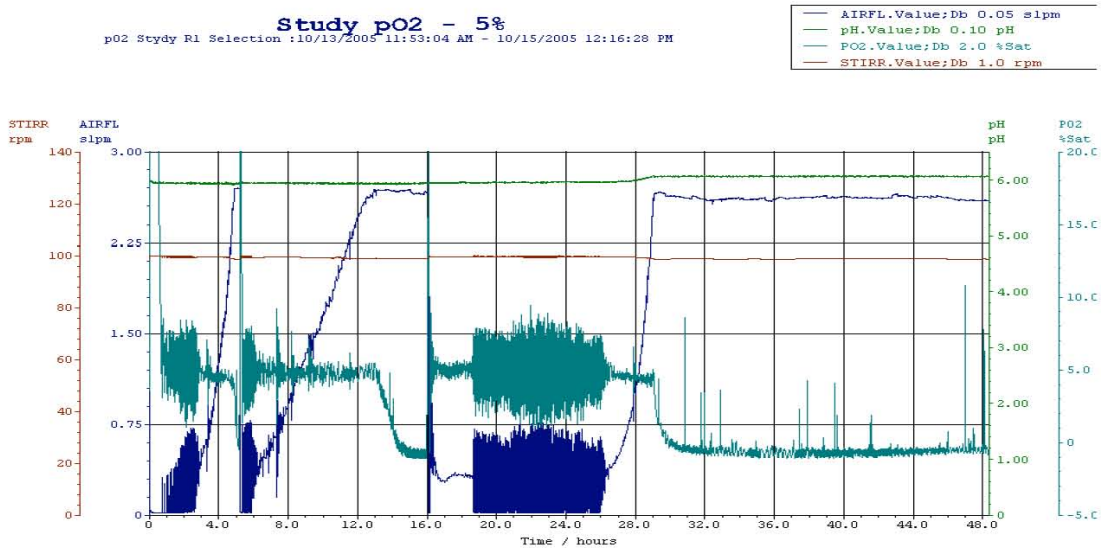

Fig. 4: On-line monitoring of pO_2 level for Run 1

2, 4 and 3. This was relatively related to the cells concentration that was discussed earlier. As Run 1 and Run 2 had the highest cells concentration, therefore both of these Runs had produced higher lactic acid than the other two Runs. However, Run 1 showed better production compared to that of Run 2. This was due to the different of pO_2 level applied to both Runs. It was proved that lactic acid bacteria live better in a condition with small amount of O_2 concentration because of their characteristic as facultative anaerobic bacteria (Teuber, 1993). Therefore, in practice low oxygen tensions should be maintained and exclusion of oxygen (air) is not an absolute requirement for this microorganism.

The cells consumed the substrates for growing together with the production of lactic acid as being observed that when the cells were rapidly grew, the rate of substrate consumption decreased rapidly and the lactic acid production was increased (data not shown). After 6 h of fermentation, the glucose was totally consumed by the cells and the cell growth tends to be in the stationary phase until 48 h of fermentation.

Online monitoring of the parameters: During the bioreactor fermentation, online monitoring of the parameters using Multi-Function Control System (MFCS) Win version 2.0 software has been carried out where the stirrer speed (rpm), pO_2 and pH have been monitored and recorded as on-line for 48 h of fermentation. Among the four Runs, the best results were obtained in Run 1 (Fig. 4). The initial pO_2 level was set at 5%, while the pH level was maintained at 6. After certain time, when the concentration of the cells was increasing, the pO_2 level was slightly dropped. At that time, airflow valve was open to supply the culture with more oxygen to support the pO_2 and maintained it at 5%. After the pO_2 level reached its initial concentration, the airflow valve stopped in order to maintain the level of the desired pO_2. This was what so called as cascade airflow or gas-flow where pO_2 was automatically supplied by the adjusted airflow.

As described earlier, *Lactobacillus* bacteria are facultative anaerobes. Therefore, pO_2 was the critical parameter that has to be maintained during the fermentation process. High level of pO_2 is not suitable for these bacteria. Thus, from the result obtained (Fig. 3), it is suggested that lower level of pO_2 (between 5 and 10%) should be used as the critical point of the pO_2 level.

CONCLUSIONS

The optimum bioreactor setting parameters are important to ensure the best culture condition for the bacteria cells. From the results obtained, the best condition for the *Lactobacillus rhamnosus* to grow and produce better amount of lactic acid was at 5% of pO_2, pH 6 and stirrer speed of 100 rpm. The highest amount of lactic acid produced was about 16.8 g L^{-1} or 1.68% production yield. In order to effectively produce the lactic acid, the pO_2 level must be adjusted within the critical pO_2 value between 5 and 10%.

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