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The Influence of Process Parameters on Lactic Acid Fermentation in Laboratory Scale Fermenter

Maizirwan Mel, Mohd Ismail Abdul Karim, Parveen Jamal,
Mohamad Ramlan Mohamed Salleh and Ruzi Aini Zakaria
Bioprocess Engineering Research Group,
Department of Biotechnology Engineering, Faculty of Engineering,
International Islamic University Malaysia, Jalan Gombak 53100,
Kuala Lumpur, Malaysia

Abstract: The purpose of this study was to study the influence of process parameters on the Lactic Acid Fermentation by *Lactobacillus rhamnosus* in laboratory scale fermenter. The experiment was designed by Taguchi Method using STATISTICA software. Three parameters have been chosen as the main parameters that affect significantly to the growth of *L. rhamnosus*; the agitation speed of the impeller, dissolve oxygen level (pO_2) and pH. The most influence and important parameter for the fermentation process is pH at acidic value of 6, followed by stirrer speed (rpm) and pO_2 even pO_2 supplied and rpm played similar function in maintaining suitable dissolved oxygen to the cells.

Key words: *Lactobacillus rhamnosus*, lactic acid, Taguchi method, optimization, laboratory scale fermentation

INTRODUCTION

Lactic acid is an organic acid widely used in industrial applications. In the recent years, the interest towards lactic acid recovery from fermentation broth increases. This interest is caused by increasing in the demand of pure, naturally produced lactic acid. The most important applications are as a preservative and acidulant in foods, as a controlled delivery of drugs in pharmaceutical agents, as a precursor for production of polymer in plastic industries and in leather tanning and textile dyeing (Stanbury and Whitaker, 1984). World demand for lactic acid is estimated as $150 million (100,000 tones). About 50% of the market is in food and beverage applications which are a mature and stable market (Shuler and Kargi, 2002).

Many fermentation parameters in bioreactor have a significant effect in the growth and metabolic production of lactic acid such as temperature, pH, agitation speed, dissolved oxygen level and etc. Growth kinetic study of the microbial cultures can be used to estimate the cost effective production of lactic acid in large scale. In this study, the influence of three process parameters (the agitation speed of the impeller, dissolve oxygen level (pO_2) and pH) on the growth of *L. rhamnosus* producing the lactic by fermentation was demonstrated. In order to confirm significant results, the Taguchi's analysis and online monitoring system (SCADA) have been used.

MATERIALS AND METHODS

Microorganism: *Lactobacillus rhamnosus*, a homo-fermentative bacterium to produce L-Lactic acid was used in this study.

Inoculum preparation: For the preparation of the starter culture, a single colony from *Lactobacilli* slant agar was transferred into a bijou bottle containing 10 mL culture medium and left overnight (24 h). After 24 h cultivation, 1 mL of *lactobacillus* liquid culture was pipetted into a bijou bottle containing 9 mL of media and incubated at 37°C for 5-6 h, the time needed for the bacteria to reach the exponential growth phase. A 10% inoculum grown in the medium was used in all fermentation processes. The 10 mL starter culture was then transferred into 250 mL shake flask containing 90 mL media. The culture was incubated in a rotary shaker set at 37°C using 150 rpm for 5-6 h. The 100 mL inoculum was then transferred aseptically into an inoculation flask for inoculation in bioreactor.

Corresponding Author: Dr. Maizirwan Mel, Bioprocess Engineering Research Group, Department of Biotechnology Engineering, Faculty of Engineering, International Islamic University Malaysia, Jalan Gombak 53100, Kuala Lumpur, Malaysia Tel: 603-61964566 Fax: 603-61964442

Table 1: Reference condition for designed experiment in bioreactor

	rpm (cascade)	pO_2 (%)	pH
Run 1	1-500 (L)	10% (L)	6 (L)
Run 2	1-500 (L)	20% (H)	7 (H)
Run 3	1-1000 (H)	10% (L)	7 (H)
Run 4	1-1000 (H)	20% (H)	6 (L)

L: Low level and H: High level

Design of experiment: Optimization process had been designed by Taguchi Method using STATISTICA software (Version 6.0) where the three significant parameters (agitation speed of impeller (rpm), partial dissolved oxygen (pO_2) and pH) were set at various values. These parameters were observed to be the most significant parameters that affected the growth of *Lactobacillus* bacteria (Teuber, 1993). The experimental conditions were set according to Table 1.

Bioreactor operation: A 2 L Stirred Tank Reactor (STR), B-Braun fermenter, with batch mode of operation was operated at the different values of parameters using Taguchi method as shown in Table 1. The agitation speed and culture temperature were controlled at 150 rpm and 37°C. Due to the fact that the used strain is facultative anaerobic, there is no need for air-sparging. The medium of the cultures had the following composition: 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}, respectively. The culture pH in the fermenter was controlled at 6 by adding Sodium Hydroxide (NaOH) and Chloric acid (HCl). All the media were sterilized at 121°C for 20 min. The conditions of operating parameters were set accordingly. A cascade control method was applied in the operation where agitation was set up to 500 rpm for low level and 1000 rpm for high level. Air flow was not supplied. All of these parameter values were controlled through online once inoculation to the bioreactor takes placed. The pO_2, rpm and pH levels were monitored during the fermentation process. The fermentation process was conducted for 24 h and at different time intervals (every 30 min), sample was withdrawn and the OD, total cells number and the concentration of glucose and produced lactic acid were analyzed.

Analytical method: Optical Density Analysis (OD), Total Cell Number (TCN) and Cell Dried Weight (CDW) were analyzed using Maizirwan (2002) protocol.

Glucose and lactate analysis: One milliliter of sample was transferred into a 1.5 mL Eppendorf tube and centrifuged at 3000 rpm for 10 min. The supernatant was transferred into a cuvette and analyzed using the YSI Biochemical Analyzer.

RESULTS AND DISCUSSION

Growth kinetic study: Table 2 shows that *L. rhamnosus* cells grow fastest in Run 1 and 4. This is because of the suitable operational condition with acidic pH and enough pO_2 level that it may have. The growth rate has been correlated with the fastest doubling time, t_d which also occurred in Run 1 and 4. Between Run 1 and 4, production of lactic acid is much higher in Run 1. Therefore, Run 1 has showed the highest productivity which is 0.1282 g^{-1} h.

Parameter influence study by Taguchi method: Taguchi method was employed as a tool for systematic experimental design. It allows several effects of parameters to be simultaneously determined effectively and efficiently. Taguchi's approach complements two important areas: 1) Clearly defines a set of orthogonal arrays (OA) and 2) Devised a standard method for analysis of results. The combination of standard experimental design techniques and analysis method in the Taguchi approach produces consistency and reproducibility rarely found in any others statistical method (Roy, 1990).

After getting the results of cell concentration, substrate and product concentration for each run, those values were used in Taguchi method to analyze which parameters that most affect the lactic acid production. There are many types of analytical method that can be used to study the correlation of each parameter (under controlled) in bioreactor such as Signal-to-Noise (S/N) ratios, Larger-the-better, Smaller-the-Better etc. Here, S/N ratios analysis method was chosen. Analysis was done by selecting lactic acid production as dependent variable.

S/N ratio is one of the analyses in Taguchi method which determined the most significant correlation between parameters. The change in the quality characteristics of a product under investigation in response to a factor introduced in the experiment design is the signal of the

Table 2: Summary of growth kinetics data for bioreactor fermentation

	Run 1	Run 2	Run 3	Run 4
μ (h^{-1})	0.5005	0.3866	0.396	0.5143
td (h)	1.38	0.79	0.74	1.35
Yx/s (g g^{-1})	0.0023	0.0031	0.0054	0.0036
Yp/s (g g^{-1})	0.7347	0.7333	1.2873	0.762
Productivity, P (g g^{-1} h)	0.1282	0.0685	0.0315	0.0775

Table 3: Effect of size for each parameter

	Expected S/N ratio under optimum conditions Mean = 10.8300 Sigma = 3.76952	
Factors	Level	Effect size
rpm (cascade)	1	1.30500
pO_2	2	0.53000
pH	1	2.94500
Expected S/N		15.61000

Table 4: Correlation between rpm and pO_2

Analysis of variance (Spreadsheet 1)
Mean = 10.8300 Sigma = 3.76952
*- Effect pooled into error term

Effect	SS	df	MS	F	p-value
*rpm (cascade)	6.81210	1			
*pO_2	1.12360	1			
*pH	34.69210	1			
1 by 2	34.69210	1	34.69210	8.743299	0.097871
Residual	7.93570	2	3.96785		

Table 5: Correlation between rpm and pH

Analysis of variance (Spreadsheet 1)
Mean = 10.8300 Sigma = 3.76952
*- Effect pooled into error term

	SS	df	MS	F	p-value
rpm (cascade)	6.81210	1			
*pO_2	1.12360	1			
*pH	34.69210	1			
1 by 3	1.12360	1	1.12360	0.054144	0.837647
Residual	41.50420	2	20.75210		

Table 6: Correlation between pO_2 and pH

Analysis of variance (Spreadsheet 1)
Mean = 10. 8300 Sigma = 3.76952
*- Effect pooled into error term

Effect	SS	df	MS	F	p-value
rpm (cascade)	6.81210	1			
*pO_2	1.12360	1			
*pH	34.69210	1			
2 by 3	6.81210	1	6.81210	0.380397	0.600245
Residual	35.81570	2	17.90785		

desired effect. It measures the sensitivity of the quality characteristic of the product which being investigated in a controlled manner, to the external influencing factors (noise factors) which is not under control (Ani *et al.*, 2002). The S/N is simply the ratio of the mean to the standard deviation.

Main effect plot: As shown in Fig. 1 the pH has a bigger main effect than rpm and pO_2. That is, the line connecting the means responses for low pH and high pH, lower rpm and higher rpm have a steeper slope than the line connecting the mean responses at the low and high setting of pO_2. Although the pH appears to affect the lactic acid yield more than rpm and pO_2. Thus, the most significant parameter that affects the production of lactic acid according to this analysis is the pH (Table 3). This proven that the lower pH which was applied to Run 1 and 4 produced better production of lactic acid. In cascade control that has been applied in bioreactor fermentation, pO_2 was categorized as a master to the rpm where the rpm have to work in order to maintain the desired pO_2. Therefore, up and down the rpm level affected the pO_2.

Analysis of variance (ANOVA): ANOVA is the quantitative measure of the influence of individual factors/parameters. It is important for determining the relative importance of the various factors/parameters. Table 4-6 show that the influences of rpm to the pO_2 is about 0.097871 or 90%, rpm to pH is 83% and pO_2 to pH is 60%, respectively. This indicated that rpm and pO_2 exert the most significant effect on the production of lactic acid. The correlation between rpm and pH has low significant effect to the bioreactor operation, thus low benefit to the production of lactic acid and can be adjusted independently. The combination of rpm and pO_2 has

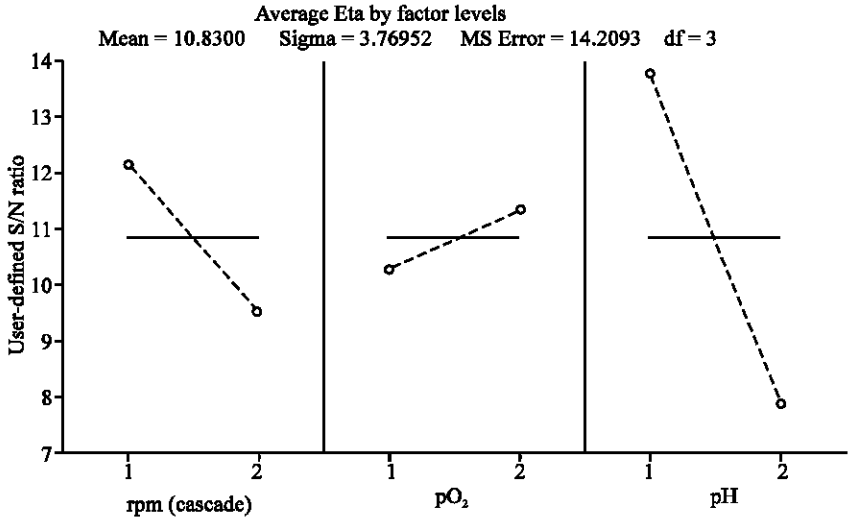

Fig. 1: Means plot using S/N ratio

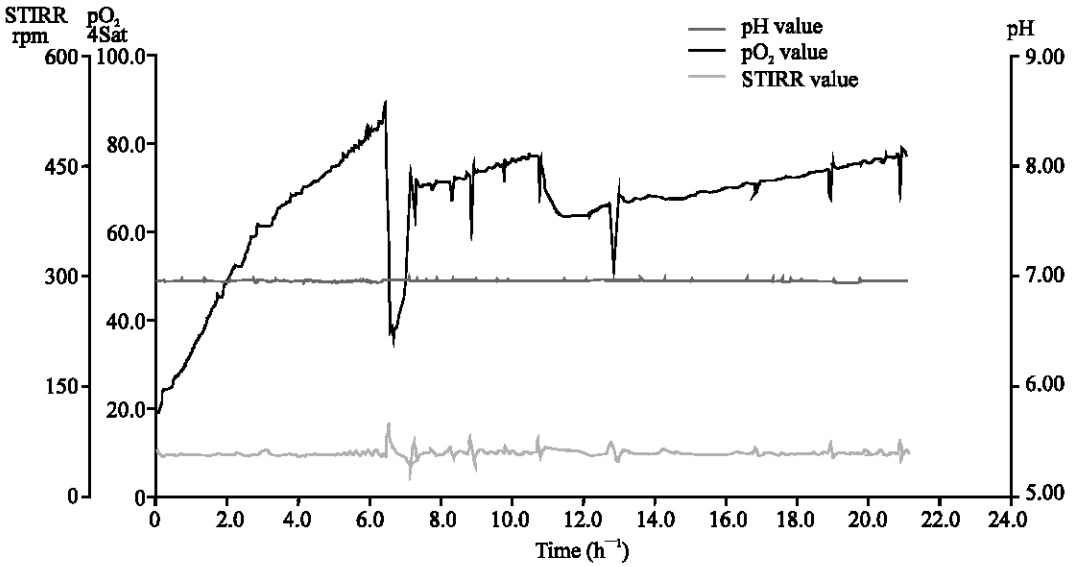

Fig. 2: Online monitoring during Run 3 fermentation

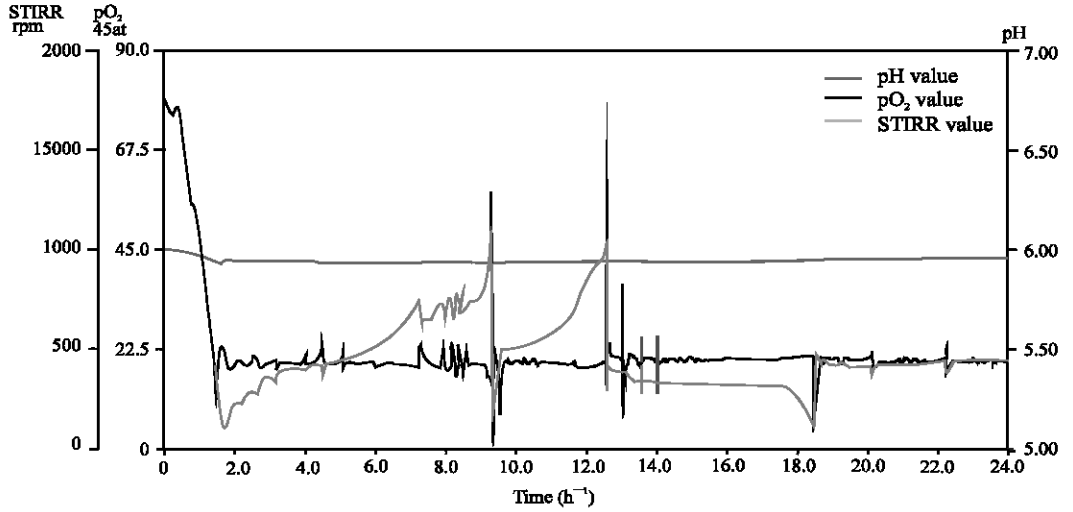

Fig. 3: Online monitoring during Run 4 fermentation

significantly affected the production of lactic acid using bioreactor. Therefore, good production of lactic acid is depending on controlling these two dependent parameters.

Bioreactor online monitoring: During the bioreactor fermentation, online monitoring using MFCS/Win has been carried out. Parameters of stirrer speed (rpm), pO_2 and pH have been monitored and recorded. Figure 2 and 3 showed the graphs of parameters monitoring for Run 3 and 4, respectively, that have been carried out for 24 h of fermentation. Between the four graphs, monitoring parameters for Run 3 and 4 showed the best. This is due to the ability of controlling the level of pO_2 at desired level without the stirrer exceeds its range which is 1-1000 rpm for both runs. For Run 3, the initial pO_2 level was set to be 10%, while pH level was maintained at 7. After certain time, when the concentration of the cells was increased, the pO_2 level was slightly dropped. At that time, stirrer was function to agitate the culture from slow to rapid agitation to support the pO_2 and maintained it at 10%. After the pO_2 level reached its initial concentration, stirrer decreased it rpm and maintained at the suitable value of pO_2 level required. This is called as cascade control where pO_2 just supplied by the rpm not from the sparger. As described earlier, *Lactobacillus* bacteria are species of facultative anaerobic. Therefore, pO_2 was the critical

parameter that has to be maintained during the fermentation. High level of pO_2 is not suitable for this species of bacteria. As for Run 4, the function of cascade (rpm) was also shown. The stirrer speed was increased when pO_2 decreased and vice versa when it want to decrease the excess pO_2. As compared to Run 1 and 2 (figure is not shown here), a bigger fluctuation occurred to the rpm. It was exceed the range of 0-500. It can be said that pO_2 and stirrer speed relate closely to each other to maintain stable performance of bioreactor.

CONCLUSIONS

In conclusion, from the three parameters controlled during the fermentation in the bioreactor, pH with acidic condition showed the most influence factor and be the important parameter to the cells to perform best growth. The process will become more productive if under the acidic pH condition, the pO_2 supplied which is closely related to the stirrer speed maintaining the suitable dissolved oxygen to the cells is controlled under suitable level. High agitation of the impeller did not affect the cells strain because this cell is not shear sensitive and also a gram positive bacterium.

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