Media screening of lactic acid fermentation using *Lactobacillus rhamnosus*

Mohd Ismail Abdul Karim, Maizirwan Mel*, Parveen Jamal, Mohamad Ramlan Mohamed Salleh, and Noraini Alamin

Bioprocess Engineering Research Group, Department of Biotechnology Engineering, Faculty of Engineering, International Islamic University Malaysia, Jalan Gombak 53100, Kuala Lumpur, Malaysia.

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The screening of media used for lactic acid production using microorganism (*Lactobacillus rhamnosus*) was successfully carried out in shake flask experiment. Initially eleven variables were screened using Placket Burman Design and the results indicated that, the main variables that affected the process were glucose and peptone. The correlation between those two variables was analyzed using Response Surface Methodology (RSM). The contour plot indicates that there is no significant interaction between these two variables.

Key Words: *Lactobacillus rhamnosus*, lactic acid, media screening, Response Surface Methodology (RSM), fermentation.

Introduction

The efficiency and economics of the lactic acid production through microbial fermentation is still a problem if considered from many points of view and among them, media composition plays a vital role in the improvement of it's production. Fermentation of lactic acid by the microbes using different media plays an important role in the final product of the process. Proper design of the media does affect the performance of microorganisms in optimizing the lactic acid production.

The growth of metabolism use in fermentation and the metabolic pathway of the process in the production of lactic acid are affected by the fermentation parameters such as temperature, pH, agitation speed, and dissolved oxygen level (Wenge *et al*., 1999). Growth kinetic study of the microbial cultures can be used to estimate the cost effective of lactic acid production in large scale.

*Corresponding author: Maizirwan Mel; e-mail: maizirwan@iiu.edu.my

Nowadays, research effort is focused on looking not only for new and effective nutritional sources but also new progressive fermentation techniques which enable the achievement of both high substrate conversion and high production yields. The improvement of lactic acid production has been studied under the control of various factors and media components (Pintado *et al*., 2002; Arasaratnan *et al*., 1996; Cheng *et al*., 1991).

In this study, the media compositions of the fermentation process for lactic acid production using *L. rhamnosus* were screened using Placket Burman Design. The correlation between the influenced compositions was analyzed using Response Surface Methodology (RSM).

Materials and Methods

Microorganism and media

The microorganism used in this study was *L. rhamnosus*, the homo-fermentative lactic acid bacteria. The culture media used was the MRS medium containing glucose, peptone, yeast extracts, lactose, Tween 80, K_2HPO_4, sodium acetate, $(NH_4)_2SO4$, MnSO4, $MgSO_4$ and distilled water.

Inoculums preparation

The preparation of inoculums started with transferring the stock culture into a liquid MRS media. After the growth of culture, the microorganisms were transferred to a plate of solid MRS medium. The plate was incubated at 37^{o}C for 48 hours in order to allow sufficient growth of colonies.

The grown colonies were either used to initiate a fermentation process or were stored back at 4^{o}C as stock culture which can be prepared by culturing the colonies in slant agar followed by adding 30% of glycerol. The *L. rhamnosus* inoculums were prepared by inoculating a single colony of them into 10 ml broth media which was then incubated at 37^{o}C for 24 hours. 1 ml of inoculums was transferred into bijou bottle containing 9 ml media. Cultures were incubated for 10 hour at 37^{o}C before being transferred into shake flask.

Fermentation in Shake Flask

For each Run, 10 shake flasks were used. In each flask, 10ml inoculums were transferred into 90ml formulated media under aseptic condition. The shake flask was capped with cotton and swabbed with 70% ethanol and then incubated in a thermo-stated rotary incubator shaker for 30 hours under setting temperature of 37^{o}C and rotation speed of 150 rpm.

Sampling

Sample in shake flasks were taken by using aseptic technique for every 2 hours by flaming the cap swabbed with 70% ethanol. 12 ml of sample was transferred into a bijou bottle, which was then being divided for measuring optical density (OD, A_{660nm}), product (lactic acid), substrate (glucose) and cell dry weight. The flasks then were transferred back to the thermo-stated rotary incubator shaker to continue the fermentation process.

Analytical Method

Optical density analysis (OD), total cell number (TCN) and cell dried weight (CDW) were analyzed using Maizirwan protocol (Maizirwan, 2002)

Glucose and Lactic acid Analysis

One ml sample was transferred into 1.5 ml Eppendorf tube and centrifuged at 3000 rpm for 10 minutes. The supernatant was transferred into a cuvette and analyzed using the YSI 2700 Biochemical Analyzer.

Experimental design for media screening

Plackett –Burmann Design using STATISTICA Software v 6.1 was used to screen and select media components which influence the lactic acid production. Media were divided by two, media that can increase the production of product or that can increase the growth rate of the microorganisms. Each component in media formulation plays a different role in the fermentation process. Therefore it is important to screen first the most significant or effective component before optimizing the media. The lower and upper level of concentration of each component was selected based on the literature information (Cejka, 1983; De Man, *et al*., 1960).

By this, the most significant component that affects the production of lactic acid can be determined and will be used for media optimization. The design experiment for screening the most significant component of MRS media such as: yeast extract, lactose, peptone, glucose, sodium acetate, K_2HPO_4, Tween 80, $(NH_4)_2SO_4$, $MgSO_4$, $MnSO_4$ and pH was performed and the complete media formulation for each experiment is shown in Table 1.

Correlation between the influenced compositions

Correlation between the influenced compositions was analyzed using Response Surface Methodology (RSM).

Results and Discussion

Media screening

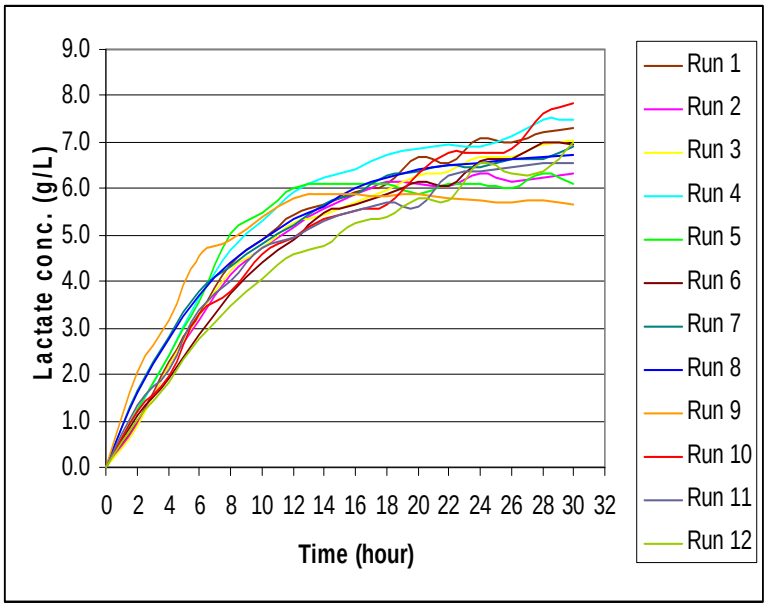

Fig. 1. Lactic acid production at different Runs

Fig. 1 shows a comparison of lactic acid produced among all Runs. The production of lactic acid was increased as the fermentation time increased, and for some conditions the product yields were still increasing even after the cell reached stationary phase.

After 30 hours of fermentation, Run 10 produced the best amount of lactic acid. The lactic acid production was increased steadily even though at the beginning, the production rate is lower compared to the Runs. For Run 9, the production was the lowest, even at the beginning the production rate was faster than others. Media formulation for these two Runs contains the same amount of glucose but different amount of peptone and yeast extract. Run 10 contain higher amount of peptone but lower concentration of yeast extract compared to Run 9.

Using the statistical analysis, the significant components were cited using Pareto chart as shown in Fig. 2. It is clearly shown that the components which showed high positive value, affected the lactic acid production and the order was peptone, glucose, ammonium sulphate, yeast extract and lactose if it is arranged in the decreasing order.

Correlations between peptone and glucose

According to Fig. 3, there is no correlation between the glucose and peptone in the production of lactic acid. From the graph, it can be seen that when the amount of glucose increased, the production of lactic acid also increased but for peptone, the low amount of lactic acid produced was not increased even though peptone concentration was increased. The reason why there is no relation between peptone and glucose is that the glucose is used as main substrate for lactic acid production. Meanwhile, peptone is required as a nitrogen source for the growth of bacteria (Hujanen, *et al*., 2001).

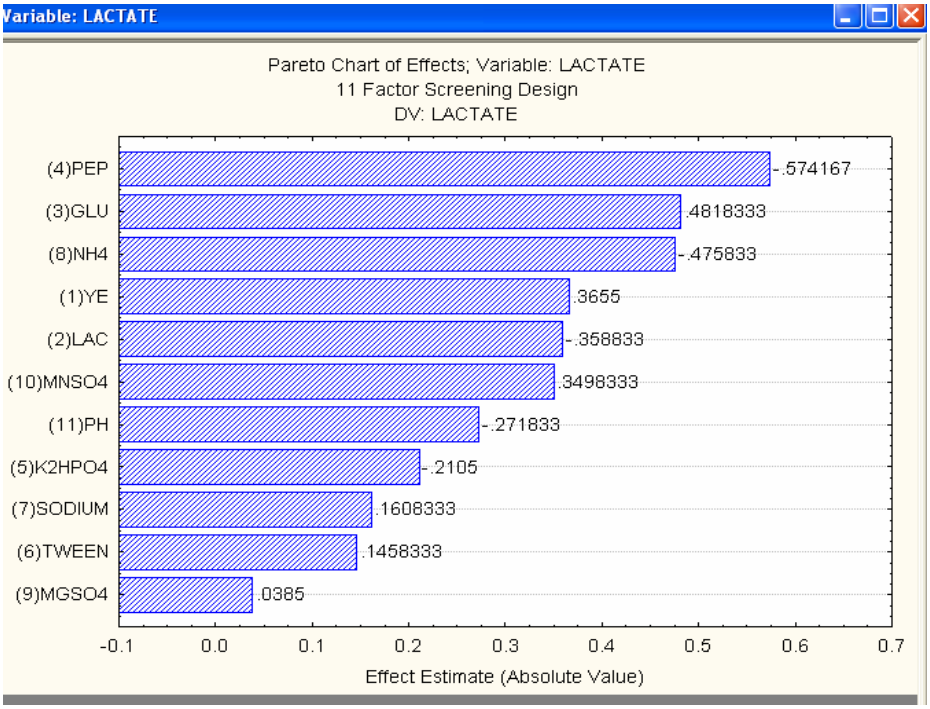

Fig. 2. Pareto Chart for Media Screening

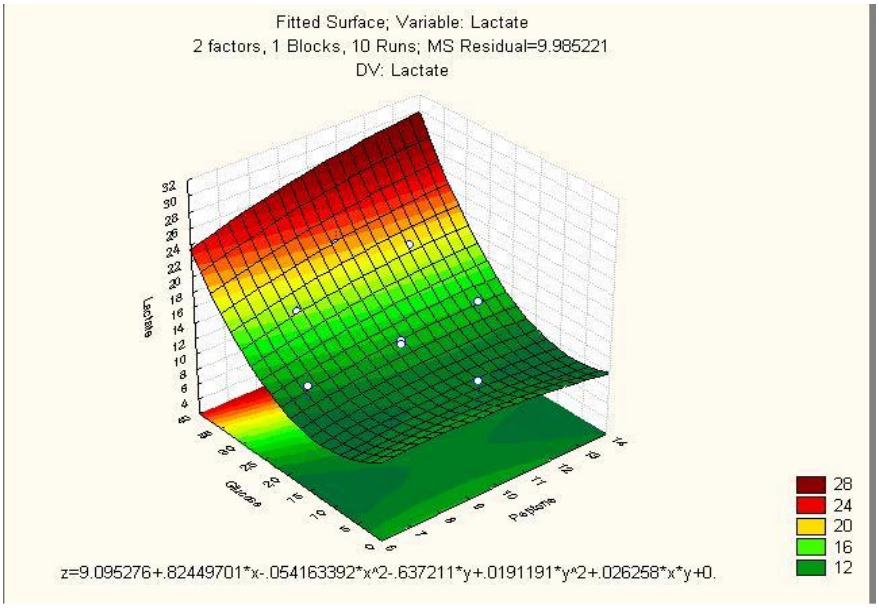

Fig. 3. Correlation between Peptone and Glucose

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