EFFECT OF *RHIZOPUS ORYZAE* FERMENTATION ON KENAF-BASED POLYLACTIC ACID'S MONOMER

NUR AIMI MOHD NASIR[1], MOHD ADLAN MUSTAFA KAMALBAHRIN[1], NURHAFIZAH SEENI MOHAMED[1], HAZLEEN ANUAR[1], MAIZIRWAN MEL[2], AND RASHIDI OTHMAN[3]

[1]Department of Manufacturing and Materials Engineering,
[2]Department of Biotechnology Engineering, Faculty of Engineering,
[3]Faculty of Architecture and Environmental Design,
International Islamic University Malaysia.

hazleen@iium.edu.my; maizirwan@iium.edu.my; rashidi@iium.edu.my

ABSTRACT: Kenaf biomass is the potential as raw materials used to produce polylactic acid's monomer which is lactic acid via fermentation by *Rhizopus oryzae*. Kenaf biomass' structure is complex due to its lignin and cellulose content. This matter had encouraged it to undergo pre- treatment process as the initial step before fermentation process can be done. In this paper, kenaf biomass was treated with dilute sulphuric acid (H_2SO_4) to hydrolyze the cellulose content in it as well as to convert the cellulose into glucose- a carbon source for *Rhizopus* to grow. Then, the fermentation process was carried out in shake flask for 3 days at pH 6. Several conditions for fermentation process had been chosen which were 25^{o}C at 150 rpm, 25 ^{o}C at 200 rpm, 37 ^{o}C at 150 rpm and 37^{o}C at 200 rpm. In this fermentation process, 0.471 g/L, 0.428 g/L, 0.444 g/L and 0.38 g/L of lactic acid was produced respectively. Sample at 25^{o}C at 200 rpm produced maximum amount of lactic acid compared to others.

ABSTRAK: Biojisim kenaf berpotensi sebagai bahan mentah dalam penghasilan monomer asid *polylactic* (poliester alifatik termoplastik diterbitkan daripada sumber boleh diperbaharu seperti kanji jagung) yang merupakan asid laktik menerusi penapaian oleh *Rhizopus oryzae* (sejenis fungus yang hidup dalam jirim organik yang telah mati*).* Struktur biojisim kenaf adalah kompleks disebabkan kandungan lignin dan selulosanya. Hal ini menyebabkan ia perlu melalui proses pra-rawatan sebagai langkah awal sebelum proses penapaian dijalankan. Dalam kertas ini, biojirim kenaf dirawat dengan asid sulfurik (H_2SO_4) yang dicairkan untuk menghidrolisis kandungan selulosa di dalamnya di samping menukar selulosa menjadi glukosa – sumber karbon bagi tumbesaran *Rhizopus.* Kemudian, proses penapaian dijalankan di dalam kelalang goncang selama 3 hari pada pH 6. Beberapa ciri proses penapaian telah dipilih iaitu 25 ^{o}C pada 150 rpm, 25 ^{o}C pada 200 rpm, 37 ^{o}C pada 150 rpm dan 37 ^{o}C pada 200 rpm. Dalam proses penapaian ini, 0.471 g/L, 0.428 g/L, 0.444 g/L dan 0.38 g/L asid laktik dihasilkan secara berturut. Sampel pada 25^{o}C pada 200 rpm menghasilkan kadar asid laktik yang maksimum dibandingkan dengan yang lainnya.

KEYWORDS: *kenaf biomass; pre-treatment; lactic acid; Rhizopus oryzae; fermentation*

1. INTRODUCTION

The environmental pollutions and global warming caused by the petroleum polymer and the variation price of oil had encouraged several researchers to find solution to the

issue. Polylactic acid has the answer. Polylactic acid (PLA) derived from its monomer which is lactic acid (LA). Lactic acid (IUPAC systematic name: 2-hydroxy propanoic acid) is a biochemical value added product which has a remarkable applications in industries. Industrial development has significantly increased interest in lactic acid. There are two ways to produce lactic acid, which are chemical synthesis and biological fermentation. The chemical synthesis method was found to be not economical because of the expensive used of petrochemical. In contrast, the biological fermentation offer the low cost of substrate and low energy consumption as well as low energy consumption [1, 2].

Lactic acid bacteria or aerobic microorganisms are commonly used in industry because of its high growth and high product yield which involved complex substrate and product recovery. In contrary, *Rhizopus oryzae* fungus can overcome the problems in bacterial fermentation [3]. *Rhizopus* can produce pure L (+)-lactic acid from complex carbohydrates from agriculture plant and biomass with a simple substrate. It is an anaerobic microorganism which needs oxygen to grow and indirectly produce lactic acid. Because of that, several factors of fermentation must be considered such as incomplete substrate, temperature and agitation speed.

Commercially, Lactic acid is via biological fermentation is derived from food sources such as beet sugar, potato, corn and barley malt [4, 5]. However, these sources are not economical especially for a long term. It has to compete with the food value which results to the expensive prices. These sources also were not sustainable and green technology. On the basis of these reasons, many researchers are looking forward for a new source that is renewable, sustainable and cheap is important. Kenaf (*Hibiscus cannabinus L*) could be candidate of choices because of its inexpensive crop, warm season annual fibre, herbaceous plant and widely available for its renewable carbon sources. It has wide application especially for its bast. In this paper, we are using kenaf biomass which was basically an agro residues obtained from the processing of kenaf fibre in order to separate core and bast. Kenaf is a lignocellulosic material which contains high lignin (15-23 %) and high cellulose (37- 57%) composition. This is similar to its biomass which has a complex structure results the difficultness of the microorganism to degrade it. Thus, it has to undergo pre-treatment process to remove or modify cellulose into fermentable carbon sources. There are many types of pre-treatment which had been investigated by several researchers for more than decades such as chemical pre-treatment [6, 7]. In earlier work, we studied the best solvent used for the pre-treatment process [6]. It was reported that dilute sulphuric acid (H_2SO_4) hydrolysis provide highest glucose conversion for kenaf biomass under optimum condition.

Sufficient glucose, oxygen requirements and condition's temperature are the crucial factor for *Rhizopus oryzae* to be a promising lactic acid producer. Thus, in this paper, the influence of temperature and agitation speed for the fermentation process via kenaf biomass based glucose by *Rhizopus oryzae* was studied.

2. MATERIALS AND METHODOLOGY

2.1 Kenaf Biomass and its Pre-treatment Process

Kenaf Natural Fibre Industry Sdn Bhd, Kelantan, Malaysia was one of the factories supplied kenaf biomass for this research. Previously, 2 g, 3 g and 4 g of kenaf biomass were added in dilute sulphuric acid (H_2SO_4) solution and conducted in a temperature 140 ^oC, 160^oC and 180^oC for 10 min, 35 min and 60 min respectively [6]. The experiment for this hydrolysis process was designed using design expert software 6.08. Anuar et al.

reported that the optimum glucose attained when the mass of biomass is 2 g at 60 min and 180^{o}C. In this study, we are trying to use such condition to produce better yield of lactic acid. Several replicates of pre-treated kenaf biomass which are at the mass of 2 g in 60 min and 180 min were prepared.

2.2 Microorganism and Medium

Rhizopus oryzae was isolated originally from the bread and grown in potato dextrose agar (PDA) and stored at 37^{o}C for 2 days. The fermentation medium used was potato dextrose broth (PDB) which consists of potato starch (4 g/L) and dextrose (20 g/L). The medium was autoclaved at 121^{o}C for 30 min. Glucose obtain from pre-treatment process was autoclaved separately and then added to 20 ml sterile PDB. The initial pH of the media in the shake flask was adjusted to pH 6 by adding sodium hydroxide (NaOH).

2.3 Lactic Acid Production via Fermentation

In this study, the fermentation process was done in a shake flask. Initially, it begin with the collection of the growth spores of *Rhizopus oryzae* using L-loop by extracting it with distilled water and added it into 250ml Erlemenyer shake flask. Next, the mixture of the sterilized medium and hydrolyzed glucose were added into it and was then incubated for 3 days. In order to yield maximum lactic acid via *Rhizopus oryzae*, the influence temperature and oxygen transfer rate which is agitation speed were chosen.

For fermentation design, the STATISTICA 7 software - two levels full factorial design was chosen. The experimental design consists of 4 runs. An average of three replicates for each experiment was conducted. The experimental design can be illustrated in Table 1. After fermentation, the obtained of lactic acid was analyzed using High Pressure Liquid Chromatography (HPLC) Agilent 1101.

Table 1: Experimental design with two varied conditions.

Run	**1 to 4**
Temperature (^{o}C)	25 and 37
Speed (rpm)	150 to 200

3. RESULTS AND DISCUSSION

3.1 Effect of Kenaf Biomass on Lactic Acid Production

Statistical analysis was done for the fermentation to be run in the varied temperature and agitation speed. Temperature between 25^{o}C and 37^{o}C were chosen in order to compare the growth of *Rhizopus oryzae* between the room temperature and human body temperature. Note that, *Rhizopus oryzae* can grow in a temperature range between 25^{o}C to 45^{o}C [8]. The temperature of 45^{o}C had not been chosen since it is beyond the growth temperature of *Rhizopus*. *Rhizopus* will die during that time. The growth of *Rhizopus* was then aided with the agitation speeds which were between 150 rpm and 200 rpm to show whether agitation speed will influence the production of lactic acid or not. All data recorded were analyzed and plotted on the graph after three (3) days of fermentation in shake flask. During fermentation process, glucose consumption was analyzed since glucose is the carbon substrate for lactic acid production. As for that, in this study, glucose content from PDB was added with hydrolyzed glucose from kenaf biomass to give more carbon substrate to *Rhizopus* to produce lactic acid.

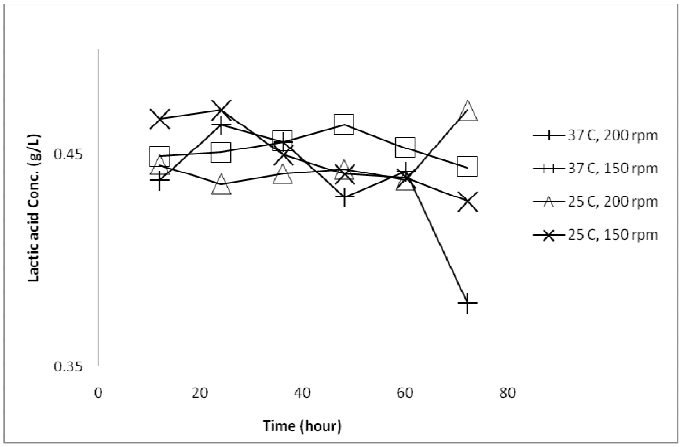

Fig. 2: Lactic acid analysis.

Figure 2 shows the rate of lactic acid production during fermentation period. In this graph, sample at 25°C in 200 rpm produced the highest concentration of lactic acid which is 0.471 g/L at the end of its production. Whereas for the sample at 25°C in 150 rpm and sample at 37 °C in 150 rpm , the values of lactic acid concentration are 0.428 g/L and 0.444 g/L respectively. Lactic acid produced at 37°C in 200 rpm results the lowest concentration compared to the other sample. It was 0.38 g/L during that condition in 72 hours of fermentation process.

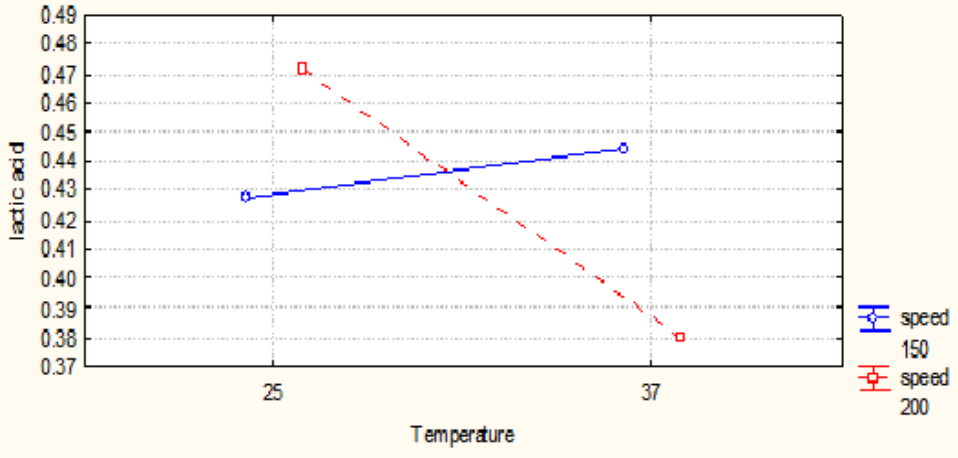

Fig. 3: Interaction between temperature and speed with production of lactic acid obtain from STATISTICA 7 software.

As illustrated in Fig. 3, when the agitation speed is increased and the temperature is lower, the production of lactic acid is increased. At 25°C, *Rhizopus* cell tend to grow faster with the aid of faster agitation speed which is at 200 rpm. Even though the cell grows faster in 25°C, the cell will not growth effectively in the low agitation speed condition which is at 150 rpm. This could be due low agitation speed will not enhance the growth of *Rhizopus* to produce lactic acid. For fermentation process at 37°C, the *Rhizopus oryzae* tend to grow smoothly but at the same time, some of it may died, thus produced low concentration of lactic acid. This matter was enhanced by the agitation speed of process,

where the faster the speed of agitation, the lower the lactic acid produced at high temperature which is at 37^{o}C.

Naranong and Poochareon claimed that the values of specific growth rate increased with increasing the agitation rate demonstrating that growth was dependent on oxygen supply. As in the case of agitation rate 200 rpm production, yield of L- lactic acid was higher than those obtained at the agitation rate of 150 rpm. This was due to the medium homogeneity and higher transfer rates of substrate and oxygen resulting from more thorough mixing [9]. In addition, an increase in agitation rate also increased oxygen supply in the medium which promoted not only *Rhizopus* growth but also lactic acid production.

4. CONCLUSION REMARKS

In this paper, we found that *Rhizopus oryzae* has the capability to produce lactic acid using kenaf biomass as the raw materials. The production of lactic acid is influenced by temperature and agitation speed of shake flask. The highest lactic acid production is sample which produced at 25^{o}C in 200 rpm. Further investigation will be required to produce maximum lactic acid especially for industrial usage.

ACKNOWLEDGEMENT

This work was supported by COMSTECH-TWAS grant. Thank to the staff of Department of Biotechnology Engineering and Department of Manufacturing and Materials Engineering, IIUM as a whole.

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