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Effect of nitrogen sources on lactic acid production from *Onggok* and Tofu liquid waste

SURIPTO DWIYUWONO[1], SUTOPO HADI[1]* and TAKAO KOKUGAN[2]

[1]Department of Chemistry, University of Lampung Bandar Lampung 35145 (Indonesia).
[2]Department of Chemical Engineering, Tokyo University of Agriculture and Technology, 2-24-26 Naka-machi, Koganei-shi, Tokyo 184-8588 (Japan).

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ABSTRACT

Onggok (dried cassava bagasse), is the by-product of the tapioca industry. It is mostly treated as industrial waste and has low commercial value. This research aims to utilize *onggok* as the substrate in the production of lactic acid in Tofu Liquid Waste (TLW) medium by *Streptococcus bovis* JM5802. The fermentation process is carried out in anaerobic conditions at pH 5.5 and temperature 39°C. The results of the fermentation using TLW media reached a productivity of 0.55 g/L.h. To increase its nutrient content, the media is then added with concentrate *maguro* waste (CMW) with concentrations of 10, 20, 30 and 40 g/L. The addition of CMW enhanced the production of lactic acid, maximum productivity of 5.18 g/L.s is reached at the addition of 30 g/L MWC.

Key words: *Onggok*; Lactic acid; Tofu liquid waste; *Streptococcus bovis.*

INTRODUCTION

Recently, the demand for lactic acid has increased due to its use as a base for poly lactic acid (PLA), a biodegradable polymer. PLA has become more appealing as this substance has better characteristics compared to other biodegradable polymers. For examples PLA has tensile strength and elongation properties that are better than those of petrochemical polymers. However, PLA is still considerably more expensive compared to conventional polymers; thus attempts to tackle this problem are really needed. In an attempt to reduce the production cost, a new medium of fresh cassava root in tofu liquid waste was used for production of lactic acid by *Streptococcus bovis*[1]. However, the productivity and specific growth rate were very low compared to the standard medium.

One of the efforts that can be done is to find an alternative substrate to the conventional substrate, corn. *Onggok* (dried cassava bagasse) is one of the substrate that is look promising as a substrate for lactic acid fermentation. *Onggok* is waste of the tapioca industry and has low commercial value. Cassava bagasse is easily degraded by microorganisms without any pre-treatment. Currently cassava bagasse has successfully been used for fermentation of citric and fumaric acid[2]. In this paper we attempt to utilize it as substrate for lactic acid fermentation. Tofu liquid waste (TLW) can be used as a medium for lactic acid fermentation by *S. bovis*[1,3,4]. The effort to replace the expensive standard basal media; trypto-soya broth would further lower the cost of lactic acid production.

There are many reports on nutrient supplements for improvement of lactic acid production using various less expensive supplements such as corn steep liquor[5], whey protein hydrolysate[6], and malt combing nuts[7]. One

of the interesting issues raised in this research is to use *onggok*-TLW, as the proposed medium. To improve the nutrient content, the addition of nitrogen sources is needed. Four different waste products are tried as the supplement to the tofu liquid waste medium. They are concentrated waste of CMW, concentrated *katsuo* (salmon) waste (CKW), skim milk (SM), and yeast extract (YE). By the use of waste products in the fermentation process it is expected that a better economical value for producing lactic acid can be achieved.

EXPERIMENTAL

Seed culture

S. bovis JCM 5802 was obtained from the Institute of Physical and Chemical Research (RIKEN Japan). *S. bovis* JCM 5802 is a facultative anaerobic and homofermentative bacteria producing mainly L-lactic acid. The strain was stored in deMan, Rogosa and Sharpe (MRS) broth with skim milk at -80°C. The medium composition was as follows (g/L): peptone, 10; meat extract, 10; yeast extract, 5; glucose, 20; K_2HPO_4, 2; sodium acetate, 5; diammonium citrate, 2; $MgSO_4.7H_2O$, 0.1; $MnSO_4.H_2O$, 0.05; Tween 80 (poly sorbit-80). In preparation for each experiment, a stock culture was inoculated into 5 mL MRS broth incubated for 18 h on a shaking water bath maintained at 37°C.

Medium preparation

Onggok tapioca was used as substrate (50 g/L), while tofu liquid waste was used as the basic medium. MWC and KWC were obtained from fish processing industry (Japan). SK and YE were obtained from Difco (USA). The basic medium and substrate solution were then sterilized by autoclaving at 12°C for 15 minutes.

Fermentation

Batch culture was carried out at 39°C in a membrane bioreactor with the volume total of 500 mL, the working volume used for experiments in this vessel was 300 mL (1). At the bottom of the bioreactor vessel, a 2 mm micro filtration membrane sheet (Advantec, Toyo Roshi Kaisha) with 76 mm diameter was incorporated. Before fermentation, the bioreactor was sterilized. Temperature of fermentation was kept constant at 39°C while pH of fermentation was maintained at 5.50 by controlling the addition of 6 M NaOH.

Analytical methods

Lactic acid and glucose concentrations were determined using Biosensor (Bio Flow BF4, Oji Scientific Instruments Ltd). The biosensor is an analytical device using a flow injection method (Bio Flow) with enzyme column and hydrogen peroxide. Two columns with different enzymes were used for the measurements. One column was packed with lactic acid dehydrogenase to measure lactic acid concentration, while the other one was packed with glucose oxide to measure glucose concentration.

RESULTS AND DISCUSSION

Variations of nitrogen source

A study on the fermentation of lactic acid using TLW has been carried out by Ghofar et al.[8] and they found that the product was lower when compared to the standard medium (Trypto-soya broth).

However it could still potentially be used as a fermentation medium. To support lactic acid production and microbial growth the medium is added with nitrogen sources, such as concentrated CMW, CKM, YE, and SM at 10 g/L. Fermentation with a media containing only *onggok* and TLW is also done for comparison.

Fig. 1 showed the results of the lactic acid production during a 84h fermentation time. The figure demonstrates results of added *Onggok*-TLW medium added with and without nitrogen sources. All the nitrogen sources were added when the concentration was 10 g/L and the fermentation was done with a membrane bioreactor where the pH is kept at 5.5 and with a temperature of 39°C. The *Onggok*-TLW medium that relies only in the nitrogen source on *onggok* and TLW showed low results of lactic acid production compared to enriched mediums. On 48 h the *Onggok*-TLW media did reach a production of lactic acid of 15.0 g/L (Fig. 1) but the fermentation process was going very slowly. This medium shows the lowest productivity of 0.75 g/L.h (Fig. 2). Lactic acid bacteria are generally fastidious organisms, which require complex nutrients such as amino acids and vitamins for their cell growth[9].

The effect variation of nitrogen sources can be seen in Fig. 1b. In the case of the TLW without the CMW supplement, the maximum viable cell count was 1.6 x 10^7 CFU mL^{-1}. When the TLW was added with supplement nitrogen sources, the maximum viable cell counts were higher than that without CMW supplement, although it is still lower than the standard nitrogen sources, YE. A similar result has also been observed by Ghoffar and Kokugan[3].

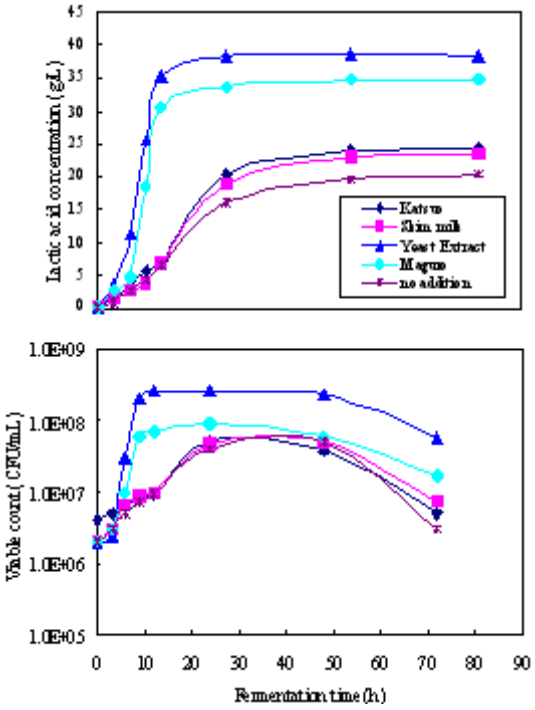

Fig. 1: (a) Lactic acid production; (b) viable count using *onggok* in TLW medium with several nitrogen sources

The *Onggok*-TLW medium added with YE, a commonly used nitrogen source, showed good results, reaching the highest value of 37.5 g/L at 24h. The addition of YE affects the production of lactic acid in the medium and also increases lactic acid productivity as it reached 3.5 g/L.h. Yeast extract, the most commonly used nitrogen source, provides complex nutrients for lactic acid bacteria[10]. Yuwono and Kokugan[1] who investigated the effect of yeast extract on lactic acid fermentation from *onggok*, also reported that lactic acid fermentation was considerably simulated by increase of YE.

Best results were obtained on the *Onggok*-TLW medium added with concentrated *Maguro* waste (CMW). This media showed rapid production of lactic acid and reached the highest point of 34.2 g/L after 24h. The highest lactic acid production was obtained by CMW in the other nutrients supplements. It can be considered that *S. bovis* has more ability to digest the crude protein in the CMW than in the other nutrient supplements[3].

Variations of CMW concentrations

In the previous part of this research, concentrate *maguro* waste (CMW) appeared to be the nitrogen source that gave the best result[11]. It even surpasses the value obtained by the OTY media that uses YE as an additional nitrogen source. In this section we attempt to find the best concentration of CMW that would give the best result

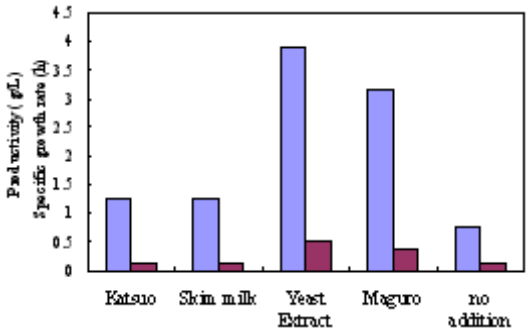

Fig. 2: Lactic acid production using *onggok* and tofu liquid waste media on different concentrations of CMW (Concentrated *Maguro* Waste)

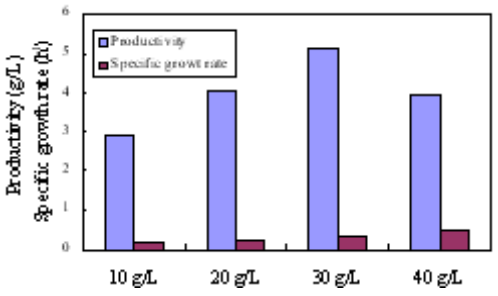

Fig. 3: Lactic acid productivity using several nitrogen sources variations of CMW concentration

in lactic acid production. Four different concentrations of CMW were tried; 10 - 40 g/L on the *Onggok*-TLW medium.

All fermentation in these mediums ran well and with good results. However the best results were found for the medium with the addition of 30 g/L CMW (Fig. 3). This medium showed a lactic acid production as high as 34.2 g/L at 72h and has a productivity of 5.18 g/L.h.

CONCLUSION

Through this study *onggok* has demonstrated as a potential substrate for lactic acid fermentation. The addition of nitrogen sources considerably accelerates and increases the production of lactic acid on the onggok-TLW medium. From the four additional nitrogen sources which were tested, *maguro* waste concentrate provided the best lactic acid production. A concentration of *maguro* waste concentrate of 30 g/L in the medium provided the best results with lactic acid productivity of 5.18 g/L.h.

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