

Production of Lactic Acid from Onggok and Tofu Liquid Waste with Concentrate *Maguro* Waste Supplement by *Streptococcus bovis*

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Abstract: The aim of this work was to study the capability of amylolytic of lactic acid bacteria to hydrolyze the onggok tapioca for lactic acid production. Of the three strains tested, *Streptococcus bovis* was selected. *S. bovis* was found to produce lactic acid directly from onggok substrate at pH 5.5. It has been demonstrated that lactic acid concentration as high as 39.98 g/l can be obtained from onggok-tofu liquid waste-20 g/l concentrates maguro waste. The yield and productivity of lactic acid in batch fermentation were 85% and 3.01 g/l.h, respectively.

Key words: Direct Fermentation, Onggok, Tofu Liquid Waste, Lactic Acid, *Streptococcus bovis*

INTRODUCTION

The processing of cassava tubers for the large-scale production of starch result in solid and liquid waste. The fibrous slurry constitutes about 15-20% of cassava chips processed, which contains around 50-70% starch on dry weight basis. Cassava bagasse (onggok), which is generally discarded to the environment without any treatment, causes serious concern to environmental pollution in areas where the starch industries are located. It is not found any useful application of onggok except little utilization as feed or combustible. The cassava bagasse can be used as animal feed after enriching its protein content using the fungi (Balagopalan and Manini, 1977; Dung *et al.*, 1994; Manilal *et al.*, 1985). Various starch-derived products like maltose, maltodextrine, corn syrup solid can also be produced from cassava bagasse (Ghildal *et al.*, 1990; Panday *et al.*, 2000). The application of agro-industrial residue not only provides alternative substrate, but it also helps to solve their disposal problem. With the advent of biotechnological innovations, mainly in the area of enzyme and fermentation technology, many new avenues have opened for their utilization.

One of the efforts that can be done is to find an alternative substrate to the conventional substrate such as corn (Hoshino *et al.*, 1991), glucose (Dermici and Pometto, 1995), and starch (Shamala and Sreekantiah, 1988). In recent years, there has been an increasing trend towards more efficient utilization of agro-industrial residues such as molasses (Wee *et al.*, 2004), corncobs (Hang and Woodams, 1998), and sugar beet pulp (Pandey *et al.*, 1998). Applications of agro-industrial residue in bioprocesses on the one hand provides alternative substrate, and on the other hand help in solving pollution problems, which their disposal may otherwise cause.

Onggok (dried cassava bagasse) is one of the substrate that looks promising as a substrate for lactic acid fermentation. Recently cassava bagasse has been used successfully for fermentation of citric and fumaric acid (Pandey *et al.*, 2000). 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* (Ghofar, 2004; Yuwono and Kokugan, 2008). The effort to replace the expensive standard basal media; trypto-soya broth would further lower the cost of lactic acid production. In the present study, we describe lactic acid production from onggok and TLW by *S. bovis*, in order to maximize the lactic acid produced and productivity.

MATERIALS AND METHODS

Seed Culture:

S. bovis JCM 5802 was obtained from 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; K₂HPO₄, 2; sodium acetate, 5;

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diammonium citrate, 2; MgSO₄.7H₂O, 0.1; MnSO₄.H₂O, 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 was used as substrate (5g/L), while tofu liquid waste was used as the basic medium. MWC was obtained from fish processing industry (YSK, Japan). The basic medium (TSB) consisted of the following (per liter of distilled water): 17 g peptone, 3 g soybean peptone, 2.5 g glucose, 2.5 g K₂HPO₄, 2.5 g KH₂PO₄, 5 g NaCl. The basic medium and substrate solution were then sterilized by autoclaving at 121 °C for 15 minutes.

Fermentation:

Batch culture was carried out at 39 °C in a bioreactor (ABLE, Japan) with volume total of 1 L, the working volume used for experiment in this vessel was 750 mL. Temperature of fermentation was kept constant at 39 °C while pH of fermentation was maintained at 5.5 by controlling the addition of 6 M NaOH.

Analytical Methods

Lactic acid and glucose concentration were determined using Biosensor (Bio Flow BF4, Oji Scientific Instruments Ltd). The viability counts during fermentation were carried out using plate counts on BCP agar medium and incubated at 39 °C for 48 h. After 48 h incubation the colonies were counted by colony counting method in Colony Forming Unit (CFU/mL).

RESULTS AND DISCUSSIONS

Screening Microorganism:

To compare the amyolytic aptitude of three available strains, cultivation was conducted in bioreactors, in cassava-TSB medium. The result obtained for three different strains grown with onggok-TSB medium shown in Figure 1. With exception of the strain *L. amilophilus*, which has low amyolytic activity, in other cases, a total consumption of carbon source occurred. In particular with *L. lactis* and *S. bovis*, starch degradation took place in the first 12 h of culture. In this screening the best lactic acid production were *S. bovis* with 85% yield.

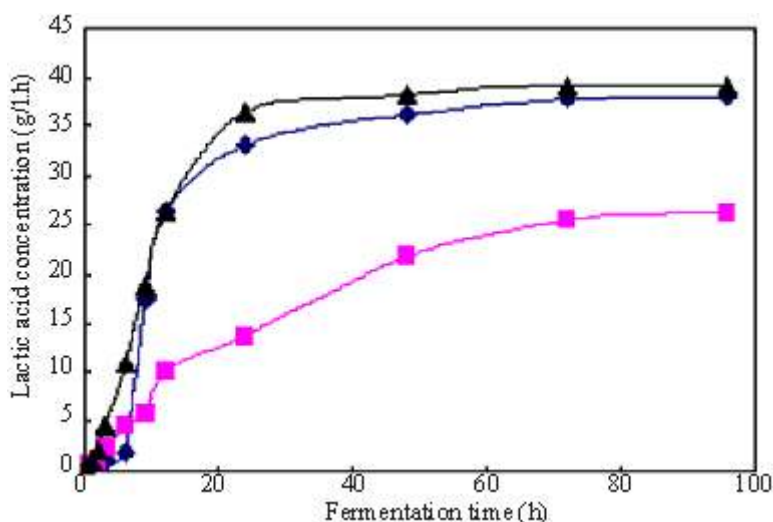


Fig. 1: Lactic acid production from onggok and TLW by *L. amilophilus* (■), *L. lactis* (□), and *S. bovis* (▲)

Comparison between Kinetics of *S. bovis* on Onggok-TSB, Onggok-TLW and Onggok-TLW with Addition of 20 g/L of CMW:

S. bovis was selected for its higher level of lactic acid production on onggok. By the use of this strain, kinetics of cultures on onggok (50 g/L) substrate with different source of medium: TSB, TLW and TLW-20 g/L CMW were analyzed (Figure 2). In onggok-TSB medium, culture kinetics shows a fast hydrolysis of onggok with a high increase in reducing sugar at 24 h. Meanwhile, in onggok-TLW medium the slope of residual starch was lower. From the results obtained on onggok-TLW-20 g/L CMW medium it is evident that the strain is able to hydrolyze the onggok and achieve an amyolytic activity similar to that obtained in onggok-TSB medium.

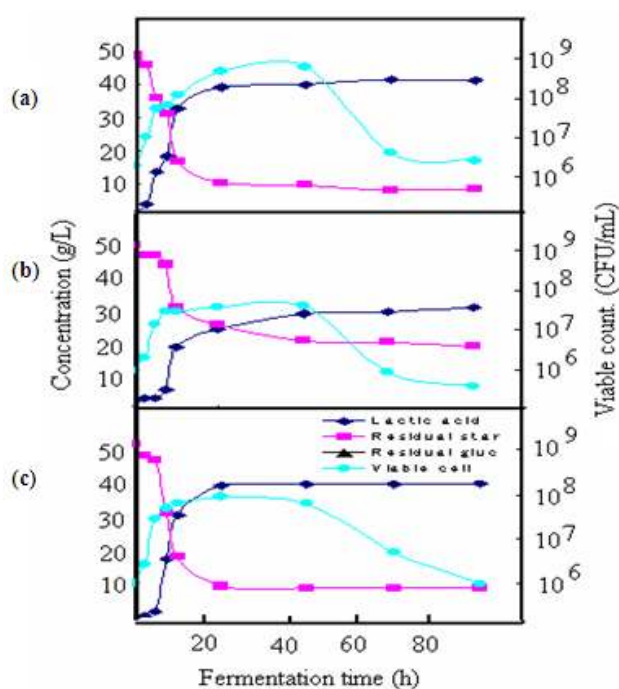


Fig. 2: Lactic acid production, residual starch, residual glucose and viable cell in onngok-TSB (a), onngok-TLW (b) and onngok-TLW-20 g/L CMW (c).

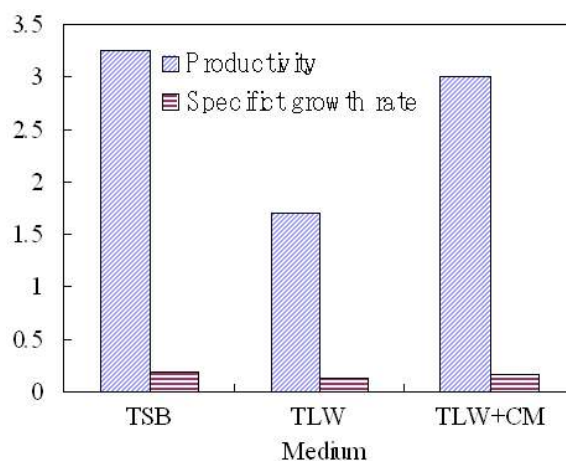


Fig. 3: Maximum productivity and specific growth rate for different media

Lactic acid production, in onngok-TSB, onngok-TLW and onngok-TLW-20 g/L CMW media was coupled with biomass production (Figure 2). Culture in onngok-TSB medium showed a rapid uptake of starch to glucose and that transformed to lactic acid and higher biomass productions than that of onngok-TLW. In onngok-TLW-20 g/L CMW, biomass and lactic acid production were higher than that of onngok-TLW medium. In onngok-TLW culture, the high level of reducing sugars remaining in the medium could be due to the limitation of growth caused by some nutrient deficiency, *e.g.* protein.

Figure 3 shows the maximum productivity and specific growth rate for assay media. It can be concluded that the amylase produce by she strain were capable of hydrolyzing onngok in TLW medium with added CMW as a nitrogen source. The major advantage of CMW is low raw material cost. The CMW raw material cost at 30 g/L supplementation is less than one-fifth of the cost of supplementation with 20 g/L YE (Yuwono *et al.*, 2005b). The volumetric productivity with TSB, TLW and TLW-20 g/L CMW of lactic acid were 3.35, 1.57 and 3.01 g/L.h, respectively. While the yield with TSB, TLW and TLW-20 g/L CMW of lactic acid were 80.2, 58.9 and 80.0 %, respectively.

Conclusions:

This work demonstrated the aptitude of amylolytic lactic acid bacteria to hydrolyse the starch in onggok. The α -amylase strain *S. bovis*, was able to use starch from onggok-TLW although at a slower than that of onggok-TSB medium. The addition of CMW in onggok-TLW medium can improve the lactic acid production.

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