REPEATED BATCH FERMENTATION FOR LACTIC ACID PRODUCTION BY *Enteroccocus faecium* IN LIQUEFIED SAGO STARCH

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Abstract

Enterococcus faecium No. 78 (BIOTECH 10375) isolated from *puto,* a type of fermented rice in the Philippines was used to produce lactic acid (LA) in repeated batch fermentation (RBF) mode. Enzymatically liquefied sago starch (LSS) was used as the sole carbon source. LSS was inoculated with *E. faecium* to perform the saccharification and fermentation processes simultaneously. Results demonstrated that *E. faecium* was reused for up to 11 fermentation cycles with an average LA yield of 36.3 g/L obtained in 15 hrs at the end of the log phase with the ability to produce 42.5 g/L LA in 30 hrs. By using RBF mode the strain was able to maintain a volumetric productivity of 1.96±.0.241 g/Lh during more than 280 hrs of fermentation. Work is in progress to maintain and increase the usability of the cells over higher fermentation cycles.

Introduction

One of the most important factors that affect the overall production cost in LAF is the raw material. The consortium CSM reported that the raw material costs for LA production, as a percentage of sales, increased from 52.9% in 2009 to 53.2% in 2010 (CSM, 2010). Among the starchy materials, sago starch is considered an attractive raw material for food and industrial exploitation because it is produced abundantly in the agricultural plant *Metroxylon spp* (Flach, 1997). On the other hand, to improve the economics of the LAF, the use of microorganisms with amylolytic activity could be preferred because of the saving of enzyme and energy in the liquefaction/saccharification process. Some strains of fungi and bacteria which are capable of producing LA directly from starchy materials using different strategies have been reported in literature (Rojan et al., 2009, Shibata et al., 2007). Shibata et al. (2007) reported the use of *Enterococcus faecium* 78 as a promising microorganism to produce L-(+)-LA using direct raw sago starch in continuous culture and using a hollow fibre cartridge to recycle the cells. *E. faecium* performed well at 30°C and pH 6.5 to have a productivity of 3.04 g/Lh with LA concentration as low as 16.6 g/L. Therefore, in this study we used the RBF for LA production, using liquefied sago starch as the only carbon source with the aim to increase the concentration of LA in the fermented broth.

Materials and Methods

Industrial grade sago starch was liquefied (LSS) using Termamyl SC, according to the enzyme manufacturer's protocol (Novozyme). *E. faecium* No. 78 (BIOTECH 10375) was used throughout this study. One ml of a stock culture frozen at -84°C was thawed and refreshed into 9 ml broth containing 20 g/L of LSS and 5 g/L of yeast extract (YE) and incubated for 24 h at 36 °C in static conditions. The media for LA production was prepared using 20-100 g/L LSS and supplemented with 5 g/L of YE, (Difco, USA). The count of cells was performed by the method of serial dilutions in agar plates containing 10 g/L of glucose, 5 g/L of YE and 1.5% of agar. The dry cell weight (DCW) was determined by a pre-established calibration curve of optical density (OD) against DCW. The fermentations were carried out in 3-L Jar fermenter, fully computer controlled system. The temperature (30°), pH (6.5), agitation (150 rpm), optical density (laser