

## Enhancing $\alpha$ -Amylase and Cellulase *in vivo* Enzyme Expressions on Sago Pith Residue Using *Bacillus amyloliquefaciens* UMAS 1002

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**Abstract:** The effect of Solid State Fermentation (SSF) parameters on the production of extracellular  $\alpha$ -amylase and cellulase (endoglucanases) by *Bacillus amyloliquefaciens* UMAS 1002 using sago pith residue hampas in shaker flasks was investigated in this study. The appropriate incubation period, temperature, pH, agitation speed, inoculum concentration, hampas concentration and additive substrate effect were optimized for enzyme production. The activity of  $\alpha$ -amylase and cellulase was at 14.19 and 13.15 IU mL<sup>-1</sup>, respectively in optimal culture medium. Maximum yield for both enzymes were achieved by employing 4% w/v hampas in 0.2 M citrate buffer at pH 6 and incubated at 40°C for 6 h with agitation speed of 100 rpm. Inoculum concentrations were found to be optimum at 3% v/v and 4% v/v for  $\alpha$ -amylase and cellulase, respectively. Enzyme activity was 2.8 (10.80 IU mL<sup>-1</sup>) and 3.2 (9.38 IU mL<sup>-1</sup>) fold higher for  $\alpha$ -amylase and cellulase respectively when 1% w/v soluble starch was applied as additive substrates with 0.5% hampas. However in optimal media that consist of 4%w/v of hampas, addition of 1% w/v soluble starch intend to inhibit both enzyme productions. Result revealed that temperature, pH and shaking condition were the most significant factors for the production of  $\alpha$ -amylase and cellulase enzyme. Temperature influenced enzyme production by affecting the other parameters including bacterial growth, pH, Dissolved Oxygen (DO) and reducing sugars. Nevertheless, shaking condition could affect DO concentration that in turn affected bacterial growth and enzymes production too.

**Key words:** *B. amyloliquefaciens* UMAS 1002,  $\alpha$ -amylase, celulase, sago pith residue hampas, solid state fermentation

### INTRODUCTION

Solid State Fermentation (SSF) can be defined as insoluble substrate fermented with adequate moisture which has numerous importance compared to submerged fermentation (SmF) or liquid state fermentation (Doelle *et al.*, 1992) due to simple technique applied with lower capital investment, lower level of catabolite repressions and finally for their better product recovery (Babu and Satyanarayana, 1993, 1991; Mulimani and Patil, 2000; Baysal *et al.*, 2002; Ikram *et al.*, 2001). According to Carmelo *et al.* (2002) effluents, especially from food processing industry, which is rich in substrates such as starch, cellulose, fats and proteins, have the potential for microbial degradation to yield products with added values.

Sago starch industry is the main food industry in Malaysia (Barrau, 1995) especially in East Malaysia, which contributes more than 90% of the country production (Bujang and Ahmad, 1999). Bujang *et al.* (1996) have

estimated the three types of sago waste, which are 15.6 tons of woody bark, 237.6 tons of wastewater and 7.1 tons of starchy fibrous sago pith residue or hampas which were generated from the processing of 600 logs of sago palm per day. In the state of Sarawak, Malaysia this hampas is usually washed off into nearby streams together with wastewater, thus contributing to pollution load, or deposited in the factory's compound, which can lead to serious environmental problems. Prior studies show that sago pith waste or hampas is composed mainly of starch (41.7-65.0%) and fiber (14.8%) including a fair amount of minerals (Wina *et al.*, 1986).

An attractive and proficient means in utilizing this waste is through a biotechnological approach in which microbial strains are employed to degrade the sago waste. Microorganisms such as fungi and bacteria are known to play a major role in the degradation of cellulose and starch components (Coughlan, 1985). The genus *Bacillus*, represent one of the important group of bacteria in secreting extracellular commercial enzymes such as