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COMPARISON OF THREE BIO-PROCESSING METHODS IN ETHANOL PRODUCTION: A POTENTIAL GREEN SOLUTION FOR FUTURE ENERGY CRISIS

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Bioethanol is an alternative energy fuel for the current fossil fuel depletion and it can be used either as a fuel enhancer or as an independent fuel. Bio-ethanol production includes two major conversions: cellulose into fermentable sugar and sugar into ethanol. There are three major bio-processing methods that convert cellulose biomass into the end product, 'ethanol'. The present study focused on evaluation of the efficiency of the three different biological pathways involved in bio-ethanol production using carboxymethylcellulose (CMC) as a raw material; Separate enzymatic hydrolysis and fermentation (SHF), Simultaneous saccharification and fermentation (SSF) and Enzyme-microbe synergy method (EMS). The crude cellulase enzyme filtrate used for SHF and SSF methods was obtained from *Bacillus* sp. which was isolated from termite gut. In the SHF method, hydrolysis was performed at 50 ^{o}C for 72 hours using cell-free crude cellulase enzyme filtrate and the fermentation was conducted by decreasing the temperature to 37 ^{o}C with supplementing 15 mL of glucose-free Yeast Extract-Peptone-Glucose (YPG) broth. Subsequently, *Achromobacter* sp., which was isolated from palm wine, was inoculated and incubated for 72 h at 100 rpm. In the SSF and EMS methods, hydrolysis and fermentation steps were conducted under the same conditions, *viz.* temperature 37 ^{o}C and incubation period 72 h, while in the SSF method, hydrolysis was conducted using cell-free crude cellulase enzyme filtrate; in the enzyme-microbe synergy method hydrolysis and fermentation steps were carried out with bacterial cells. The stirring rate was kept at 100 rpm in a shaking incubator for all the processes and ethanol percentage was quantified through solid phase micro-extraction (SPME) by selected ion mode (SIM) method coupled with gas chromatography-mass spectrometry (GC-MS). Bioethanol production from the SSF method was recorded as the highest (1.67%) followed by the SHF method (1.25%) and the EMS method (1.17%). Out of these different bioprocesses, the SSF method was the most efficient for bioethanol production for further studies involving combination of potential bacterial candidates, *viz. Bacillus* sp. with *Achromobacter* sp.

Keywords: Bioethanol, Enzyme-microbe synergy method (EMS), Separate enzymatic hydrolysis and fermentation (SHF), Simultaneous saccharification and fermentation (SSF)