Thesis Title	Microbial Cellulase Saccharification of Oil Palm Empty Fruit Bunch and Its Acid Hydrolysis Residue for Ethanol Production
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Abstract

Twelve samples of soil and plant materials for isolation of microorganisms were collected at locations associated with the palm oil industry. The fifty eight isolates of microorganisms that could be isolated and identified were consisted of 49 isolates of bacteria and 9 isolates of fungi. From 49 isolates of bacterial isolates, 10 isolates were actinomycetes group. Cellulase activity of all isolated microorganisms was determined on carboxymethylcellulose (CMC) agar and demonstrated the enzyme activity by staining the agar with Congo red. The cellulase production was calculated from the ratio of unstained zone surrounding the colony to colony diameter. Actinomycetes, isolate 5.1.A, 11.2.A and 12.3.A had the highest cellulase production. The production of cellulase and reducing sugar from CMC medium of the three isolates were investigated and compared. The isolate 11.2.A and 12.3.A were selected for degrading of oil palm empty fruit bunch (OPEFB) and its acid hydrolyzed residue. The isolate 12.3.A produced higher cellulase and reducing sugar from both substrates than isolate 11.2.A. The optimal conditions for cellulase and reducing sugar production from OPEFB of the isolate 12.3.A were pH 7, temperature at 30°C, and OPEFB concentration at 1% with the highest cellulase activity at 0.71 U/mL and reducing sugar at 3.83 mg/mL. For the acid hydrolyzed residue of OPEFB, the similar conditions were observed, but the cellulase and reducing sugar production were only at 0.56 U/mL and 3.04 mg/mL, respectively. The suitable nitrogen sources for the culture medium made of OPEFB and its residue were also verified. Ammonium sulfate was a proper nitrogen source for OPEFB, while peptone was for the residue. A partial purification of cellulase by 60% ammonium sulfate precipitation and subsequently dialysis had raised the specific activity 57% (specific activity at 7.38 U/mg). The zymogram assay with polyacrylamide amended with CMC demonstrated that the isolate 12.3.A produced 2 CMCases (endo β-glucanases). The optimal conditions for the cellulase activity were at pH 6.5 and 45^{o}C. The saccharified products of the isolate 12.3.A cellulase were then fermented in batch cultivation by *Saccharomyces cerevisiae*. With initial value of reducing sugar at 1.77 g/L and 1.01 g/L, *S. cerevisiae* produced maximum ethanol at 0.76 g/L and 0.41 g/L for OPEFB and the residue, respectively, in 72 hours. This is indicated that *S. cerevisiae* could ferment sugar from OPEFB and the residue and then convert sugar to ethanol. The 16S-rRNA gene analysis of the isolate 12.3.A was 99% similarity with *Streptomyces hirsutus* strain NRRL B-2713. Therefore, the isolate 12.3.A was preliminary appointed to be *Streptomyces hirsutus*.

Keywords : *Streptomyces* / Cellulase Activity / Reducing Sugar / OPEFB/ Acid Hydrolyzed Residue