Topic: Development of Efficient Fungal Biomass-Degrading Enzyme Mixtures for Saccharification of Local Lignocellulosic Feedstock

Name of student: Ms. Wanwitoo Wanmolee **Student ID:** 55300700514

Name of supervisor: Assoc. Prof. Dr. Navadol Laosiripojana

Name of co-supervisor: Dr. Verawat Champreda

ABSTRACT

Conversion of agricultural biomass to biofuels and value-added chemicals is the basis of the sustainable and environmentally-friendly biorefinery industry. The development of effective lignocellulolytic microbial and enzyme systems is a challenge for the successful establishment of cost-effective biomass conversion processes. The culture media parameters such as carbon, nitrogen sources and types of medium were investigated using one factor at a time (OFAT) analysis. The optimization of fermentation conditions including concentration of carbon source, inducer and initial pH were further studied using multivariate analysis; besides, addition of oil palm empty fruit bunch (OPEFB) was observed as co-substrate for enhancing yield of cellulases. Among various complex biomass and synthetic substrates for carbon and nitrogen sources contained in fermentation media, 1% (w/v) OPEFB, 2% microcrystalline cellulose (AVICEL®) and 1% soybean meal (SBM) in distilled water were found to be potent sources for the production of cellulase by *Chaetomium globosum* BCC5776. The incubation temperature, time and initial pH were 30 ºC, 6 days and 5.8, respectively. The maximum volumetric productivity of cellulase was 0.40 FPU/ml with specific activity of 0.79 U/mg proteins from submerged fermentation. Further optimized culture fermentation condition, the BCC5776 fungal enzyme extract was produced in 1-L and 5-L bioreactor. Comparable yield of cellulase activity (0.359 FPU/ml, 0.77 U/mg) was obtained when the enzyme production was up-scaled in a 5-L bioreactor. The crude cellulose enzyme from BCC5776 worked optimally in temperature range of 50-60ºC with pH range of 5.0-6.0. A synergistic enzyme system for the hydrolysis of alkaline pretreated rice straw was then optimized based on mixture design approach based on the synergy of the BCC5776 crude fungal enzyme extracts with commercial enzymes β-glucosidase Novozyme® 188 and hemicellulase Accellerase® XY. Using the full cubic model, the optimal formulation of the enzyme mixture was predicted to the percentage of BCC5776:Novozyme® 188: Accellerase® XY = 44.4:20.6:35.0, which

produced 572.68 mg reducing sugar/g pretreated rice straw enzymes. The highest total reducing sugar was approximately 764.69 mg reducing sugar/g pretreated rice straw using enzyme loading equivalent to 15.53 FPU/g when amount of enzyme dosage was increased four folds. The work showed potential of cellulase from *Chaetomium globosum* BCC5776 for lignocellulose hydrolysis for further conversion to fuels and chemical in sugar-platform biorefineries.

Keyword: Biomass, cellulase, *Chaetomium globosum*, saccharification