Development of Biofuel and Biomaterial Production Technologies for Cassava Pulp as Starchy and Cellulosic Biomass

澱粉混在セルロース系バイオマス・キャッサバパルプからのバイオ燃料及びバイオマテリアル生産技術開発

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Abstract

Cassava pulp (CP), a byproduct from cassava starch industry, that consists not only cellulose and hemicellulose as fiber (20-30%) but also starch (50-60%). It has a large potential as renewable bioresources for bio-based product production. This thesis describes that development of biofuel and biomaterial production technologies from CP.

In chapter 1, the energy crisis has been occurred because almost chemical products and fuels had been produced from fossil fuel such as petroleum. Fossil fuels are depleting, unsustainable in the long term and unfriendly with environmental. To reduce this problem, utilization of renewable energy resources has been developed. Bioethanol is renewable biofuel and environmentally friendly which can produce from CP. Anyway, starch in CP is trapped and embedded inside of complex structure of fiber. Suitable pretreatment is required to liberate the starch from CP for ethanol production. Biochemical such as 1,3-propanediol (1,3-PD) can produce by chemical method which requires expensive catalyst, high pressure and also generate toxic intermediates during reaction. Glycerol can be converted to 1,3-PD by microbes in biological method but growth, yield and productivity rate are limited. So, co-substrate fermentation is required to improve this problem. The other way to utilize CP is biogas production. However, the biogas production cost is high because pretreatment is

necessary step for lignocellulosic utilization. To reduce production cost, direct utilization technology by anaerobic microbes is developed.

In Chapter 2, to enhance saccharification of CP by amylolytic and cellulolytic enzymes, effective pretreatment is desired to use for raw CP before enzymatic treatment. Ammonia gas absorption fiber expansion (AGAFE) was developed to accomplish efficient saccharification of CP. AGAFE was designed to be used under moderate pressure by directly adding compressed ammonia gas before rapidly releasing the pressure. Scanning electron microscopy analysis showed that the cell wall of AGAFE-pretreated CP was severely disrupted, while the fiber of the raw CP remained largely intact. Additionally, the concentration of glucose released from the hydrolysis of pretreated CP was higher than that obtained from raw CP using α-amylase and glucoamylase. The optimum pretreatment conditions for AGAFE were 0.2 MPa and 70 °C for 10 min, which resulted in saccharification of 83.2% of the total starch in CP which higher than raw CP (53.7%). To confirm whether the AGAFE-pretreatment influenced ethanol fermentation, a fermentation test using the hydrolysate from AGAFE-pretreated CP was carried out *using Saccharomyces cerevisiae* NCYC3233 and *Kluyveromyces marxianus* TISTR5925. The result showed that >85 % of the theoretical yields were achieved without inhibition from any mixture components.

In Chapter 3, to establish efficient bioproduction process, CP was utilized to fermentation process for biomaterials. 1,3-PD is one of the important products in industry and mainly used as a substrate for biopolymers such as polytrimethylene terephthalate. 1,3-PD is commonly known to produce from glycerol by microorganism such as *Clostridium butyricum*, however the productivity and yield are quite low due to its poor growth by low glycerol assimilation. To obtain *C. butyricum* with high starch utilization ability, strain I5-42 was isolated from compost. This study shows that supplementation of 10 g/L glycerol medium with soluble starch was able to improve the productivity and yield of 1,3-PD production by *C. butyricum* I5-42, and 1 g/L starch supplementation was the best by increasing productivity rate and yield. In addition, 1,3-PD production could also improve by using starch free cassava pulp fiber (CPF) from CP. The 1,3-PD production and growth of *C. butyricum* were considerably higher in glycerol plus xylan (a major hemicellulosic component of CPF) than in glycerol alone, although xylan is not utilized as a carbon source. CPF and xylan supplementation strongly enhanced the transcription levels of the major enzymes of 1,3-PD production (glycerol dehydratase, 1,3-propanediol dehydrogenase, and glycerol dehydrogenase). The intracellular redox reactions maintained equal balance in the supplemented media, suggesting that CPF and xylan promote 1,3-PD production in the reductive pathway by effectively regenerating NADH.

In chapter 4, to propose more direct utilization technology of CP, anaerobic microbes which can solubilize CP were developed to production process of biogas as biofuel. Power generation by biogas is very important to maintain zero-emission on starch factory. Anaerobic thermophilic microorganisms were successfully isolated from biocompost. Isolated strain W21.10 that has high degradation ability for raw CP was formed bacterial communities composed to cellulolytic anaerobic microbes and amylolytic anaerobic microbes. This consortium could degrade not only CP but also various types of biomass such as rice straw, erianthus, CPF, corn hull, corn, bagasse and oil palm residual. To reveal bacterial member consisted of the consortium, metagenomic approach was carried out for the consortium. *Clostridium thermocellum* and *Cullosibacter alkalithermophilus* was dominantly occupied in the consortium. To understand role for each bacteria, the two anaerobic microbes were successfully isolated from the consortium. Co-culture of isolated *C. thermocellum* strain F and *C. alkalithermophilus* AP could show a high synergistic effect for CP degradation.

These results indicate that *C. thermocellum* F and *C. alkalithermophilus* AP play as lignocellulose degradation and starchy degradation for CP, respectively.

Based on the results, CP is promising substrate to produce bio-based production. AGAFE is a useful pretreatment option for ethanol production from biomass containing starch and fiber such as CP. CP (starch, CPF) and their polysaccharide (xylan) can also be used as co-substrate of 1,3-PD production to improve growth of *C. butyricum*, yield and productivity rate of 1,3-PD. Finally, CP can be used as substrate for biogas production by direct degradation using isolated strain W21.10. The combination of *C.thermocellum* F and *C.alkalithermophilus* AP showed variety degradation of biomasses that contains starch and fiber at the same time.