Abstract of the Dissertation:

ADVANCED BIOETHANOL PRODUCTION FROM NIPA PALM SAP VIA ACETIC ACID FERMENTATION

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Nipa, scientifically known as *Nypa fruticans*, is a non-threatened and underutilized palm found along river estuaries, coastal areas and mangrove forests. It produces sugar-rich sap which can be used for various purposes but has never been commercialized due to limited understanding of the chemical composition of the sap and lack of adequate technologies. With high potential and sustainability, nipa sap has recently been explored as a raw material for bioethanol production. However, conventional bioethanol production through alcoholic fermentation by *Saccharomyces cerevisiae* releases CO_2 as by-product and consequently reduces the total conversion efficiency of sugars to ethanol. Under such circumstances, a novel process for bioethanol production from nipa sap without CO_2 emission is proposed. It involves hydrolysis of nipa sap, fermentation of the hydrolyzate to acetic acid by *Moorella thermoacetica* and hydrogenolysis of the obtained acetic acid into bioethanol. Thus, the goal of the current study is to apply nipa sap for advanced bioethanol production.

Chapter 1 reviewed the availability of palm saps, their methods of collection, chemical composition and various uses [1]. Comparison of various palm saps and their potential lead to the selection of nipa sap as promising raw material for the advanced bioethanol production via

acetic acid fermentation by *M. thermoacetica* and subsequent hydrogenolysis.

Because the effects of gas conditions on acetic acid fermentation are not well understood yet, the growth and activity of *M. thermoacetica* under different gas conditions were explored in Chapter 2 using glucose as substrate [2]. As a result, sparged N_2 caused severe inhibition to the growth of *M. thermoacetica*, leading to almost no sugar consumption and no acetic acid production. In contrast, both non-sparged N_2 and sparged CO_2 could support rapid cell growth and effective acetic acid production. Although the growth of *M. thermoacetica* under sparged CO_2 was slightly stronger and could be maintained longer than that under non-sparged N_2, both of these gas conditions provided similar conversion efficiencies of around 82% after 97 h of fermentation. Thus, non-sparged N_2 and sparged CO_2 were concluded to be the most appropriate gas conditions for acetic acid fermentation of nipa sap by *M. thermoacetica*.

Nipa sap is predominantly composed of glucose, fructose and sucrose. Among these sugars, *M. thermoacetica* cannot directly ferment sucrose. To overcome this issue, in Chapter 3, acid catalysts and invertase as enzyme were investigated for hydrolysis of sucrose in nipa sap to fermentable glucose and fructose [3]. The results showed that acetic acid was too weak to hydrolyze sucrose rapidly and completely, whereas hydrochloric acid was too strong and caused monosaccharides decomposition. In contrast, both oxalic acid and invertase could hydrolyze sucrose in nipa sap to the corresponding yields of monosugars. Nipa sap hydrolyzates from oxalic acid and invertase treatments were fermented by *M. thermoacetica*. The results revealed that the two hydrolyzed nipa saps provided similar conversion efficiencies of around 98% and acetic acid concentration of 9.9 g/L. This confirms that both catalysts do not show negative effects on the subsequent fermentation step. Notably, oxalic acid was also found to be consumed by *M. thermoacetica*; avoiding the necessity to separate it after acetic acid fermentation.

In Chapter 4, batch fermentations of high substrate concentrations were undertaken to improve acetic acid concentration [4]. However, increasing substrate concentration in batch fermentation was found to cause low conversion efficiency and reduced acetic acid productivity. Therefore, fed-batch fermentation with different feeding rates was investigated. Low and high feeding rates provided similar final acetic acid concentrations of 42.3 and 42.6 g/L, and conversion efficiencies of 86 and 87%, respectively. However, fed-batch fermentation with high feeding rate reduced the total fermentation time and therefore provided 1.7-fold higher acetic acid productivity than that with low feeding rate. Compared with batch fermentation of hydrolyzed nipa sap, fed-batch fermentation technique could increase acetic acid concentration

by approximately 4.3-fold. These results show that fed-batch fermentation with high feeding rate is the appropriate method to increase acetic acid concentration from nipa sap.

In Chapter 5, minor compounds present in nipa sap were explored as potential nutrients for *M. thermoacetica* [5]. The results revealed that nipa sap could be fermented to acetic acid with and without addition of external inorganics, demonstrating that the inorganic elements present in nipa sap could have been used as nutrients for *M. thermoacetica*. Since fermentation process usually requires nutrients, acetic acid fermentation without inorganic supplement is economical for the final bioethanol production. This property makes nipa sap a valuable alternative feedstock for bioethanol production.

Chapter 6 aims to evaluate bioethanol production from nipa sap. For that purpose, the overall conversion efficiency of nipa sap to bioethanol using the advanced process was estimated. Based on the result from hydrogenolysis, the conversion efficiency can potentially reach 0.62-0.74 g ethanol/g sugars which is 29-54% higher than the result from traditional alcoholic fermentation. Subsequently, the advanced bioethanol process was designed and simulated in Pro/II^{TM} software to estimate energy consumption. As a result, energy consumption was found to be 38 MJ/L bioethanol, which is as competitive as that for the traditional alcoholic process. The preliminary results from this process simulation can be useful for further pilot-plant process design of bioethanol production from nipa sap via acetic acid fermentation.

Finally, the above-mentioned investigations were summarized and concluded in Chapter 7. Future prospects of this research were proposed.

References

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