UTILIZATION OF LIGNIN IN EMPTY FRUIT BUNCH FOR PRODUCTION OF FINE CHEMICALS: DEVELOPMENT OF SUBCRITICAL WATER TECHNOLOGY AND Δ*pcaHG*-Δ*catA* *Rhodococcus jostii* RHA1

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SUMMARY: Lignin is an alternative source of chemicals particularly phenolic compounds if it could broken down into smaller molecular units. Subcritical water (SCW) is a known technology that has the ability to break down lignin by hydrolysis. In this study, the SCW depolymerizes empty fruit bunch derived lignin into a mixture of aromatic compounds. The mixture, however, is impractical to be utilized and the cost to separate each of the components is relatively high. *Rhodococcus jostii* RHA1 is a bacterium that can degrade a wide range of aromatic compounds. Specific gene deletion of RHA1 has shown that the RHA1 loses its ability to catabolize specific chemicals. This research project aims to utilize empty fruit bunch derived lignin via SCW technology and subsequently biological process using mutant *Rhodococcus jostii* RHA1, Δ*pcaHG*-Δ*catA* mutant RHA1.

Keywords—*Empty fruit bunch (EFB), Lignin, Mutant* Rhodococcus jostii *RHA1, Subcritical water, Catechol/protocatechuic acid (PCA)*

INTRODUCTION

Fossil fuel such as petroleum is an important energy resource and a raw material for chemical production [1,2]. Currently, the production of chemicals for fine chemicals manufacture, polymer synthesis and food (or flavour) chemistry is a by-products of the petrochemical industry [3]. Due to the high reliance on the non-renewable fossil fuels, most countries confront several major dilemmas which are global warming, acid rain, and depletion of fossil fuel [1]. The dilemmas have forced researchers to investigate alternative to fossil fuel as well as new routes to produce chemical feedstock.

Currently, biomass seems to be a promising alternative source of energy and chemicals particularly for countries that promote agricultural activities where biomass is abundantly available and inexpensive [1]. *Elaeis guianensis*, also known as oil palm is one of the important agricultural crops in Malaysia [1]. The estimated amount of oil palm biomass waste produced in 2008 was approximately 37.0 million tonnes; weight percentages of empty fruit bunch (EFB), fruit press fibre (FPF), and shell were 22%, 13.5% and 5.5% respectively [4]. EFB can be clearly seen as the main by-product produced from oil palm industry and in 2014 the amount of EFB generated has reached approximately 17.08 million ton [5].

The EFB is a lignocellulose material which consists of 44.20%, 33.50%, and 20.40% of cellulose, hemicellulose and lignin respectively [6]. Current biorefinery schemes utilize lignocellulose materials only for bioethanol production while lignin remains underutilized to its potential or is used as a low grade boiler fuel [7]. The structure of lignin, however, suggests that it can be a valuable source of chemicals.

SCW process decomposes lignin into aromatic compounds. These compounds exist in the form of mixture in liquid phase. It is not only unfeasible to utilize the mixture of aromatic compunds, but the cost to separate each of the components is rather high. Biological process seems to be a promising approach to solve the unfeasible and high cost concerns. Δ*pcaHG*-Δ*catA* mutant RHA1 has the ability to degrade most of the aromatic compounds to 1,2-dihydroxybenzene (catechol) and 3,4-dihydroxybenzoic acid (protocatechuic acid) as well as accumulating these two chemicals.

Therefore, the aim of this study is to utilize the empty fruit bunch derived lignin for production of fine chemicals via combination of subcritical water technology and biological process; the candidate bacterium used is Δ*pcaHG*-Δ*catA* *Rhodococcus jostii* RHA1.

2. MATERIALS AND METHODS

2.1 Sampling and Sample Sreparation

EFB(s) were collected from the Felda Maokil Labis, Johor. The EFB were kept in a refrigerator (-20ºC) to avoid any kind of bacteria growth and deterioration. EFB(s) were cut and ground to 1000µm prior to being dried. Drying of raw EFB was done at 105°C for 24 hours.

2.2 Characterization of Empty Fruit Bunch

Dried EFB was characterized in terms of its moisture, ash, and organic matter contents. The procedures were done by following the APHA Method 2540G.

2.3 Subcritical Water Process

The SCW process was performed using Pressurized Micro-Reactor. Dried EFB was weighed (5g) and loaded into the reactor and mixed with 100mL of deionized water. The assessed parameters for the SCW process are temperature, reaction time and types of solvent used.

2.4 Separation Process

Products after subcritical water process were divided into two phases which are liquid (water) and solid (residue) phases. The products were filtered using Whatman qualitative filter paper to separate the two phases.

2.5 pH Value and Total Organic Carbon (TOC) Analysis

The pH values of liquid products at different temperatures were done ex-situ using the benchtop pH meter (Mettler Toledo). The TOC was measured using the Shimadzu TOC Analyzer

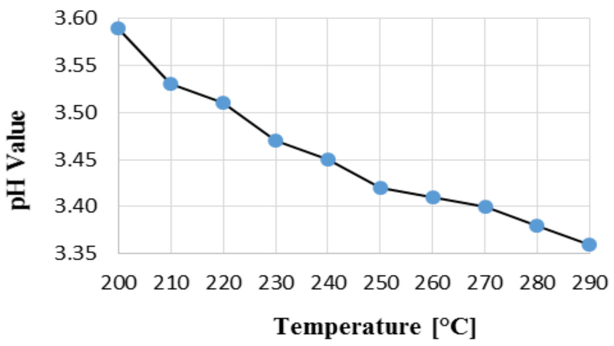

Fig 1: pH value analysis of liquid products after SCW process

3. RESULTS AND DISCUSSION

3.1 pH Value Analysis

Fig. 1 showed the pH value decreased as temperature of reactor increased. This implied that at higher temperature, there was a probability that recalcitrant lignin was decomposed into phenolic compounds (acidic compounds). The acidic value could also be caused by the broken down of homocellulose to form some acids (e.g. acetic acid). Further analysis needs to be done to identify type of compounds that contribute to the acidic value.

3.2 TOC Analysis

Fig.2 showed that the value of TOC increased as temperature increased from 200°C to 270°C and decreased after 270°C. An optimum value of TOC was found to be 211.44mg/gEFB. This result implied that at high temperature, many organic compounds formed. However, the type of organic compounds formed were to this point unknown. Further analysis needs to be perfomed to identify type of organic compunds formed.

3.3 Future Analysis and Planning

In order to determine what type of compounds formed after SCW process, analysis by High Performance Liquid Chromatography (HPLC) is necessary. The HPLC analysis will be done afterward. The effect of reaction time and types of solvent used will also be done afterward. As mentioned in the introduction, this study combines subcritical water process with biological process. In biological process, Δ*pcaHG*-Δ*catA Rhodococcus jostii* RHA1 will be used. This will also be done subsequently.

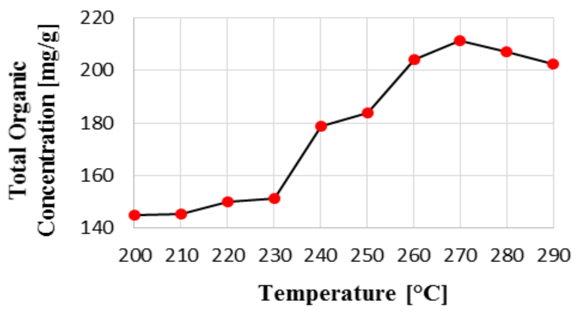

Fig. 2. TOC value analaysis of liquid products after SCW process

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