Depolymerization of Microbial-Derived Polyhydroxybutyrate (PHB) in an Aqueous Environment

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**Introduction:** Polyhydroxybutyrate (PHB) is a biopolymer produced by bacteria as an energy storage compound, and processes for obtaining and using PHB focus on its value as a biodegradable-derived plastic. However, the process for culturing and extracting PHB with high enough quality for plastic applications is often expensive, requiring pure bacterial culture conditions. In addition, extraction and processing of PHB is energy intensive and commonly involves use of toxic solvents. Both problems limit PHB use in industrial applications. This project aims to examine an alternative accumulation method and application for PHB, integrating it into wastewater treatment and producing a renewable hydrocarbon fuel. The proposed plan is to cultivate PHB-rich biosolids from a mixed culture during wastewater treatment in a process that also works well for nitrogen removal, then extract and depolymerize the PHB before converting the resulting monomers to propylene, all under aqueous conditions. Ultimately, the volatile propylene can be oligomerized into gasoline-grade hydrocarbons. The product fuel would be more energy dense than biogas, the traditional energy product recovered during wastewater treatment, and the use of a mixed culture alongside aqueous extraction and reaction conditions would lower processing costs considerably. The process would also reduce overall costs of wastewater treatment by recycling the carbon in the waste stream and producing an energy dense fuel.

**Research Goals and Procedure:** This REU project focused specifically on characterizing the aqueous PHB depolymerization step in the overall process described above. PHB is ideally depolymerized into a mixture of 3-hydroxybutyric acid (3HB) and crotonic acid (CA) through hydrolysis at high temperatures. The objective for the research was to optimize conditions for the depolymerization by controlling reaction time, temperature, and PHB loading to maximize conversion of PHB into its component monomers while minimizing energy costs. Previous research indicated that at a 4.7 wt% loading, a 4 hour reaction at 200°C achieved the highest extent of conversion of PHB (Saeki, Tsukegi, Tsuji, Daimon, & Fujie, 2005); however, they did not report on a distribution of CA and 3HB. This study analyzed product distribution from reactions using 5 wt% and 10 wt% loadings of PHB and explored a range of temperatures and reaction times centered around 4 hours at 200°C. These loadings were considered because processing lower loadings is not practical due to the energy requirement for heating water.

Reactions were carried out in sealed 3 inch long, ½ inch diameter steel tubes placed inside a furnace for the desired temperature and time. Approximately 0.4 grams of PHB and 3.6 grams deionized water were added to each reaction tube before sealing, to yield a ~10 wt% PHB mixture. After the reaction, gas production was quantified by the weight difference prior to and after opening the tubes. Residual solids were then collected and weighed to estimate the extent of PHB depolymerization. Aqueous reaction products (3HB and CA) were analyzed using high performance liquid chromatography (HPLC) with UV detection. The goal for the reaction was to fully depolymerize PHB selectively to CA and 3HB with minimal gas product production.
Results: Product yields from various reaction conditions can be seen in Figures 1 and 2. Figure 1 shows the effect of time in each chart and the effect of loading by comparing the charts side by side. From these results, a base case of 4 hours and 10 %wt loading was chosen for further testing alongside 2 cases of potential energy sacrifices, 4 hours and 5 wt% loading and 6 hours and 10 wt% loading. These results across a temperature range of 175-225 °C can be seen in Figure 2. Figure 3 shows the molar ratio of CA to 3HB over different reaction conditions. While it does increase with more severe conditions and longer reaction times, gas production does increase alongside it if you refer to the product yield charts for the higher points. Analysis of the gas was not completed, but it is hypothesized to be propylene and carbon dioxide.

Discussion: The results of this study reflect previous research as the optimum conditions still center around 4 hours at 200°C (Saeki et al., 2005). However, this study shows conversion can be achieved at 10 wt% loading as well as, if not better than, the 5 wt% loading in order to achieve higher water and energy efficiency. Additionally, a longer time of 6 hours and a lower temperature of 190°C achieve similar results. Since the reactions occurred in aqueous conditions, it was expected that 3HB be more prevalent than CA across the board as it results from hydrolysis of PHB while CA results from pyrolysis (Kang & Yu, 2015). The increase in the CA/3HB molar ratio at longer times and higher temperatures indicates that this ratio can be controlled by reaction conditions to potentially benefit downstream processing. However, the implications of the gas production at these conditions are still unclear. If the gas is actually propylene and carbon dioxide, it may be ideal to optimize the gas production to obtain propylene in this step, eliminating the need for dehydration and decarboxylation in the catalytic operations unit later on in the process. Overall, these results support the goal of an integrated process to convert PHB to propylene all under aqueous conditions, a process that will derive value and recycle carbon from a wastewater stream.
References: