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Biological production of a hydrocarbon fuel intermediate polyhydroxybutyrate (PHB) from a process relevant lignocellulosic derived sugar

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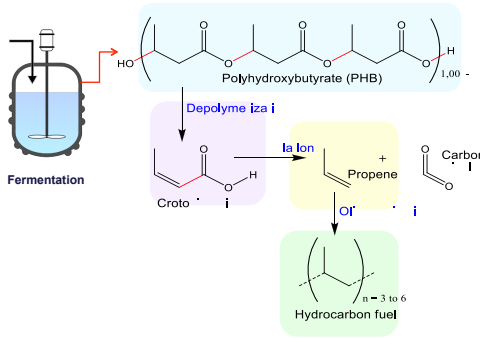
Abstract

PHAs are synthesized by many microorganisms to serve as intracellular carbon storage molecules. In some bacterial strains, PHB can account for up to 80% of cell mass. In addition to its application in the packaging sector, PHB also has great potential as an intermediate in the production of hydrocarbon fuels. PHB can be thermally depolymerized and decarboxylated to propene which can be upgraded to hydrocarbon fuels via commercial oligomerization technologies. *Alcaligenes eutrophus* is the microorganism that has been most extensively studied and used for PHB production on an industrial scale; However the substrates used for producing PHB are mainly fructose, glucose, sucrose, fatty acids, glycerol, etc., which are expensive. In this study, the strain was grown on pretreated lignocellulose hydrolyzate slurry and evaluated in terms of cell growth, sugar utilization and PHB accumulation. In addition, the mechanism of inhibition in the toxic hydrolysate generated by the pretreatment and saccharification process of biomass, was also investigated.

Sugar to hydrocarbon fuel process flow diagram



Materials and methods

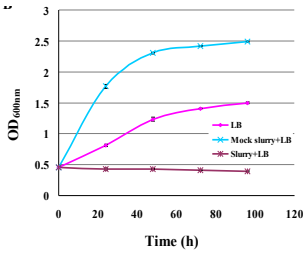
Microorganisms

Alcaligenes eutrophus NCIMB 11599

PHB analysis

The PHB content of bacterial cells was determined by a quantitative method that uses HPLC to measure the CA formed by acid-catalyzed depolymerization of PHB. Briefly, freeze dried bacterial cells (15 to 50 mg) were digested in 96% H_2SO_4 (1 mL) at 90°C for 1 hr. The reaction vials were then cooled on ice, after which, ice-cold 0.01N H_2SO_4 (4 mL) was added followed by rapid mixing. The samples were further diluted 20- to 150-fold with 0.01N H_2SO_4 and analyzed by HPLC.

Growth of A. eutrophus in pretreated saccharified lignocellulosic slurry*

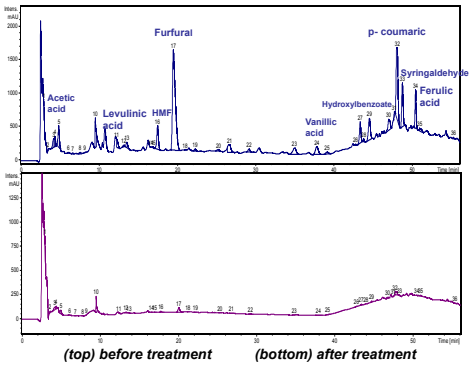


* Hydrolysate slurry was saccharified with Ctec2 Htec2 enzyme from Novozyme

A. eutrophus did not grow in the original saccharified slurry or even in the 2-fold diluted slurry. This low viability of cells suggests that the strain is not tolerant to the high concentration of toxic compounds in the hydrolysate slurry

Treatment of acid hydrolysate slurry with activated carbon

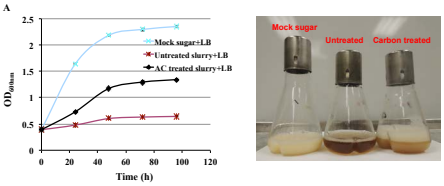
LC-MS analysis



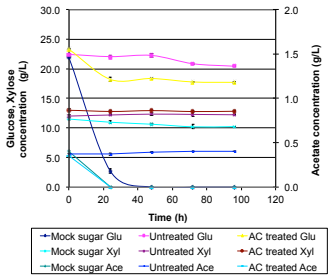
(top) before treatment *(bottom) after treatment*

LC-MS results showed that most of the inhibitors in the slurry including furfural, HMF, vanillic acid, coumaric acid etc. have been removed by carbon treatment

Cell growth in carbon treated hydrolysate slurry (4x)

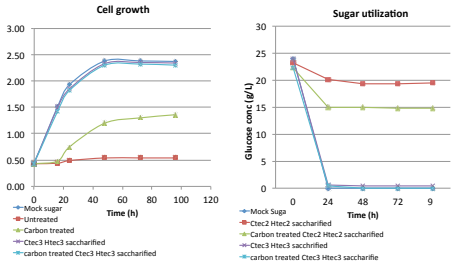


Sugar utilization



Better cell growth was found in carbon treated slurry however glucose utilization was much slower than sugar control indicating other inhibition still existed.

Growth in slurry saccharified with advanced enzyme



* Hydrolysate slurry (4x diluted) was saccharified with Ctec3 Htec3 enzyme from Novozyme

A.eutrophus grew well in new enzyme saccharified slurry (4x diluted). Similar cell growth and sugar utilization rate, and almost same PHB content of 33% as in mock sugar medium were achieved.

Conclusions

- Carbon treatment was effective in removing most chemical inhibitors in the acid hydrolysate slurry. Although these chemicals proved to be inhibitory to *A. eutrophus* they were not the vital factor leading to inhibition for the microorganism;
- The good performance of strain in slurry saccharified with advanced enzyme indicated some compounds generated in saccharification process in first slurry, most likely oligo-saccharides, accounted for much of the inhibition;
- Lignocellulosic hydrolysate can be used as substrate for biological production of PHB.

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