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

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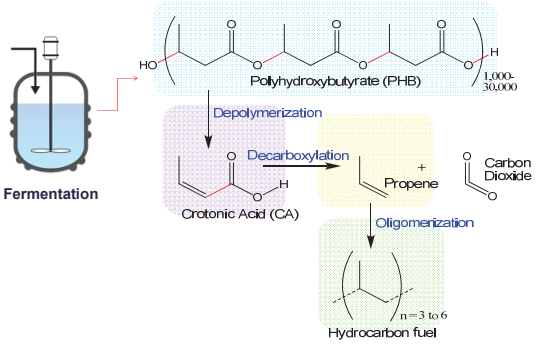
Abstract

PHAs are synthesized by many microorganisms to serve as intracellular carbon storage molecules. In 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. Although PHB has been produced by microorganisms at a large scale in industry, the substrates used for producing PHB are mainly fructose, glucose, sucrose, glycerol, etc., which are expensive. In this study, the strain was grown on pretreated lignocellulose hydrolyzate and evaluated in terms of cell growth, sugar utilization and PHB accumulation. The PHB content reached 70% of dry cell weight when *Alcaligenes eutrophus* was grown on 2X diluted saccharified solids of pretreated corn stover.

Schematic diagram of sugar to hydrocarbon fuel



Materials and methods

Microorganisms

Alcaligenes eutrophus NCIMB 11599

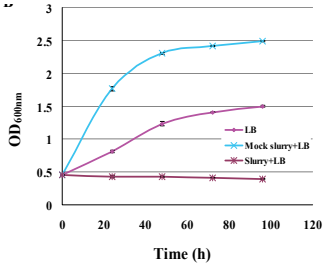
Substrate material

Saccharified slurry/solids of acid-pretreated corn stover

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.

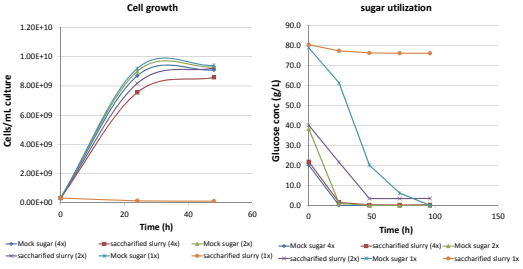
Growth of *A. eutrophus* in pretreated saccharified lignocellulosic slurry*



* Hydrolysate slurry was saccharified with Ctec2 Htec2 enzyme from Novozyme

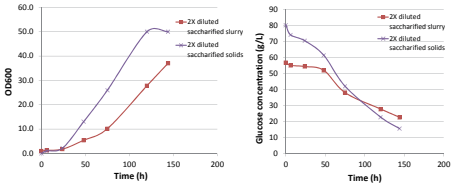
A. eutrophus did not grow in the original saccharified slurry or even in the 2x diluted slurry. This low viability of cells suggests that the strain is not tolerant to the high concentration of toxic compounds in the hydrolysate slurry.

Growth in slurry saccharified with advanced enzymes



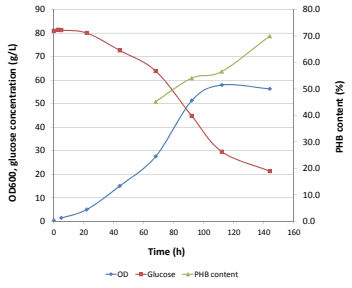
Significant growth of *A.eutrophus* was observed in flask using slurry saccharified with advanced enzymes. Similar cell growth was achieved as on mock sugar, and PHB content of 41% was obtained as compared to mock sugar (52% PHB content).

Fermentation in 1-L bioreactor



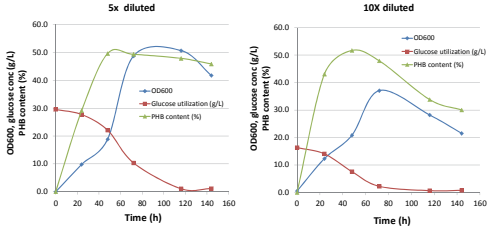
Good cell growth was achieved on 2X diluted saccharified slurry however a 48h lag phase was observed for glucose utilization. Growth was much better when saccharified washed solids were used instead of saccharified slurry, which indicates something in the acid hydrolysate liquor, most likely xylo-oligomers, inhibited the cell growth.

On 2X diluted saccharified solids



70% PHB accumulation was achieved at 120h when grown on 2x diluted saccharified solids, which was very close to growing on mock sugar; however the glucose utilization rate needs improvement, so a lower concentration of saccharified solids was tested

On 5X and 10X diluted saccharified solids



A. eutrophus grew well on 5X diluted saccharified solids and had a better glucose utilization profile than 2X diluted solids. At 116h, the cell mass reached 9.6 g/L with PHB content of 50%. This can serve as a baseline for our next step, fed-batch fermentations.

Conclusions

- The better performance of *A. eutrophus* on saccharified solids than saccharified slurry indicated compounds in the hydrolysate liquor generated in pretreatment, most likely xylo-oligomers, accounted for much of the inhibition;
- Different fermentation modes, such as fed-batch and continuous culture will be investigated based on the batch results above, aiming at high cell density and PHB yield;
- Lignocellulosic hydrolysate can be used as a substrate for biological production of PHB based on our studies.

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