Studies on acetone-butanol-ethanol fermentation from designed renewable substances

趙, 涛

Name　：Zhao Tao

Title　：Studies on acetone-butanol-ethanol fermentation from designed renewable substances (デザイン化した再生可能原料から効率的なアセトン-ブタノール-エタノール発酵プロセスの構築に関する研究)

Category：Kou

Thesis Summary

Biobutanol is an outstanding biofuel because it has many chemical and physical features compared with traditional biofuels. The biobutanol can be produced from acetone-butanol-ethanol (ABE) fermentation using inedible substrates, such as lignocellulosic materials and organic acids. However, the fermentation process suffers from 3 major problems, namely, high material costs of pretreatment liquor, enzymes, etc., insufficient utilization of lignocellulosic biomasses and products inhibition. To construct efficient ABE fermentation, we need to select appropriate biomasses and design the processes. Therefore, I focused on the establishment of highly productive fermentation systems for butanol production from hydrolysate of paper pulp, rice straw and mixed acetic acid and lactic acid by using butanol hyper-producer, *Clostridium. saccharoperbutylacetonicum* N1-4 (ATCC 13564).

I successfully hydrolyzed paper pulp, producing high concentrations of cellobiose (13.9 g/L) and glucose (21.3 g/L) due to low lignin content. Sequentially, I performed ABE fermentation using semi-hydrolysate of paper pulp, which was as efficient as fermentation using commercial sugars without inhibitors. Furthermore, I compared fermentation performances of paper pulp hydrolysate in separated hydrolysis and fermentation, simultaneously repeated hydrolysis and fermentation and a novel non-isothermal simultaneous saccharification and fermentation with in-situ butanol recovery. Finally, the non-isothermal simultaneous saccharification and fermentation with in-situ butanol recovery improved butanol production, that is 20.6 g/L, butanol yield to consumed sugar (0.847 C-mol/C-mol), butanol yield to solid loading (197 g/kg-pulp), butanol yield to enzyme loading (0.0360 g/U-cellulase), butanol yield to medium (20.6 g/L-medium), butanol productivity (0.343 g/L/h) and overall butanol productivity (0.286 g/L/h).

ABE fermentation of the semi-hydrolysate of rice straw (containing cellobiose, glucose, and xylose) was performed with low cellulase loading which repressed carbon catabolite repression, unlike a glucose-oriented hydrolysate with high enzyme loading. Furthermore, inoculation with high cell density was initially used to overcome inhibition in ABE fermentation by inhibitors formed from the pretreatment and enzymatic hydrolysis. Finally, an effective simultaneously repeated hydrolysis and fermentation for butanol production was established from semi-hydrolysate by recycling cellulases without enzyme supplementation or cell inoculation, showing the highest butanol yield to enzyme loading (0.0898 g/U) among the studies reported so far.

I initially used mixed acetic acid and lactic acid for ABE fermentation. Addition of mixed acids made up deficiencies of inefficient substrate consumption using lactic acid and low ratio of butanol

to acetone using acetic acid. This result was verified by ^{13}C tracer experiment that addition of lactic acid improved carbon distribution to butanol instead of acetone and acetic acid was more readily consumed than lactic acid at initial fermentation stage. In addition, the ABE fermentation was influenced by pH and compositions of glucose and acids. The pH-stat fed batch fermentation at pH 5.5 produced 12.4 g/L butanol and 6.50 g/L acetone with consumption of 28.9 g/L glucose, 6.34 g/L acetic acid and 9.06 g/L lactic acid. Thus, efficient ABE fermentation can be constructed by using mixed acids.