

## Studies on extractive butanol fermentation by immobilized cells with large extractant volume

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(新たな抽出剤を使用した固定化菌体によるブタノール抽出発酵プロセスの構築に関する研究)

Category : Kou

### Thesis Summary

Product inhibition by butanol and acetone is a known drawback in acetone-butanol-ethanol (ABE) fermentation. Extractive fermentation improves butanol production by several ABE-producing *Clostridium* spp., but only low volume ratios ( $< 4$ ) of extractant to broth ( $V_e/V_b$ ) have been studied. Here, a novel extractive fermentation process was developed using *Clostridium saccharoperbutylacetonicum* N1-4 and a large  $V_e/V_b$  ratio.

ABE fermentation with large  $V_e/V_b$  ratio using immobilized cells was studied. A mixture of oleyl alcohol-tributyryn (1:1 (v/v)) yielded high distribution coefficients ( $K_d$ ) for both butanol (3.14) and acetone (0.660) that extract higher amount of both butanol and acetone from broth. Although a fed-batch culture using free cells and the oleyl alcohol-tributyryn mixture at a  $V_e/V_b$  ratio of 5 had a lag phase of  $> 24$  h, it produced a higher concentration of total butanol (i.e. butanol produced in all the phases per broth volume used) of 24.2 g/L-broth after 96 h compared with 14.4 g/L-broth at a  $V_e/V_b$  ratio of 1, resulting in a low butanol concentration in the aqueous phase. The use of cells immobilized with calcium alginate beads shortened the lag phase to  $< 12$  h. Butanol production was achieved not only in a 3-phase mode (extractant, beads, and Tryptone-Yeast extract-Acetate (TYA) medium) but also in a 2-phase mode (extractant and beads containing TYA medium, without an aqueous phase) at a  $V_e/V_b$  ratio of 5, resulting butanol concentrations of 30.9 g/L-broth and 27.7 g/L-broth, respectively. The 3-phases fed-batch extractive fermentation at a  $V_e/V_b$  ratio of 10 showed a better performance compared with published reports: a total butanol and acetone concentration of 64.6 g/L and 30.6 g/L, respectively and respective butanol and acetone yield to consumed sugar of 0.378 C-mol/C-mol and 0.166 C-mol/C-mol.

Further study was performed to utilize esters as co-substrate in fermentation. Previous reports have been published mentioning the ability of the strain to consume organic acid as co-substrate of a low concentration ( $< 10$  g/L) in the media. In order to reduce toxicity effect of the acids, extractive fed-batch fermentation with immobilized cells was conducted using organic acid as co-substrate. Butyric acid to glucose ratio of 0.1 produced total butanol concentration 59.3 g/L-broth. However higher ratio led to the growth inhibition to the strain. Therefore, organic ester was used to reduce the toxicity effect of dissociated acid. This is the first study to report the utilization of ester in ABE fermentation. Ethyl butyrate addition of 20 g/L-broth enhanced the total butanol concentration compared with only glucose. In addition, ethyl butyrate has a potency to be used as extractant with

high  $K_d$  of 4.45 and 0.804 for butanol and acetone, respectively. These results confirmed the ability of ester utilization for ABE fermentation by the strain and its potency to be used extractant.

Energy and economical aspect of this fermentation technique were studied. Overall production process was designed using rice straw as raw material which is semi-hydrolyzed to produce cellobiose, glucose, xylose, and arabinose mixture. Concentrated sugar was then fed to extractive fed-batch fermentation using immobilized cells. Finally, extractant was recovered and product were purified by distillation column. By evaluating this process design for the small scale capacity of 238 kg-butanol and acetone/day, the energy requirement was 41.3 MJ/kg-butanol and acetone and the cost was 1.83 \$/kg-butanol and acetone. Although the cost was higher than butanol produced by petrochemical process of 1.08 \$/kg-butanol, it may reduce if the scale is increased.