Studies on highly efficient butanol production from exogenous acetate by Clostridium saccharoperbutylacetonicum N1-4 (ATCC 13564)

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論文題名　：Studies on highly efficient butanol production from exogenous acetate by *Clostridium saccharoperbutylacetonicum* N1-4 (ATCC 13564)
（酢酸のサルベージ合成による効率的ブタノール生産に関する研究）

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論　文　内　容　の　要　旨

Butanol is one of the most promising biofuels, which can be produced from renewable feedstock in a process referred to as the acetone-butanol-ethanol (ABE) fermentation. Acetate is readily produced from hemicelluloses by extensive degradation during acid hydrolysis. In consideration of the reutilisation pathways of ABE-producing clostridia, acetate is considered to be an available and feasible substrate for ABE fermentation. Therefore, the objectives of this study are to investigate the metabolism of acetate in ABE fermentation, and then establish a highly efficient butanol production system from exogenous acetate.

Firstly, we investigated the characteristics of ABE production from acetate and analysed the metabolism of acetate by *Clostridium saccharoperbutylacetonicum* N1-4. Supplementation of 4 g/L exogenous acetate to media containing glucose, increased not only concentrations of butanol (48.3%) and acetone (90.5%), but also the ratio of acetone to butanol (27.1%), which suggested that acetate addition altered the metabolic flux. Acetate could not be metabolized in the absence of glucose, thus glycolysis appeared to be necessary for acetate utilisation. In order to clarify the metabolism of exogenous acetate, ^{13}C tracer experiments were performed by supplementing [1, 2-$^{13}C_2$] acetate in culture medium. Based on the results of gas chromatography-mass spectroscopy analysis, we first confirmed both butanol and acetone formations from acetate. Further, the acetate-to-butanol efficiency will significantly decrease when more acetate than 2−4 g/L is added to the fermentation, while acetate-to-acetone efficiency may remain high (up to a ratio of 2 mol acetate per 1 mol glucose fed). Moreover, the culture supplemented with acetate exhibited an increase in conversion efficiency of glucose to butanol and acetone, from 0.196% to 19.5% and from 0 to 7.64%, respectively, even during acidogenesis. Thus, we first revealed quantitatively that acetate addition induced solvent production during early growth phase, and increased metabolic flux to acetone and butanol production from both acetate and glucose.

Subsequently, enzyme assays suggested that acetate addition enhanced the activities of essential enzymes in acetate uptake (acetate kinase, phosphate acetyltransferase and CoA transferase) and solvents formation pathways (acetoacetate decarboxylase and butanol dehydrogenase) during both of acidogenesis and solventogenesis. Thus, acetate could be considered to improve the ability of solvents production by using strain N1-4. Further, batch cultures with feeding acetate at different culture phases indicated that acetate addition increased the volumetric and specific butanol production rates, compared with control (without acetate addition). In order to achieve high butanol production, several fed-batch cultures in which acetate is fed have been investigated. It was found that when fed a mixture of acetate and glucose, the final concentration of butanol produced by a fed-batch culture was greater than that produced by a batch culture. In addition, a pH-controlled fed-batch culture resulted in not only stimulation of acetate consumption but also a further increase in butanol production rate. Further, we obtained 15.1 g/L butanol at a production rate

of 1.04 g/L/h using a fed-batch culture with a pH-stat continuous acetate and glucose feeding method.

Finally, compared with butyrate and lactate, acetate was found to be an ineffective carbon source with lower butanol yield of total substrates, because acetate requires more reducing equivalents from glycolysis to produce butanol. In order to establish a highly efficient butanol production system from acetate, electron carriers such as neutral red, methylene blue and methyl viologen (MV) were supplemented to regulate the electron flows. As a result, MV was selected as an appropriate electron carrier to improve butanol yield, especially by using non-growing cell of strain N1-4. Further, addition of 1.0 mM MV resulted in the highest yield of butanol of 0.518 C-mol/C-mol (acetate and glucose) by non-growing cell with pH control at 5.5.

In conclusion, we successfully verified butanol production from acetate and illustrated the metabolism of exogenous acetate in ABE fermentation. Moreover, high productive butanol production was gained with supplementing acetate as co-substrate. Further, highly efficient butanol production from acetate using non-growing cells with supplement of election carriers was established.