Clostridium tyrobutyricum CULTURE TECHNIQUE

Rebalancing Redox to Improve Biobutanol Production by Clostridium tyrobutyricum

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Abstract

***Clostridium tyrobutyricum* was engineered to co-express formate dehydrogenase (fdh) and aldehyde/alcohol dehydrogenase (adhE2) to supply the reducing power butanol synthesis needs. In a 2 L FS-01-A stirred-tank bioreactor at 37 °C and pH 6.0, the fdh pathway raised butanol titer 2.72-fold, and fed-batch free-cell fermentation reached 12.34 g/L butanol.**



Winpact fermentation system

Introduction

Motivation: n-butanol can substitute for gasoline and serves as a feedstock for bio-jet fuel, but conventional ABE fermentation gives low butanol yield, titer and productivity because of butanol inhibition and a metabolic pathway split across acidogenesis, solventogenesis and sporulation.

Gap: *C. tyrobutyricum* is attractive for butanol production because of its high butanol tolerance, relatively simple metabolic pathway and efficient conversion of sugars to butyryl-CoA, but after carbon flux had been redistributed by metabolic engineering, the shortage of reducing power (NADH) remained the limiting factor.

Objective: rebalance redox in *C. tyrobutyricum* by introducing a heterologous NADH-producing pathway, screen medium supplements that shift metabolic flux toward butanol, and evaluate the combination in bioreactor fed-batch fermentation.

Materials and Methods

Strain construction and cultivation:

A metabolically-engineered mutant CTC-fdh-adhE2 was constructed by introducing heterologous formate dehydrogenase (fdh) and bifunctional aldehyde/alcohol dehydrogenase (adhE2) simultaneously into wild-type *C. tyrobutyricum*, with CTC-adhE2 as the control mutant. Cells were grown anaerobically in CGM medium; cell extracts were prepared by sonication at 40% power for 15 min with 30 s cooling intervals.

Bioreactor fermentation:

Kinetic studies of fed-batch fermentation by CTC-adhE2 (control) and CTC-fdh-adhE2 were performed in a 2 L FS-01-A stirred-tank bioreactor (Major Science, Saratoga, CA, USA) at 37 °C, pH 6.0 and 100 rpm on CGM medium supplemented with the identified components. Four conditions were compared, combining the two mutants with sodium formate, vitamin B12 and methyl viologen hydrate. Fermentations were run in duplicate.

Analysis:

Formate dehydrogenase activity in cell extracts, with total protein by Bradford assay; intracellular NADH and NAD+ by extraction kit to give the NADH/NAD+ ratio; glucose, butyrate, acetate, ethanol and butanol concentrations by the previously published analytical methods.

Results

The fdh-catalysed NADH-producing pathway raised butanol titer 2.15-fold in serum bottles and 2.72-fold in the bioreactor, confirming that supplying reducing power, not carbon flux, had been the bottleneck. Screening identified vanadate, acetamide, sodium formate, vitamin B12 and methyl viologen hydrate as supplements that shift flux toward butyrate or butanol. Combining the engineered strain with these supplements, fed-batch free-cell fermentation produced 12.34 g/L butanol from glucose, 3.88-fold the 3.18 g/L given by the control mutant CTC-adhE2. Fermentor relevance: the improvement only appears under controlled fed-batch conditions — pH held at 6.0, 37 °C and continuous feeding — because the redox shift and the supplement effects depend on a stable pH and a maintained glucose supply, which flask culture cannot provide.

References

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