Evaluation of Metabolic Engineering Strategies on 2-Ketoisovalerate Production by *Escherichia coli*

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Abstract

2-Ketoisovalerate, a key metabolic intermediate for pharmaceuticals and biofuels, suffers from low fermentation yields due to redox imbalance. By systematically engineering *E. coli*—deleting competing pathways, overexpressing key enzymes, tuning NADPH supply, and enhancing aerobic respiration—researchers achieved 46.4 g/L and 0.644 mol/mol glucose yield.

Further modifications to acetolactate synthase and degradation tagging of PDH reduced by-product formation and NADH accumulation.

Under a two-phase (aerobic–microaerobic) strategy, PDH degradation improved redox balance, reaching 55.8 g/L, 2.14 g/L·h, and 0.852 mol/mol glucose.

These findings outline an effective metabolic engineering approach for high-yield production of redox-imbalanced fermentative metabolites.

Winpact FS-02 Fermentation System

Introduction

2-Ketoisovalerate is a valuable intermediate for pharmaceuticals and biofuels, serving as a precursor for branched-chain amino acids and isobutanol. Chemical synthesis is unsustainable, making microbial fermentation from glucose a preferred route. To enhance yield, *E. coli* metabolism must be engineered—removing competing pathways, optimizing enzyme specificity (especially AlsS), and managing redox balance. Since 2-ketoisovalerate formation generates excess NADH and requires NADPH, strategies such as NADH-to-NADPH conversion and controlled aeration are essential. Because PDH activity exacerbates NADH accumulation and diverts pyruvate flux, a timed PDH shutdown after biomass buildup offers a balanced solution for efficient 2-ketoisovalerate production.

Materials and Methods

For each bioreactor experiment, cells were precultured in LB medium, as described in the shake-flask experiments. The 100-mL LB broth was used as a seed culture to inoculate 2-L medium with 30 g/L glucose, which was contained in a 5-L bioreactor (Winpact FS-02; Major Science, Saratoga, CA, USA). A two-phase fermentation with a cell growth phase was maintained at 37°C, and then a 2-ketoisovalerate production phase maintained at 30°C was initiated. Dissolved O2 concentration was controlled at the indicated saturation by stirring at 200 to 1,000 rpm and sparging air into the bioreactor at 3 to 10 L/min. To adjust the pH to 7, concentrated NH4OH was added automatically. At the initiation of the second phase, 0.38 g IPTG was added to the broth. Six batches of 5 g yeast extract and 5 g peptone were fed into the bioreactor as an organic nitrogen source to obtain a higher cell density. Glucose was fed into the bioreactor to maintain the residual glucose concentration above 10 g/L.

Results

In advanced metabolic engineering, every percent of dissolved oxygen and every degree of temperature matters.

Using the **Winpact FS-02 bioreactor**, researchers successfully performed two-phase fermentation of **2-ketoisovalerate**—from aerobic growth to microaerobic production—maintaining pH 7 and precise aeration (200–1000 rpm, 3–10 L/min).

This level of control enabled the redox-balance strategy to be realized, reaching a titer of 55.8 g/L..

References

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