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Reaction engineering studies for the production of 2-hydroxyisobutyric acid with recombinant *Cupriavidus necator* H16

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Abstract:

Recombinant *Cupriavidus necator* H 16 with a novel metabolic pathway using a cobalamin-dependent mutase was exploited to produce 2-hydroxyisobutyric acid (2-HIBA) from renewable resources through microbial fermentation. 2-HIBA production capacities of different strains of *C. necator* H 16 deficient in the PHB synthase gene and genetically engineered to enable the production of 2-HIBA from the intracellular PHB precursor (R)-3-hydroxybutyryl-CoA were evaluated in 48 parallel milliliter-scale stirred tank bioreactors (V = 11 mL). The effects of media composition, limitations, pH and feed rate were studied with respect to the overall process performances of the different recombinant strains. 2-HIBA production was at a maximum at nitrogen limiting conditions and if the pH was controlled between 6.8 and 7.2 under fed-batch operating conditions (intermittent fructose addition). The final concentration of 2-HIBA was 7.4 g L^{-1} on a milliliter scale. Best reaction conditions identified on the milliliter scale were transferred to a laboratory-scale fed-batch process in a stirred tank bioreactor (V = 2 L). Two different process modes for the product ion of 2-HIBA, a single-phase and a dual-phase fermentation procedure, were evaluated and compared on a liter scale. The final concentration of 2-HIBA was 6.4 g L^{-1} on a liter scale after 2 days of cultivation.

Key-words: microbioreactor, 2-hydroxyisobutyric acid, *Cupriavidus necator* H 16, industrial biotechnology, 2-hydoxyisobutyryl-CoA mutase, PHB