

Supplementary Information

Development of efficient poly(3-hydroxybutyrate) production platform from lignocellulosic hydrolysates using a robust *Cupriavidus necator* RXI22 strain

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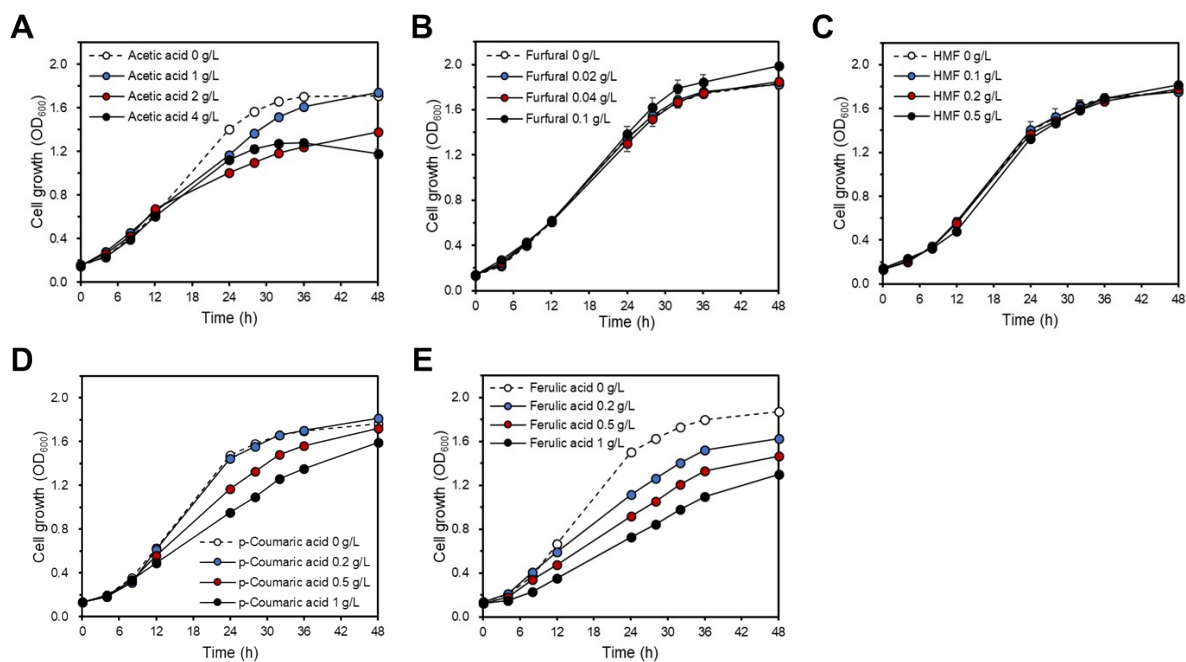


Fig. S1 Growth effects of lignocellulosic hydrolysate-derived inhibitors under glucose-only conditions. *C. necator* RXI22 was cultivated in 96-well plates using glucose as the sole carbon source, and cell growth was monitored for 48 h in the presence of individual inhibitors at varying concentrations (0–4 g L⁻¹ acetic acid, 0–0.1 g L⁻¹ furfural, 0–0.5 g L⁻¹ HMF, 0–1 g L⁻¹ *p*-coumaric acid, and 0–1 g L⁻¹ ferulic acid). Data are presented as mean ± standard deviation of three biological replicates. Abbreviations: HMF, 5-hydroxymethylfurfural.

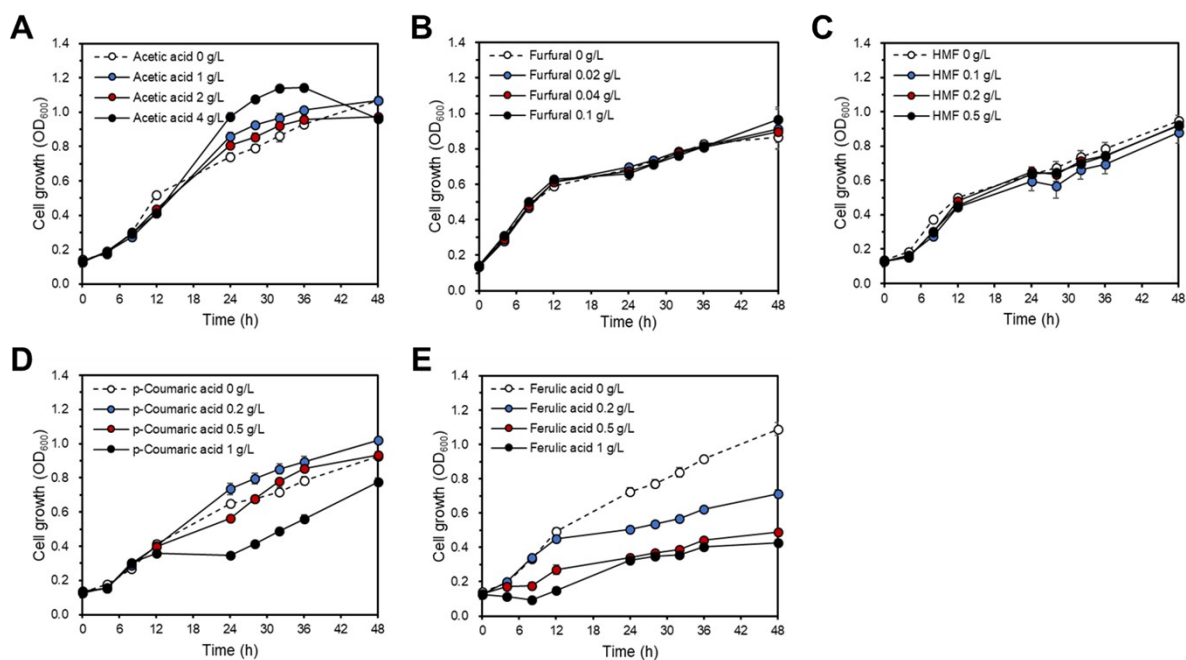


Fig. S2 Growth effects of lignocellulosic hydrolysate-derived inhibitors under xylose-only conditions. *C. necator* RXI22 was cultivated in 96-well plates using xylose as the sole carbon source, and cell growth was monitored for 48 h in the presence of individual inhibitors at varying concentrations (0–4 g L⁻¹ acetic acid, 0–0.1 g L⁻¹ furfural, 0–0.5 g L⁻¹ HMF, 0–1 g L⁻¹ *p*-coumaric acid, and 0–1 g L⁻¹ ferulic acid). Data are presented as mean ± standard deviation of three biological replicates. Abbreviations: HMF, 5-hydroxymethylfurfural.

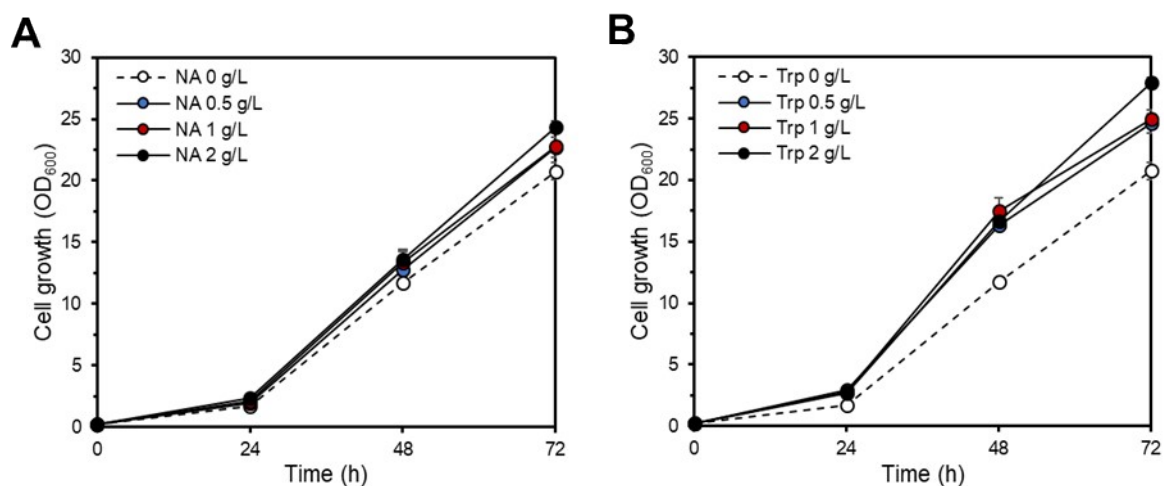


Fig. S3 Effect of nicotinic acid or tryptophan supplementation on the growth of *C. necator* RXI22 under inhibitor-stressed mixed-sugar conditions. *C. necator* RXI22 was cultivated in culture tubes (working volume 3 mL) supplemented with a defined mixture of lignocellulosic hydrolysate-derived inhibitors and either (A) nicotinic acid or (B) tryptophan at final concentrations of 0, 0.5, 1, and 2 g L⁻¹. The inhibitor mixture consisted of 1.5 g L⁻¹ acetic acid, 0.02 g L⁻¹ furfural, 0.5 g L⁻¹ HMF, and 0.5 g L⁻¹ ferulic acid, formulated to closely reproduce the fermentation performance observed in hydrolysate-based cultures. Data are presented as mean \pm standard deviation of three biological replicates. Abbreviations: NA, nicotinic acid; Trp, tryptophan.

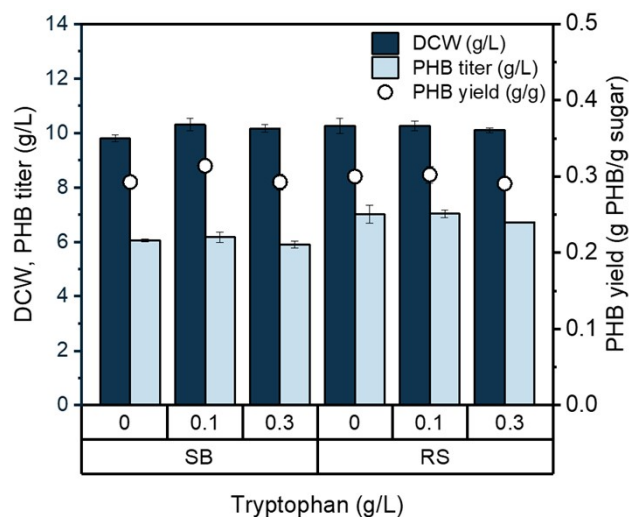


Fig. S4. Effect of tryptophan supplementation on the growth and PHB production of *C. necator* RXI22. The strain was grown in media containing either sugarcane bagasse hydrolysate or rice straw hydrolysate as the carbon source, supplemented with tryptophan (0–0.3 g L⁻¹). Dry cell weight (DCW), PHB titer, and PHB yield were measured after 96 h of cultivation. Data are presented as mean ± standard deviation of three biological replicates. Abbreviations: SB, sugarcane bagasse; RS, rice straw.