Sustainable production of cis,cis-muconic acid production from vanillin by an engineered E. coli strain

ShT-02.5-2

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The efficient valorization of the lignocellulosic biomass components, and particularly the lignin fraction, could serve as a starting point for the establishment of a circular bioeconomy model aimed at recycling and reusing industrial by-products over the exploitation of virgin feedstock. In a previous work, we developed an engineered E. coli strain co-expressing 4 different recombinant enzymes (the dehydrogenase LigV, the demethylase VanAB, the decarboxylase AroY and the dioxygenase C12O) to convert lignin-derived vanillin (Van) into cis,cis-muconic acid (ccMA), a valuable precursor for the production of plastic materials [Previously published in: Molinari et al. (2023) ACS Sustainable Chem. Eng. 11, 6, 2476–2485]. The whole-cell biocatalyst used with a resting cells approach converted >95% of 10 mM Van in ccMA in 2 h. In this work, the scaled-up production of ccMA from Van using the engineered E. coli growing cells was performed. The bioconversion reaction was carried out in a bioreactor, providing improved control of reaction conditions such as pH, dissolved oxygen, and substrate pulse-feed rate, streamlining the biocatalytic process and enhancing scalability. The optimized growth medium composition (0.5 g/L glucose and 2 g/L lactose) and the substrate addition strategy (1 mmol/h pulse-feed) enabled the engineered strain to produce 5.2 ± 0.4 g/L of ccMA in 48 h, corresponding to 0.86 g_{ccMA}/g_{Van}. The purification of the produced ccMA from the fermentation broth was achieved through crystallization, yielding 2.6 ± 0.1 g_{ccMA}/L of broth, corresponding to a ≈50% purification yield. Noteworthy, the proposed process proved to be more efficient and environmentally sustainable compared to the previous resting cells approach. To our best knowledge, this is the first reported production of ccMA from Van with an engineered E. coli strain using a growing cells approach in a bioreactor.