World Academy of Science, Engineering and Technology
International Journal of Biotechnology and Bioengineering
Vol:16, No:06, 2022

Development of Broad Spectrum Nitrilase Biocatalysts and Bioprocesses for Nitrile Biotransformation

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Abstract : The enzymatic conversion of nitriles to carboxylic acids by nitrilases has gained significance in the green synthesis of several pharmaceutical precursors and fine chemicals. While nitrilases have been characterized from different sources, the industrial application requires the identification of nitrilases that possess higher substrate tolerance, wider specificity and better thermostability, along with the development of an efficient bioprocess for producing large amounts of nitrilase. To produce large amounts of nitrilase, we developed a fed-batch fermentation process on defined media for the high cell density cultivation of E. coli cells expressing the well-studied nitrilase from Alcaligenes fecalis. A DO-stat feeding approach was employed combined with an optimized post-induction strategy to achieve nitrilase titer of 2.5*105 U/l and 78 g/l dry cell weight. We also identified 16 novel nitrilase sequences from genome mining and analysis of substrate binding residues. The nitrilases were expressed in E. coli and their biocatalytic potential was evaluated on a panel of 22 industrially relevant nitrile substrates using high-throughput screening and HPLC analysis. Nine nitrilases were identified to exhibit high activity on structurally diverse nitriles including aliphatic and aromatic dinitriles, heterocyclic, □-hydroxy and □-keto nitriles. With fed-batch biotransformation, whole-cell Zobelia galactanivorans nitrilase achieved yields of 2.4 M nicotinic acid and 1.8 M isonicotinic acid from 3-cyanopyridine and 4-cyanopyridine respectively within 5 h, while Cupravidus necator nitrilase enantioselectively converted 740 mM mandelonitrile to (R)–mandelic acid. The nitrilase from Achromobacter insolitus could hydrolyze 542 mM iminodiacetonitrile in 1 h. The availability of highly active nitrilases along with bioprocesses for enzyme production expands the toolbox for industrial biocatalysis.

Keywords : biocatalysis, isonicotinic acid, iminodiacetic acid, mandelic acid, nitrilase
Conference Title : ICBEB 2022 : International Conference on Bioprocess Engineering in Biotechnology
Conference Location : London, United Kingdom
Conference Dates : June 27-28, 2022