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Enzyme Optimization and Process Development for a Scalable Synthesis of Methoxymandelic Acid

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PURPOSE OF THE ABSTRACT

Enzyme engineering and process development are two key tools towards industrial biocatalytic solutions. Almac has experience of developing commercially successful biocatalytic solutions.[1-3] Herein we detail a parallel approach to process development and engineering of a nitrilase enzyme (Burkholderia cenocepacia J2315 (BCJ2315)) to achieve an economically viable process for the one-pot, enantioselective, dynamic kinetic resolution towards (R)-2-methoxymandelic acid.[4] Through a combination of molecular docking and B-factor analyses, a focused library of mutants was identified and screened to improve nitrilase selectivity, activity, and stability. Initial optimization revealed that the addition of sodium bisulfite prevented enzyme deactivation by the aldehyde starting material, removing the need to isolate the cyanohydrin and allowing for development of a one-pot process for mutant screening. Further optimization of the process with the preferred mutants revealed subtle interactions between temperature, pH, substrate loading, and enzyme source which ultimately led to development of a suitable whole cell process for scale-up, affording (R)-2-methoxymandelic acid in 97% ee and 70% isolated yield on multigram scale.

FIGURES

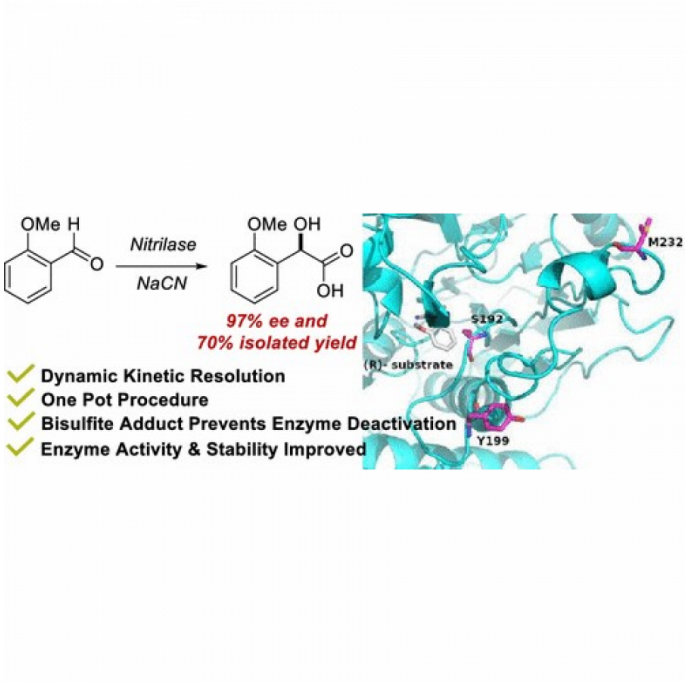

FIGURE 1

Nitrilase-mediated DKR for (R)-2-methoxymandelic acid

FIGURE 2

KEYWORDS

Nitrilase | Enzyme Engineering | Process Development | Biocatalysis

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