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海因酶法耦合原位分离技术制备N-氨甲酰-D-苯丙氨酸

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摘要 利用自行筛选的海因酶高产菌株Burkholderia cepecia njut01发酵后，经过硫酸铵分级沉淀、phenyl sepharose FF、DEAE sepharose FF等纯化步骤，得到初步纯化的D-海因酶；利用碳二亚胺法建立了一种基于EAH sepharose 4B的D-海因酶共价固定化方法，酶活回收率达到79.44%，固定化酶经100d 25个批次转化后仍可保持63.2%的初始酶活。将固定化酶填充为固定床反应器，考察了不同条件下固定床与固定床耦合离子交换原位分离（in situ product removal，ISPR）两种反应体系在转化过程中pH值变化及转化率的差异。结果表明，耦合离子交换原位分离技术可大幅度提高D，L-苄基海因转化率，经24h转化，N-氨甲酰-D-苯丙氨酸转化收率最高达62.725%，较采用单一固定床酶转化体系的转化率提高了89.3%。

关键词 固定化D-海因酶；固定床反应器；原位分离；N-氨甲酰-D-苯丙氨酸

分类号

Production of N-carbamoyl-D-phenylalanine by hydantoinase method coupled with in situ product removal

Abstract

The purified D-hydantoinase was immobilized on EAH sepharose 4B via the carbodiimide method with a yield of enzyme activity up to 79. 44%. The immobilized hydantoinase showed remarkable stability at 4℃.An integrated process of N-carbamoyl-D-phenylalanine (N-D-Phe) synthesis from D,L-5-benzylhydantoin(D,L-BH) catalyzed by immobilized D-hydantoinase coupled with an ion-exchange unit for in situ product removal(ISPR) was established. The variation of pH and conversion in the fixed-bed reactor with or without ISPR was compared at different temperatures, initial substrate concentrations and volumes of adsorbent. Within 24 h, the pH value in the reactor with ISPR could be kept at the alkaline range, which was beneficial to the enzymatic conversion and racemization of L-5-benzyl hydantoinase. This led to a higher overall conversion of 62. 725% under optimal operation conditions, an increase of 89. 3% compared with the fixed-bed reactor without ISPR.

Key words immobilized D-hydantoinase；packed-bed reactor；in situ product removal（ISPR）；N-carbamoyl-D-phenylalanine

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