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FINDINGS

In the present thesis, CA from *Bacillus halodurans* (BhCA) has been immobilized using different approaches, i.e. surface display of BhCA on *E. coli* cells (*E. coli*-BhCA), entrapment of *E. coli*-BhCA cells in calcium alginate beads to develop CA biocatalytic beads, and the synthesis of BhCA hybrid nanoflowers (BhCA-HNFs) using purified BhCA. *Bacillus halodurans* is an extremophile (alkaliphilic), producing enzymes that are stable across a wide pH range. Faridi and Satyanarayana (2016) reported that BhCA is alkalistable and moderately thermostable. To further enhance the thermostability, reusability, and operational stability of BhCA, while maintaining its alkaliphilic properties, various immobilization approaches have been employed.

Surface display of BhCA on *E. coli* cells (*E. coli*-BhCA cells) is done using the AIDA-I (Adhesin involved in diffuse adherence) autotransporter system. The BhCA encoding gene was subcloned into the pAIDA-I vector, which translocated the BhCA on the outer surface of the *E. coli* cells, which was confirmed using FACS (Fluorescence Activated Cell Sorting) and cellular fractionation approach, followed by SDS-PAGE and zymogram analysis. Further, these *E. coli*-BhCA cells were entrapped in the calcium alginate beads to develop CA biocatalytic beads for enhanced stability. After successful entrapment of *E. coli*-BhCA cells, another immobilization approach, i.e. the hybrid nanoflowers synthesis was implicated. To synthesize the BhCA hybrid nanoflower, BhCA was first purified using pET28a-BhCA as per the previously reported literature, followed by the one-pot synthesis of the nanoflowers using phosphate buffer saline

(PBS) and Copper sulphate solution. SEM (scanning electron microscopy) analysis was done to confirm the formation of BhCA hybrid nanoflowers (BhCA-HNFs).

Biochemical characterization, including thermostability, pH stability, effects of metal ions, reusability, and operational stability of all three immobilized BhCA forms (*E. coli*-BhCA cells, CA biocatalytic beads, and BhCA-HNFs), were performed. It was observed that immobilization of BhCA significantly enhanced stability compared to free BhCA. Among the three approaches used, CA biocatalytic beads were the most thermostable ($t_{1/2}$ ~ 40 minutes at 80 °C), followed by BhCA-HNFs ($t_{1/2}$ ~ 24 minutes at 80 °C) and *E. coli*-BhCA cells ($t_{1/2}$ ~ 18 minutes at 80 °C). The immobilized forms of BhCA retained the alkaliphilic nature of their native form, maintaining stability across a broad pH range in all three immobilization methods. Immobilization also increased the reusability and storage stability of BhCA. It was observed that CA biocatalytic beads and BhCA-HNFs can be reused for multiple reaction cycles. BhCA-HNFs retained 95% residual activity after 42 days, whereas 80% residual activity after 42 days was observed for CA biocatalytic beads and E. coli-BhCA cells retained 80% residual activity after 12 days.

Furthermore, biomineralization of CO_2 was done using immobilized forms of BhCA to produce calcium carbonate ($CaCO_3$). The resulting $CaCO_3$ precipitates were further examined with FESEM and EDS analysis, revealing that biomineralization with immobilized forms produced calcite (rhombohedral) morphology, which is the most thermodynamically stable form. Therefore, it can be concluded that immobilising BhCA resulted in a robust biocatalyst suitable for industrial-scale carbon capture and sequestration. Future research should focus on scaling up, integration with renewable energy systems, and policy frameworks for the industrial deployment of immobilised BhCA forms.