**Biocatalyst development for CO2 capture and utilization by enzymatic cascade process**

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Biocatalytic processes present new opportunities for CO2-based production by combining CO2 with renewable raw materials, including waste biomass. This study introduces a novel enzymatic technology for synthesizing bio-based carboxylic acids, which serve as building blocks for the pharmaceutical, food, and biopolymer industries. We propose a two-step enzymatic cascade for the efficient utilization of CO2. The process consists of enzymatic CO2 reactive absorption followed by the fixation of bicarbonate into carboxylic acids derived from phenolics and catalyzed by non-oxidative decarboxylases. The feasibility of the process was assessed for a pilot scale CCU process where the CO2 from flue gas was absorbed in K2CO3 solvent supplemented with immobilized carbonic anhydrase (CA) [1] and phenolic substrates (*e.g.* catechol, orcinol) - derived from the fractionation of pyrolytic bio-oils - were considered for enzymatic carboxylation in the bicarbonate-rich solvent [2]. Immobilization of both enzymes is crucial for the development of CO2 capture and conversion units. Thermostable CAs were successfully immobilized by several techniques in previous studies [1]. Now we are focusing on the immobilization of the cofactor-free 2,3 dihydroxybenzoic acid decarboxylase (2,3 DHBD) to unlock the design and set up of a continuous lab-scale bioreactor. To this aim, the analytical chromatographic methods defined to assess the decarboxylation activity were adapted to conditions that mimic the CO2 absorption solvents (*i.e.* KHCO3/K2CO3 solutions). Second, the carboxylation activity of free 2,3 DHBD was assessed against a model substrate (catechol) in KHCO3/K2CO3 solutions. Finally, a wide set of enzyme immobilization techniques have been selected for screening and optimization against the homo-tetramer structure of 2,3 DHBD. These techniques include cross-linking on granular solids activated with amino groups; direct binding with epoxy-activated resins; and cross-linked enzyme aggregates. Immobilized enzyme loading and activity data will inform a theoretical model of a lab-scale bioreactor that accounts for both the reversible enzymatic carboxylation kinetics and the physical properties of the biocatalyst support.

**Keywords**: *CO2 capture and utilization, carbonic anhydrase, heterogeneous biocatalysis*

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