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Cell-free Bioconversion of n-Octane to n-Octanol via a Heterogeneous and Bio-Catalytic Approach

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Abstract : Linear alkanes are produced as by-products from the increasing use of gas-to-liquid fuel technologies for synthetic fuel production and offer great potential for value addition. Their current use as low-value fuels and solvents do not maximize this potential. Therefore, attention has been drawn towards direct activation of these aliphatic alkanes to more useful products such as alcohols, aldehydes, carboxylic acids and derivatives. Cytochrome P450 monooxygenases (P450s) can be used for activation of these aliphatic alkanes using whole-cells or cell-free systems. Some limitations of whole-cell systems include reduced mass transfer, stability and possible side reactions. Since the P450 systems are little studied as cell-free systems, they form the focus of this study. Challenges of a cell-free system include co-factor regeneration, substrate availability and enzyme stability. Enzyme immobilization offers a positive outlook on this dilemma, as it may enhance stability of the enzyme. In the present study, 2 different P450s (CYP153A6 and CYP102A1) as well as the relevant accessory enzymes required for electron transfer (ferredoxin and ferredoxin reductase) and co-factor regeneration (glucose dehydrogenase) have been expressed in E. coli and purified by metal affinity chromatography. Glucose dehydrogenase (GDH), was used as a model enzyme to assess the potential of various enzyme immobilization strategies including; surface attachment on MagReSyn® microspheres with various functionalities and on electrospun nanofibers, using self-assembly based methods forming Cross Linked Enzymes (CLE), Cross Linked Enzyme Aggregates (CLEAs) and spherezymes as well as in a sol gel. The nanofibers were synthesized by electrospinning, which required the building of an electrospinning machine. The nanofiber morphology has been analyzed by SEM and binding will be further verified by FT-IR. Covalent attachment based methods showed limitations where only ferredoxin reductase and GDH retained activity after immobilization which were largely attributed to insufficient electron transfer and inactivation caused by the crosslinkers (60% and 90% relative activity loss for the free enzyme when using 0.5% glutaraldehyde and glutaraldehyde/ethylenediamine (1:1 v/v), respectively). So far, initial experiments with GDH have shown the most potential when immobilized via their His-tag onto the surface of MagReSyn® microspheres functionalized with Ni-NTA. It was found that Crude GDH could be simultaneously purified and immobilized with sufficient activity retention. Immobilized pure and crude GDH could be recycled 9 and 10 times, respectively, with approximately 10% activity remaining. The immobilized GDH was also more stable than the free enzyme after storage for 14 days at 4˚C. This immobilization strategy will also be applied to the P450s and optimized with regards to enzyme loading and immobilization time, as well as characterized and compared with the free enzymes. It is anticipated that the proposed immobilization set-up will offer enhanced enzyme stability (as well as reusability and easy recovery), minimal mass transfer limitation, with continuous co-factor regeneration and minimal enzyme leaching. All of which provide a positive outlook on this robust multi-enzyme system for efficient activation of linear alkanes as well as the potential for immobilization of various multiple enzymes, including multimeric enzymes for different bio-catalytic applications beyond alkane activation.

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