O.233. Low-cost and eco-friendly downstream processes for single-step purification and immobilization of recombinant proteins

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Given the simplicity, versatility and high-efficiency of affinity tag-based separation and recovery systems, affinity tags have become indispensable tools for the purification of recombinant proteins produced by microbial hosts. Since downstream processing is the main contributor for the overall recombinant protein production costs, for applications such as enzyme immobilization and material functionalization, higher economic and ecological efficiencies can be obtained if purification and immobilization is integrated in a single-step [1]. Moreover, purification/immobilization schemes relying on low-cost, earth-abundant and eco-friendly purification matrices, such as silica and metal oxides, are highly desirable.

Solid-binding peptides (SBPs) are short peptides that show selectivity and bind with high affinity to the surfaces of specific solid materials and thus have emerged as interesting alternatives to conventional immobilization methods [1]. Moreover, they allow the combined purification and immobilization of proteins/peptides onto natural, synthetic or hybrid materials in a single step.

Envisioning the development of low-cost and eco-friendly single-step protein purification and immobilization processes, this study aimed at assessing the efficiency of selected SBPs as tags for affinity purification/immobilization of recombinant proteins onto silicon dioxide (SiO2) and iron oxide (Fe3O4) micro/nanoparticles. For that, three SBPs (His-tag, CotB1p, Car9 [1]) were fused (alone or in combination) to a monomeric EGFP, which was used as model protein. Non-tagged EGFP was used as control. After recombinant production in *Escherichia coli*, the cellular lysates containing the tagged/non-tagged EGFP were incubated with different amounts of solid matrices, namely SiO2 and Fe3O4 micro/nanoparticles, until reaching the maximum load. After optimization of the binding, washing and elution steps, the performance of the tags were compared by determining the % of binding, recovery yield and purity of the tagged proteins. All tags bound specifically to both SiO2 and Fe3O4 particles. Depending on the tag-matrix system used, proteins with up to 96 % purity could be obtained with recovery yields up to 70 %. The combination of tags improved the binding strength, allowing more stable immobilization. Thus, for single-step purification and immobilization purposes the combination of tags is preferable, while the use of a single tag is more suitable for purification purposes.

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References

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