**Optimization of culture conditions for the production of active inclusion bodies using high-throughput technologies**

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**Highlights**

* Optimization of culture conditions to produce active inclusion bodies
* Application of various high-throughput cultivation technologies
* Investigation of cultivation temperature using a mini reactor-based temperature profiling system

**1. Introduction**

It is of primary importance to develop new generic methods for simple and cost-efficient production of large amounts of stable enzymes to facilitate industrial applications. Although immobilization methods can improve enzyme stability, the additional processing steps are laborious and thus expensive. In contrast, catalytically active inclusion bodies (CatIBs) are in vivo, carrier-free enzyme aggregates that can be purified in a simple two-step purification process and retain beneficial features of conventional immobilization preparations. CatIBs can be produced in *E. coli* by the fusion of the target enzymes with certain aggregation tags [1, 2].

**2. Methods**

Active inclusion bodies, consisting of fluorescent reporter proteins or target enzymes, were produced with *E. coli* BL21(DE3) using the pET system. *E. coli* was cultivated in the Respiration Activity MOnitoring System (RAMOS) shake flasks for the online determination of the oxygen transfer rate (OTR). Additionally, different high-throughput online monitoring cultivation devices were used to measure scattered light (biomass, morphology) and fluorescence (product). A mini reactor-based temperature profiling system was used to investigate the impact of the cultivation temperature in a single experimental setup. To prepare IBs for activity determination, *E. coli* cells were lysed and insoluble cell fractions were isolated and washed. The activity of IBs was determined by fluorescence measurements or enzyme activity assays.

**3. Results and discussion**

First, the cultivation technologies were applied to develop a standard cultivation protocol. A streamlined methodology was developed combing the cultivation protocol, CatIB purification and the activity determination of CatIBs at µL-scale to facilitate process characterization and optimization. Subsequently, the impact of important cultivation parameters like medium composition, oxygen availability, temperature, and induction strength was investigated. Since the expression temperature strongly influenced the CatIB quality, a mini reactor-based high-throughput temperature profiling system was applied to study this effect in-depth. Results show that the cultivation temperature has to be adjusted carefully depending on the target protein and the chosen aggregation tag.

**4. Conclusions**

Various small-scale high-throughput technologies were applied to study CatIBs in depth. Results show that cultivation conditions have to be carefully adjusted for the production of active IBs. Furthermore, the results underline the great potential for industrial applications, as well as the necessity for further investigations.

**References**

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