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Process optimization for scalable E. coli extract preparation for cell-free protein synthesis

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Highlights

- E. coli extract is most productive when harvested at 58.5% of their growth extent
- T7 RNA polymerase can be induced at 28% of the growth extent
- Dilute cells should be lysed with a single pass through homogenizer at 25000 psi
- Lyophilization should be at 15°C and four hours
- The large volume process produced extract with equal activity to sonication method

Abstract

Cell-free protein synthesis (CFPS) is a versatile tool for protein research and biomanufacturing. In this work, large volume cell extract preparation methods from Escherichia coli were optimized with a unit operations approach. Using face centered cubic designed experiments, optimized time points for IPTG induction (to produce T7 polymerase) and cell harvest were determined. These were at times corresponding to 28 and 58.5% the overall growth extent as measured by absorption; for 1 L cultures in 2.5 L shake flasks in our incubator, this amounted to 201 min and 255 min respectively. The cell pellet was washed in S30 buffer once for 10 minutes and stored at -80°C without flash freezing in nitrogen. Cell lysis was simplified by diluting the cells to one part S30 buffer, one part DI water, and one part cells by mass and then passing through a continuous French press for one cycle at 25,000 psi. The resulting suspension is clarified with one low speed centrifugation step at 12,000 xg for 10 minutes (4°C). All extract is then immediately frozen in a pilot-scale lyophilizer (-70°C) and dried under optimal conditions determined as 4 hours under vacuum (200 mTorr) while holding a shelf temperature of 15°C. The lyophilized extract showed no statistically significant difference in sfGFP expression levels when compared to an optimized, lower-volume sonication protocol and improved performance over extract from commercial kits. As an increasing number of efforts in CFPS expand beyond the bench top, scalability and reliability become increasingly important if it is to become a viable tool for biomanufacturing.
Keywords: Cell-free protein expression (CFPS), E. coli, extract, protein expression, lyophilization, design of experiments