Optimization of Chemical Cell Lysis Conditions for Determination of Protein Titer

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Abstract

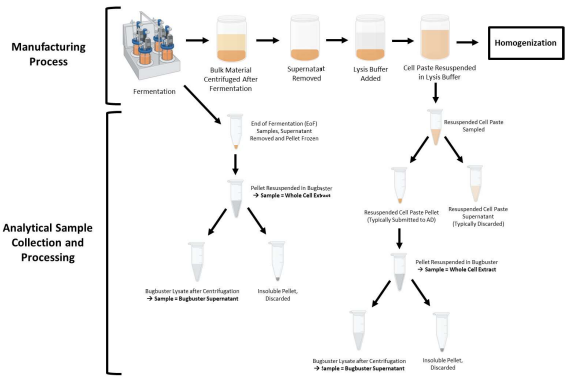
During large-scale manufacturing of protein by *E. coli* fermentation, cells are typically lysed by pressure homogenization to release the protein for further purification. To evaluate the efficiency of protein recovery by this method, a sample of cells is collected for analysis before homogenization and lysed by applying Bugbuster reagent and Lysonase in combination with sonication. This chemical treatment is assumed to cause 100% lysis and release all protein for quantitation. However, it has been observed that the relative amount of protein recovered by pressure homogenization of the bulk material was greater than that from the chemically lysed samples. We hypothesize that differences in lysis efficiency between pressure homogenization and chemical treatment causes this discrepancy. We conducted a study of different chemical lysis parameters with the goal of optimizing those conditions to achieve total protein recovery from analytical samples. This was evaluated by comparing the titer results from the tested sample processing conditions to titer values from lysis via bulk-scale pressure homogenization. No significant improvement in recovery using increased amounts of Bugbuster and Lysonase was observed. However, a comparison of recovery from whole cell extract analytical samples to such samples clarified by centrifugation demonstrated that this step causes protein loss that is exacerbated by increased Bugbuster volumes. Protein stability could be impacted by Bugbuster detergents, resulting in insoluble misfolded protein that pellets during centrifugation. Such species were not included in previous titer determinations which accounts for the lower recovery by chemical lysis. We also demonstrated that higher concentrations of lysate may have protective effect on the stability of the protein. These results show the importance of optimizing lysis protocols for analytical samples for a specific protein of interest.

Introduction

- ❑ **Problem**: Protein material was generated by *E. coli* expression in fermentation bioreactors. This was followed by mechanical lysis of the cells to release the protein for bulk recovery. To track the step yield, cell samples before mechanical lysis were lysed by chemical treatment to release the protein for quantitation. It was observed that the titer of these proteins were consistently lower than the titers of proteins from the bulk recovery process.
- ❑ **Goal:** Generate data to support, and investigate reasons for, lower lysis efficiency when using chemical (Bugbuster) versus mechanical (pressure homogenization) lysis. Address the lower lysis efficiency of chemical lysis by optimizing the treatment conditions and sampling at various processing stages

Methods

Fermentation Sampling and Processing



Sample Collection and Processing for Fermentation Material Moving forward into Primary Recovery. Pellet samples are taken for analysis from fermentation and after resuspension of cell paste in lysis buffer. These samples are chemically lysed according to the 13 conditions, followed by protein titer determination by SDS-PAGE band quantitation. The resulting protein amount is compared to samples taken after the homogenization process.

Analytical Sample Lysis Treatment Conditions

Lysis Treatment	Bugbuster	Lysonase	Sonication
1	250 µL	1.5 µL	Yes
2	250 µL	1.5 µL	No
3	500 µL	3 µL	No
4	1000 µL	6 µL	No
5	250 µL	3 µL	No
6	0.5x by pellet mass	0.5x by pellet mass	No
7	1X by pellet mass	1X by pellet mass	No
8	2X by pellet mass	2X by pellet mass	No
9	3X by pellet mass	3X by pellet mass	No
10	4X by pellet mass	4X by pellet mass	No
11	250 µL 1:2 dilution	1.5 µL	No
12	2000 µL	12 µL	No
13	250	7.5 µL	No

- Pellets were collected from 5 ml of material either at the end of fermentation or after resuspension of cell paste in lysis buffer.
- Green box is around original sample processing conditions.
- Each pellet was processed with a lysis treatment (outlined in the table to the left) that was based on either set volumes of processing reagents or set proportions relative to the sample pellet mass.
- 1X Conditions were based on manufacturer's recommendations (5 mL Bugbuster and 1.5 µL Lysonase:1 gram pellet

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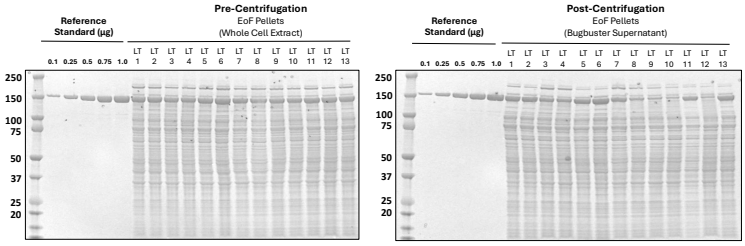
Results

1. Total Protein Recovery Consistent Across Conditions

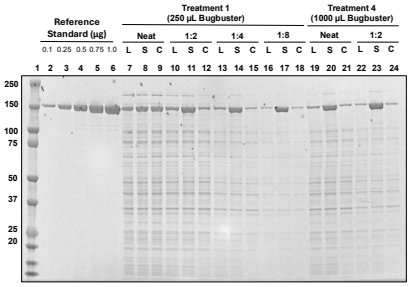


SDS-PAGE of End of Fermentation (EoF) samples processed using the defined conditions. Recovery of the protein of interest was consistent in pre-centrifugation samples but more variable in post-centrifugation samples Other protein bands from the lysate samples appeared consistent across all lysis treatments (LT). These results suggested that increasing the amount of Bugbuster used did not have an impact on lysis efficiency, but may have an impact on the stability of the protein of interest.

2. Higher Concentration of Cell Lysate has a Protective Effect on Protein of Interest

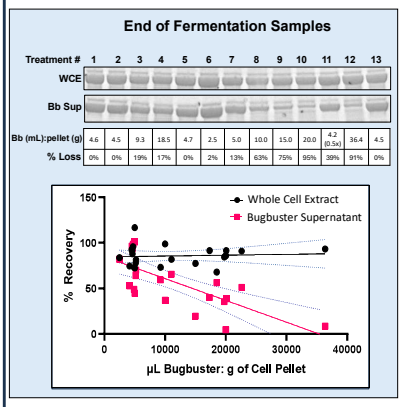
Spike Recovery

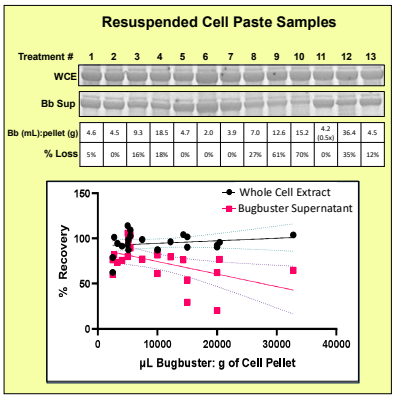
Bugbuster Processing Vol. (µL)	End of Fermentation Bb Sup Lysate Treatment (LT) #	Lysate:Total Volume (Diluted in Bugbuster)	Processing After Spike	Recovery (mg/ml) (Spiked w/ 1 mg/ml final)
250	LT 1	Neat	N/A	1.03
250	LT 1	Neat	Centrifugation	0.90
250	LT 1	1:2	N/A	1.02
250	LT 1	1:2	Centrifugation	<LOQ
250	LT 1	1:4	N/A	1.14
250	LT 1	1:4	Centrifugation	<LOQ
250	LT 1	1:8	N/A	1.32
250	LT 1	1:8	Centrifugation	<LOQ
1000	LT 4	Neat	N/A	1.39
1000	LT 4	Neat	Centrifugation	<LOQ
1000	LT 4	1:2	N/A	1.85
1000	LT 4	1:2	Centrifugation	<LOQ



Spike recovery from diluted lysate samples. Bugbuster (Bb) supernatant (Sup) samples were either used neat or diluted out further in Bugbuster (L). These samples were then spiked to 1 mg/ml with reference standard (S). The spiked sample was then centrifuged to determine if spiked protein was lost during this step (C). Spiked protein in all pre-centrifugation samples recovered ≥ 1 mg/ml. Notably, the spiked protein only recovered in the most concentrated lysis sample after centrifugation, while all others were <LOQ. This suggest that the more highly concentrated lysate may provide a protective effect for the protein of interest.

3. Loss of Protein of Interest is Exacerbated by Higher Bugbuster:Cell Paste Ratio





Analysis of protein of interest recovery before and after centrifugation. No significant trend was seen for the recovery of protein for end of fermentation or resuspended cell paste samples pre-centrifugation whole cell extracts (WCE). With increased Bugbuster (Bb) used, less protein of interest was recovered in post-centrifugation Bugbuster Supernatants (Bb Sup) for both end of fermentation samples (P=0.0166) and resuspended cell paste samples (P=0.0001) indicating that the increase in Bugbuster exacerbated this loss during centrifugation.

Conclusions

- No apparent improvement in recovery was seen using increased volumes of Bugbuster and Lysonase
- Clarification by centrifugation of analytical samples processed using Bugbuster may result in loss of protein of interest that is exacerbated by increased volumes of Bugbuster
  - Loading whole cell extract had more consistent higher recovery than post-centrifugation samples, and may be a more appropriate sample for SDS-PAGE.
  - Protein stability could be impacted by Bugbuster detergents, resulting in protein in insoluble non-native conformation that separates out during centrifugation.
  - Higher concentrations of cellular macromolecules in the smaller volume of lysate may have protective effect on the stability of the protein of interest
- Based on these data, lysis efficiency may not be the root cause of lower titers in chemically lysed samples vs. homogenized sample
- Optimization of chemical lysis for analytical samples is likely protein-dependent and should be investigated on a case-by-case basis.