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Application Note

High-Pressure Cell Disruption for Functional Pichia pastoris Cell-Free Protein Synthesis

Aw, R. & Polizzi, K. M. (2019) Biosensor-assisted engineering of a high-yield Pichia pastoris cell-free protein synthesis platform Biotechnology and Bioengineering, 116, 656–666 DOI: 10.1002/bit.26901

Preparation of concentrated, functional P. pastoris extracts for cell-free protein synthesis (CFPS)

Cell-free protein synthesis (CFPS) uses the biological machinery of a cell to manufacture proteins outside the living cell. The quality of the cell extract is therefore critical: the aim is not simply to break as many cells as possible, but to release a high concentration of functional translational machinery while minimising dilution, chemical contamination and thermal damage. In their study, Rochelle Aw and Karen M. Polizzi developed a Pichia pastoris CFPS platform and compared several approaches for cell disruption. Although enzymatic spheroplasting followed by chemical lysis produced the most complete cell disruption, the authors selected high-pressure disruption using a Constant Systems disrupter for subsequent extract production. The reason was biological as much as mechanical: high-pressure disruption provided a rapid, detergent-free route to a concentrated extract while avoiding the prolonged 37°C incubation associated with spheroplasting. Importantly, the process could be performed with cooling – a critical consideration when the objective is to preserve the activity of ribosomes, enzymes and other components of the translational machinery.

Cell disruption for CFPS: efficiency is more than cell breakage

For CFPS, the objective is to generate a functional, concentrated cell extract. Aw and Polizzi therefore assessed both the number of viable cells
remaining after lysis and the protein concentration of the resulting extract.

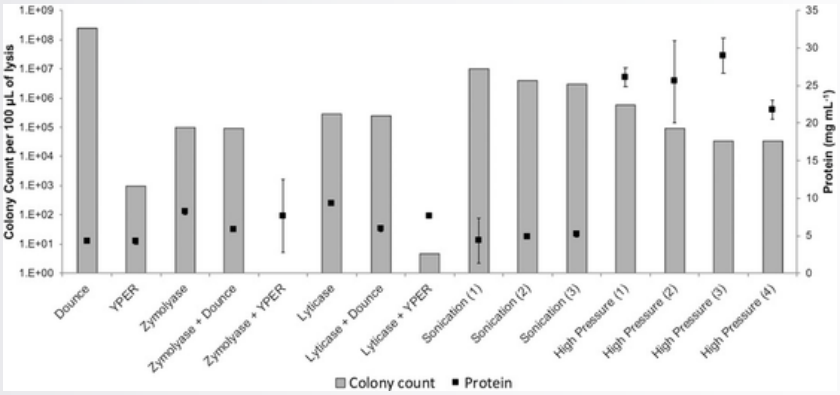

Figure 2 highlights an important distinction. Spheroplasting with zymolyase or lyticase followed by Y-PER produced the most complete lysis, but the resulting extract contained only 7.5 mg/mL protein. The authors also noted that detergent components of Y-PER could inhibit CFPS. High-pressure disruption did not completely lyse the culture, yet it produced a more attractive extract for the downstream biological application. The authors therefore selected it for subsequent extract preparation

Why high-pressure disruption was selected

1. Faster processing

Spheroplasting: 1 h at 37°C before chemical lysis. Sonication: repeated 5-min cycles, up to three cycles. Dounce: 10–15 strokes. High pressure: direct mechanical processing; final extract used two passes at 30 KPSIG. The paper does not provide total elapsed times, so a numerical time saving should not be claimed.

2. Concentrated extract

Extract concentration matters because the extract supplies the machinery for protein synthesis. The spheroplasting/Y-PER route produced only 7.5 mg/mL protein despite its high lysis efficiency. High-pressure disruption avoided detergent-based chemical lysis and unnecessary dilution.

3. Cleaner downstream processing

Y-PER introduced detergent that the authors identified as a potential CFPS inhibitor. Mechanical disruption avoids this lysis chemistry. Clarification and dialysis were still performed, but the workflow began with a detergent-free mechanical lysate.

4. Cooling protects biological function

The target is not simply protein release: it is active ribosomes, enzymes and translation factors. Cold processing helps preserve functionality. Controlled pressure combined with cooling therefore supports the biological objective of the extract.

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Time, concentration, clean-up and cooling: why the process matters

RAPID PROCESSING
Removes the 1-hour 37°C spheroplasting incubation and converts disruption into a controlled mechanical step.

CONCENTRATION
Avoiding excessive lysis dilution supports a higher concentration of the cellular machinery needed for CFPS.

CLEANER LYSATE
No detergent-based lysis reagent is required, reducing one potential downstream inhibitor.

COOLING
Maintaining low temperature helps protect the functional translational machinery released during disruption.

Processing time and standardisation

The paper does not report a single total elapsed time for each lysis method, so it is not appropriate to assign a precise percentage or fold time saving to the Constant Systems process. The process difference is nevertheless important. Spheroplasting required a 1-hour 37°C incubation before chemical lysis; sonication was performed in repeated 5-minute cycles; Dounce homogenisation required manual strokes. High-pressure disruption instead processes the suspension directly through the disrupter at a defined pressure. The final CFPS extract preparation used two passes at 30 KPSIG with a one-shot head.

Practical implication: fewer incubation and manipulation steps make the disruption stage easier to standardise and integrate into a repeatable extract-manufacturing workflow.

Sample concentration and downstream clean-up

In CFPS, the extract itself is a reagent: it supplies ribosomes, translation factors, enzymes and other cellular components. Unnecessary dilution therefore reduces the concentration of the biological machinery available to the reaction. Figure 2 illustrates this clearly: the most complete spheroplasting/Y-PER treatment generated only 7.5 mg/mL protein.

Mechanical disruption provides a detergent-free route to the lysate. The authors still clarified the extract by centrifugation and performed dialysis before final preparation, so the Constant Systems process should not be presented as eliminating downstream clean-up. Its advantage is that it avoids introducing Y-PER detergent at the point of cell breakage, reducing one potential source of CFPS interference.

Cooling: protecting the product of disruption

Cooling is a process requirement in this application, not simply an equipment convenience. The product of disruption is functional biological machinery. Aw and Polizzi's workflow incorporated cold handling: cells were kept cold before processing, buffers were chilled, and post-lysis clarification and dialysis were performed at 4°C. This is consistent with the authors' emphasis on preserving protein functionality.

Mechanical disruption introduces energy into the sample and can generate heat. Maintaining controlled temperature during processing therefore helps protect the ribosomes, enzymes and translation factors that ultimately determine CFPS performance. For this application, controlled pressure and controlled temperature work together: pressure provides reproducible mechanical disruption, while cooling helps preserve the activity of the released translational machinery.

From disruption to productive CFPS

Following the lysis comparison, Aw and Polizzi selected high-pressure disruption for preparation of the P. pastoris extracts used in their CFPS platform. The published workflow used 30 KPSIG ® two passes ® cold clarification ® dialysis ® CFPS extract. The resulting extract supported productive cell-free protein synthesis and ultimately enabled production of human serum albumin at 48.1 mg/mL.

KEY TAKEAWAY

For CFPS, the best lysis process is the one that protects the extract – not necessarily the one that breaks the most cells.

Constant Systems CF1

The CF1 provides controlled continuous-flow disruption with precise hydraulic pressure control and integrated sample cooling. It is designed for bacteria, yeast and algae. The published CFPS study used a Constant Systems high-pressure disrupter at 30 KPSIG, with two passes through a one-shot head for the final extract preparation.

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