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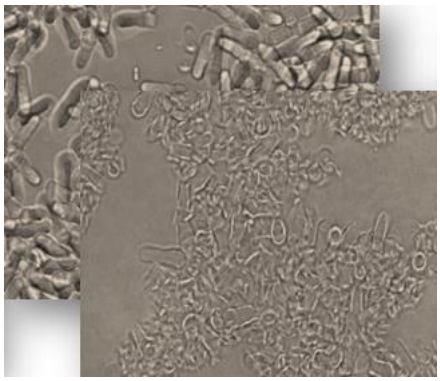
Application Note

Microfluidizer® Technology for Cell Disruption –don't just take our word for it

INTRODUCTION

This paper compiles a number of peer reviewed publications and research reports that explore the performance and advantages of using Microfluidizer technology for cell disruption.

In each case we present an abridged summary of the paper and its conclusions.



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PUBLICATION SUMMARY #1

Evaluation of the Microfluidizer for Cell Disruption of Yeast and Chlorella by E. Uera-Santos, C.D. Copple, EA Davis and WG. Hagar

Conditions such as temperature, pressure, pH, and medium composition are critical to the integrity and yield of products isolated after cell disruption.

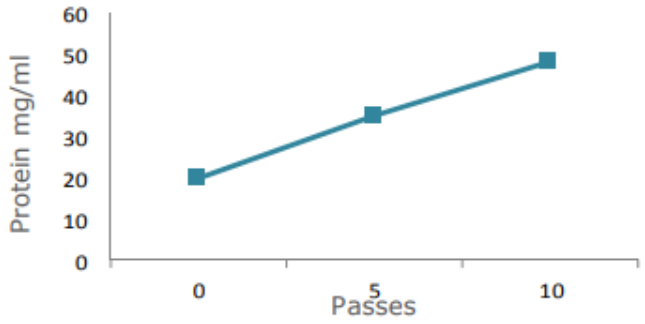
Methods originally designed for small-scale processing are often difficult and expensive to scale-up and fail to meet critical process control criteria. When a project is ready for full production, the selection of the cell disruption process used in the early development stage can significantly affect the projected value and viability of that project.

PROCESSING

The yeast (saccharomyces cerevisiae) used in this study was supplied in frozen cake form Miller Brewing Company and re-suspended at 11% solids in phosphate buffered saline. Cells were then processed through a Microfluidizer processor and a French Press side-by-side at the same pressure.

Conclusion

Faster and easier to use. The Microfluidizer processor was able to achieve more than 80% higher rupture rate after similar number of passes and gently break cells to release as much as 2.5 times higher yields of solubilized protein.



Solubilization of protein with Increasing passes at 20,000psi and 10°C number of passes (Microfluidizer at 20,000 psi)

Chart 1 demonstrating solubilization vs number passes

	Microfluidizer M-110Y	French press
Pressure	20,000 psi with back pressure	20,000 psi
Number of Passes	8	7
Conditions	Increased cooling temp. from 5°C to 10°C improved solubilized protein yield	
Results	92% breakage	50% breakage

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PUBLICATION SUMMARY #2

Characterization of E. coli Cell Disintegrates from a Bead Mill and High Pressure Homogenizers by Irene Agerkvist and Sven-Olof Enfors.

This publication compares the performance of three cell disruption methods: Microfluidizer processor, Bead Mill and High Pressure Homogenizer (HPH) across a range of parameters:

Viscosity

Viscosity varies significantly depending on which method is used. The viscosity of the cell disintegrate after one pass through the HPH is very high but decreases rapidly on further passes. Cell disruption with the Microfluidizer technology gives a disintegrate with a viscosity that is low after just one pass and which decreases further after subsequent passes.

Filtration

The cell disintegrate from the Microfluidizer processor gives the shortest filtration times and an overall better separation at centrifugation compared to the HPH. Further passes through the HPH actually make the separation worse.

Protein Release

The reason for disrupting the cells in the first place. The Microfluidizer technology gave the highest overall protein release, effectively plateauing at around 5 passes.

Particle Size Distribution

Residence time in the Bead Mill did not make a difference to the particle size distribution. The Bead Mill created comparable large and medium sized peaks. The HPH created a medium sized peak but also a peak of smaller particles that create viscosity and filtration problems. The Microfluidizer technology produced a single medium sized peak resulting in low viscosity and better separation.

Temperature

Higher temperatures denature proteins. Agerkvist and Enfors reported significantly higher temperatures after processing in the HPH vs the Microfluidizer technology. Consequently the Microfluidizer technology offers the highest yield of ß galactosidase enzyme. Exit temperatures of 40-50°C need not always be unacceptable because heat denaturation of proteins is dependent on time as well as temperature. The residence time in the Microfluidizer processor of 25ms-40ms is much shorter than in an HPH.

	Dry Weight BioMass g/L	Protein (%)	ß galactosidase (%)
Bead Mill			
2 min	49.5	62	62
3 min	49.5	72	74
4 min	49.5	79	79
HPH			
1 pass	48.4	66	58
2 passes	48.4	76	75
3 passes	48.4	82	78
Microfluidizer			
1 pass	47.6	63	61
2 passes	47.6	79	76
3 passes	47.6	88	87
5 passes	47.6	96	97
10 passes	47.6	100	100

The HPH heats the sample higher & longer – hence the increased denaturation that can be seen in the yield data

°C	Microfluidizer	HPH
Inlet	8-10	6-8
1 pass	23	21
2 passes	27	31
3 passes	28	40

Conclusion

Among the three methods, Microfluidizer technology not only yielded the highest protein and enzyme release (close to 100%) but also considerably different cell disintegrates, which enables much easier downstream purification processes. Small particles and wide particle size distributions created by the other two methods led to problems during purification. Smaller particles are difficult to separate from proteins by centrifugation and the wide particle size distribution causes blocking of filters.

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PUBLICATION SUMMARY #3

Evaluation of the Microfluidizer for Cell Disruption Purification of Cytochrome C Oxidase from Microfluidizer Processed Yeast by E. Llera-Santos, E.A. Davis, S. Ackerman and W.G. Hagar

The most efficient operating conditions for disrupting Yeast (saccharomyces cerevisiae) were developed and reported in Publication Summary 1.

In this study #3, the temperature sensitive enzyme cytochrome c oxidase (EC 1.9.3.1.) was isolated and its activity assayed by standard procedures. The solubilized protein was assayed using both the Bio-Rad Standard Assay and the cytochrome c oxidase according to a standard spectrophotometric method.

Processing

These experiments began with a semi-industrial volume of 10 liters of Baker's yeast in culture medium. Temperature sensitive enzyme cytochrome c oxidase was isolated and its activity assayed by standard procedures. Suspension cooled at 4-6°C before processing through a Microfluidizer processor and a High Pressure Homogenizer side-by-side under the same conditions.

Conclusion

The Microfluidizer yielded approximately 3 times as much sub-mitochondrial protein (SMP) compared to the HPH despite slightly less initial breakage indicating loss or damage of fewer particles during processing.

	Microfluidizer M-110Y	High Pressure Homogenizer
Pressure	10,000 psi	10,000 psi
Number of Passes	5	2
Conditions	20 foot cooling coil immersed in ice water	
Results	Yielded 5.87g of sub-mitochondrial particle (SMP) protein per kg. of dry yeast after 53% disruption.	Yielded 1.4 to 2.2g. SMP per kg. dry yield at 58% disruption

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PUBLICATION SUMMARY #4

Research report by Dr. Paul Sallis, University of Kent, England

This report shows the effect of the Microfluidizer technology on protein recovery from three different bacterial: Agrobacterium Sp., C. Glutamincum, and B. Lichenformulis.

Experiment #1

In Experiment 1A the protein in the extract after disruption increases with each pass through the Microfluidizer technology (chart 1). Note that the experiment with Agrobacterium Sp. cells was done at lower pressure. The resulting lower protein release even after many more passes suggests that process conditions should be optimized based on the requirement from different cells. In Experiment 1B (chart 2) a comparison between the Microfluidizer technology and the French Press is featured. Results clearly showed that Microfluidizer technology achieved much higher (6x) protein recovery compared to the French Press, which led to product losses even after the first pass.

Experiment 2

The table below shows that the Microfluidizer technology can be used to optimize total protein recovery, maximize Specific Activity of released enzymatic protein, and increase the total mass of recoverable, usable enzymatic protein.

The M-120E Microfluidizer processor was used in these applications at 15,000 psi. It is important to note that the Microfluidizer M-110Y operating at 20,000-23,000 psi in one cycle releases the same amount of protein as the Microfluidizer M120E operating at 15,000 psi in 21 passes.

Note also that specific activity of the protein enzymes is the same regardless of number of passes, but more of the protein enzyme can be recovered with increased number of passes.

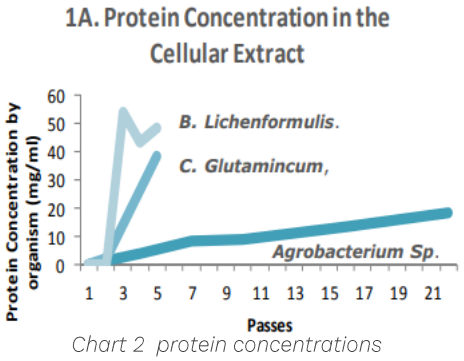

Chart 2 protein concentrations

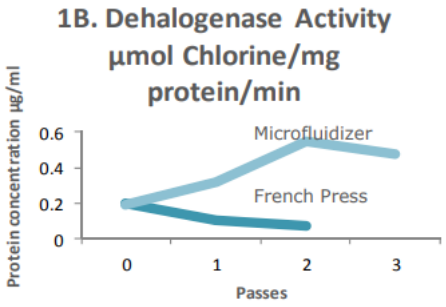

Chart 3 protein concentrations

Number of passes	Protein Concentration (Mg/ml^{-1})	Specific activity (µmol Chlorine)	Dehalogenase Units Released Protein/min
1	16.3	0.363	4734
2	20.5	0.341	5529
3	24.3	0.341	6629
4	28.0	0.323	7243
5	28.3	0.3	6792

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PUBLICATION SUMMARY #5

Research report by Maurice Gaucher, University of Calgary, Canada.

The following data compares three mechanical disruption techniques - the Microfluidizer technology, the Braun Cell Homogenizer and the APV/ Gaulin Homogenizer.

Experiment #1

m-Hydroxybenzylalcohol dehydrogenase activity in cell free extracts as produced by three different methods of disruption.

Experiment 1 shows that all three methods appear to be similar in their ability to disrupt cells as determined by protein release (mg. protein/g. dry cells).

However, the Microfluidizer technology and Braun Bottle are associated with higher levels of enzymatic activity (approximately 40% higher) as well as efficient product temperature control. Given that the Braun Bottle method is only suitable for small scale preparations (10-20 ml), the method of choice for larger preparations would be the Microfluidizer technology.

Method of Disruption	Temperature of Extract [1]	Protein Content	Enzymatic Activity	Units/mg. Protein [2]
Microfluidizer Processor	5°C	253	216	0.82
Braun Cell Homogenizer	3°C	224	219	0.98
Manton Cell Homogenizer	15.5°C	225	128	0.57

Experiment #2

6-Methylsalicylic Acid Synthetase activity in cell free extracts as produced by three different methods of cell disruption.

Experiment 2 reveals that the Microfluidizer technology disruption is associated with a higher yield of enzymatic activity in extracts (100- 200% higher) as compared to the Braun Bottle and APV/Gaulin Homogenizers.

Method of Disruption	Temperature of Extract	Protein Content	Enzymatic Activity	Units/mg. Protein [3]
Microfluidizer Processor	7°C	138	14.2	103
Braun Cell Homogenizer	5°C	179	5.4	29.5
Manton Cell Homogenizer	16°C	138	7.4	160

[1] Temperature was increased immediately after cell disruption

[2] One unit of activity is the amount of enzyme required to catalyze the formation of 1 µmole of NADP 1 per minute at 30°C.

[3] One unit of activity is amount of enzyme required to catalyze the formulation of 1 µmole of 6-methyl-salicylic acid per minute at 30°C.

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PUBLICATION SUMMARY #6

Evaluation of the Microfluidizer for Cell Disruption of Yeast and Chlorella· by E Llera-Santos, C.D. Copple, E.A Davis and W G. Hagar

This application presents a difficult extraction for photosynthetic pigments since it is resistant to standard extraction methods which involve grinding with a mortar and pestle.

A 2,6 dichlorophenol indophenol (DCPIP) reduction assay was used to evaluate the functionality of the isolated thylakoid membranes. The assay dye is blue in its oxidized state and acts as a "sink" for the electron flow of Photosystem II; thus, the reduction of the dye is a measure of the integrity of the chloroplast preparation.

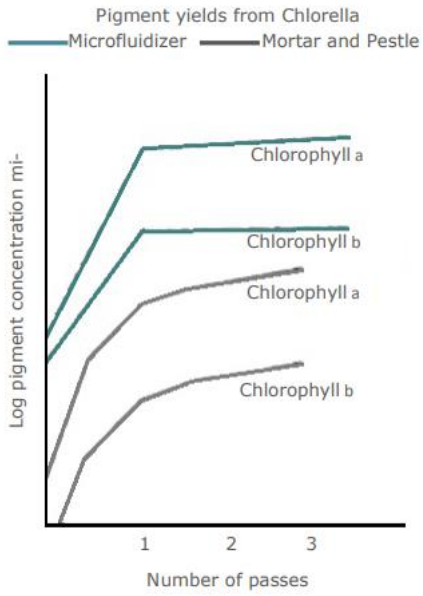
Conclusion

- 1 pass through the Microfluidizer technology achieved 95% disruption with almost complete extraction of both chlorophyll a and b.
- Yield of pigment/cell using the Microfluidizer processor was more than 6 times that achieved by grinding for 10 minutes with a mortar and pestle.

Results showed desired reduction of DCPIP was achieved in 10 minutes using a 1 pass preparation through the Microfluidizer technology.

Processing

Cell suspensions of the algae, Chlorella pyrenoidosa. diluted in phosphate buffered saline.



Results showed desired reduction of DCPIP was achieved in 10 minutes using a 1 pass preparation through the Microfluidizer.

	Microfluidizer M-110Y	Mortar and Pestle
Pressure	11,000 psi	
Number of passes	1	1-10 minutes grinding with mortar and pestle
Conditions	ice water continuously running in the cooling coils	

See next page for further results....

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Sample Description	Processing Conditions		Results and Comments
	Pressure PSI	# Passes	
Meningicoccal cell paste (0.4%) in buffer	1000	1	Cooling used to maintain 10°C temperature. Complete rupture achieved in one pass.
Mammalian cells	3000-5000	1	Complete rupture of cells and easy separation of parasite within the cell achieved in 1 pass on laboratory model H-5000
***E. coli* (10%) in buffer**	12000-18000	1	90% rupture achieved. Results scaled linearly from lab to production
Penicillium urticae	13000		40% more activity achieved than when processed on conventional homogenizer for m-hydroxybenzyl alcohol and 100 to 200% more activity for 6-methyl-salicylic acid.
Arthropod blood cells	5000 15000	3 1	Cooling used. · 10 micron spheres throughout gelatin matrix shattered with no visible cellular fragments. · Same as above but required only 1 pass so continuous processing possible.
Baker's yeast	20000	10	Cooling used. 95% rupture of yeast cells achieved.
Brewer's yeast (10%) in water	10000 20000	1 to 10 1 to 10	· Cooling used to keep temp below 5°C, in water 35% rupture at 1 pass and 63% at 10 passes, 71 % rupture at 1 pass and 98% at 10 passes. · Yield of enzyme significantly better than that achieved with other processing techniques including French press.
***M. lysodeiktuis* (1%) in deionized water**	25000	25	Cooling used. Approximately SO% rupture achieved. Superior to other mechanical techniques.