Microfluidics

Application Note

Cell Disruption—Don't take our word for it.

Here is a compilation of peer reviewed publications that highlight the performance and advantages of using a Microfluidizer™ for cell disruption.

Publication Summary #1

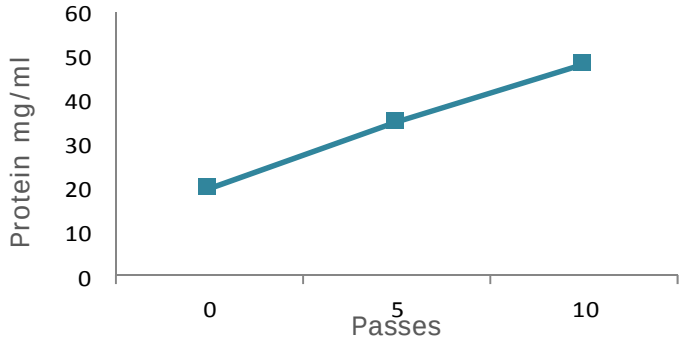
This report is an abridged summary of a paper entitled

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

Such conditions as temperature, pressure, pH, and medium composition are critical to the integrity and yield of products isolated after cell disruption. Processing methods originally designed for small scale processing are often difficult and expensive to scale-up and fail to meet these critical process control criteria. When a project is expected to lead to full production, the selection of the cell disruption process in early development can significantly affect the projected value and viability of that project.

Processing

Yeast (*Saccharomyces cerevisiae*) used in this study was kindly supplied in frozen cake form by Miller Brewing Company and re-suspended at 11% solids in phosphate buffered saline .



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

Conclusion

Faster and easier to use. The Microfluidizer™ was able to gently break cells to release as much as 2.5 times higher yields of solubilized protein.

	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

IDEX MATERIAL PROCESSING

Publication Summary #2

This report is an abridged summary of a paper entitled:

Characterization of E. coli Cell Disintegrates from a Bead Mill and High Pressure Homogenizers

Irene Agerkvist and Sven-Olof Enfors. Institute for Surface Chemistry, Box 560Z S-114 86 Stockholm, and Department of Biochemistry and Biotechnology, The Royal Institute of Technology, S-100 44 Stockholm, Sweden

This publication compares the performance of three cell disruption methods: a Microfluidizer, bead mill and high pressure homogenizer (HPH). The evaluation studied four parameters:

- **protein release**, typically the whole point of disrupting the cells.
- **particle size distribution**, which influences purification of the required protein.
- **viscosity**, also a factor in filtration and centrifugation steps.
- **temperature** history of the cells suspensions. Higher temperatures denature proteins.

Viscosity

There is a big difference in the viscosity 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 gives a disintegrate with a viscosity that is quite low already after one pass and which decreases even more on further passes.

Filtration

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

Protein Release

The Microfluidizer gave the highest overall protein release, effectively plateauing at around 5 passes.

	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

Temperature

Agerkvist and Enfors reported significantly higher temperatures after processing in the HPH vs. the Microfluidizer, consequently the Microfluidizer gave 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 of 25ms-40ms is much shorter than in an HPH.

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

The HPH heats the sample higher and longer—hence the increased denaturation that can be seen in the yield data.

IDEX MATERIAL PROCESSING

Particle Size Distribution

The residence time in the bead mill did not make a big 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 produced a single medium sized peak resulting in low viscosity and better separation.

Conclusion

All three disintegration methods give approximately the same protein and enzyme release but considerably different cell disintegrates, which influences centrifugation and filtration. Small particles and wide particle size distribution create problems during purification. Smaller particles are difficult to separate from proteins by centrifugation. Wide particle size distribution causes blocking of filters.

The Microfluidizer gives

- **Highest protein yield**
- **Shortest filtration times**
- **Lowest viscosity**
- **Better overall separation than the HPH**
- **Lower temperatures than the HPH**

Publication Summary #3

This report is an abridged summary of a paper entitled

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 Research Report Summary # 1. In this study 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. In this study, temperature sensitive enzyme cytochrome c oxidase was isolated and its activity assayed by standard procedures. Suspension cooled at 4-6^{o}C before processing.

Conclusion

The Microfluidizer yielded approximately 3 times as much sub-mitochondrial protein (SMP) 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

Pubication Summary #4

The following is from data developed by ***Dr. Paul Sallis of the Research and Development Center, University of Kent England.***

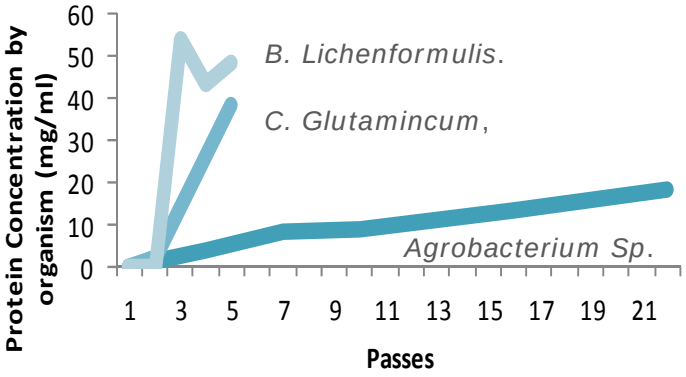
This application series shows the effect of the Microfluidizer on protein recovery from *Agrobacterium Sp.*, *C. Glutamincum*, and *B. Lichenformulis*.

Experiment 1

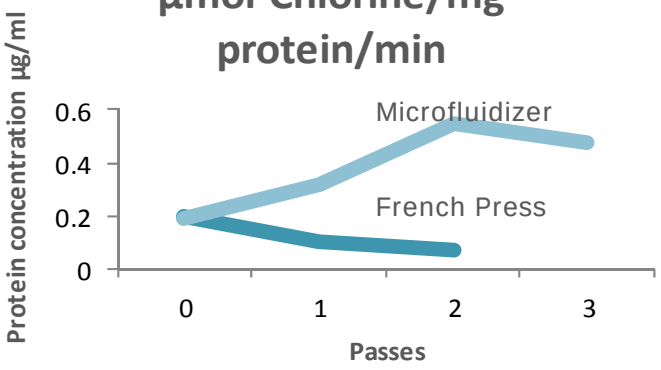
In Experiment 1A, the protein in the extract after disruption increases with each pass through the Microfluidizer.

In Experiment 1 B , a comparison between the Microfluidizer and the French Press is featured.

1A. Protein Concentration in the Cellular Extract



1B. Dehalogenase Activity µmol Chlorine/mg protein/min



Experiment 2

In Experiment 2, figures show the Microfluidizer 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 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 M-120E operating at 15,000 psi in 21 passes. Note also that specific activity of the protein enzymes is the same at both higher and lower pressure but more of the protein enzyme can be recovered at higher pressure.

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

IDEX MATERIAL PROCESSING

Publication Summary #5

This report is taken from data developed by ***Maurice Gaucher, University of Calgary, Canada.***

The following data compares three mechanical disruption techniques - the Microfluidizer, the Braun Cell Homogenizer and the Manton 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 and Braun Bottle are associated with higher levels of enzymatic activity (approximately 40% higher). 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.

Method of Disruption	Temperature of Extract[1]	Protein Content	Enzymatic Activity	Units/mg. Protein[2]
Microfluidizer	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 disruption is associated with a much higher yield of enzymatic activity in extracts (100-200% higher) as compared to the Braun Bottle and Manton Gaulin Homogenizers.

Method of Disruption	Temperature of Extract	Protein Content	Enzymatic Activity	Units/mg. Protein[3]
Microfluidizer	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.

IDEX MATERIAL PROCESSING

Publication Summary #6

This report is *an abridged summary of* a *paper entitled*

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.

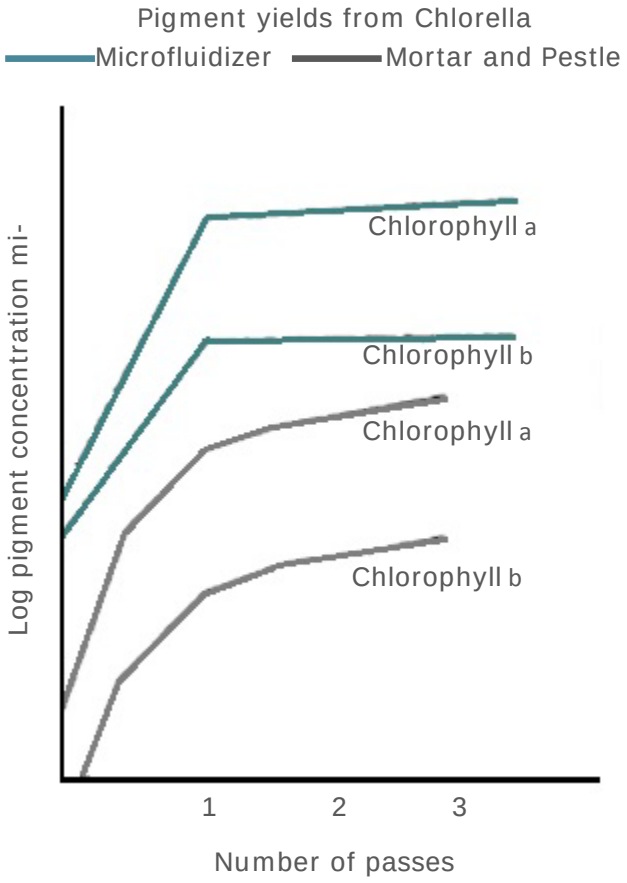
Conclusion

- **1 pass through the Microfluidizer achieved 95% disruption with almost complete extraction of both chlorophyll a and b.**
- **Yield of pigment/cell using the Microfluidizer 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.

Processing

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



	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	

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Sample Description	Microfluidizer Data		Results and Comments
	Pressure PSI	# Passes	
Meningicoccal cell paste (0.4%) in buffer	1000	1	Cooling used to maintain I0°C temperature. Complete rupture achieved in one pass.
Mammalian cells	2000	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	6000 10000 10000	3 1 3	90% rupture achieved. Results scaled linearly from lab to production • 90% rupture achieved. • 95% rupture achieved.
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.