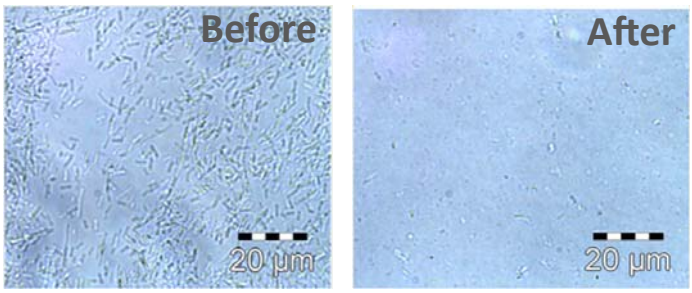
Microfluidics

Application Note

Use of Microfluidizer™ technology for cell disruption.

This Application Note gives an overview of the techniques used for cell disruption. In addition this is a summary of why a Microfluidizer is best suited for this application and the specific advantages Microfluidics technology has over alternative cell disruption methods. Also included are tips for optimal cell processing with a Microfluidizer

All cell disruption methods are not created equal. Results published in the scientific literature show that the disruption method strongly influences the physical-chemical properties of the disintegrate, such as particle size, disruption efficiency, viscosity and protein release.[1,2] For all of these important parameters the Microfluidizer comes out tops.



*Microfluidizers typically rupture **>95%** of E. coli cells in 1 pass*

Commonly used technologies

Lab scale:

French Press: generates high pressure in a pressure cell. A manually controlled valve releases the pressurized fluid from the pressure cell, resulting in cell rupture. Not scalable or repeatable; needs strength to close and open the valve. There are numerous hazards involved with French Presses. They are difficult and time consuming to clean, which has to be done for every sample. Most manufacturers of French Presses have discontinued production but they are still in use, available from small companies and second hand.

High pressure homogenizers (HPH) These devices are the next best alternative to Microfluidizers for cell disruption. Prices are typically equal to, or lower, than Microfluidizers. Cooling, cleaning, wear (valves!) and scalability can be issues. In particular if we look beyond simply the % of cells ruptured to the quality and usability of the ruptured suspension the Microfluidizer is the clear winner. Table 1 highlights the increased yield from a Microfluidizer compared to an HPH.

Ultrasonication: utilizes cavitational forces. Often used for very small sample volumes, the cell suspension is sonicated with an ultrasonic probe. Local high temperatures, resulting in low yields[2,4], scalability and noise are the main issues with this technology. Advantages are price of equipment and sample volumes (from µl) that can be processed.

Freeze-thawing: Subjecting the cell suspensions to variable temperatures results in rupture of the walls. This is not a very reproducible method, result will vary, is only suitable for very small samples in the ml range, but it is very cheap.

Chemical lysis: adding chemicals that soften and rupture the cell walls. Chemicals can be costly and thus scalability is limited. These chemicals contaminate the preparation which may be undesirable.

Mortar and Pestle: grinding the cell suspension. This is laborious manual work that can take several minutes, therefore not scalable and not very repeatable, only suitable for small lab samples.

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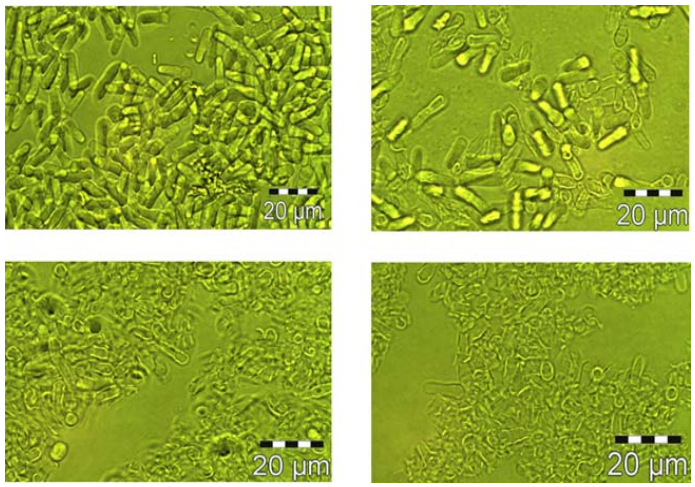
Media Milling: e.g. with Dynomills or similar equipment (*c.f.* Willi Bachhofen, www.wab.ch). Contamination by media and temperature control are difficult, other than that this tends to be an effective way of rupturing many cell types.

Enzyme pre-treatment: It is common practice to pre-treat cell suspensions with enzymes that soften the cell walls prior to mechanical disruption. It has been reported that this technique can still be valuable when using a Microfluidizer as it can reduce the pressure or number of passes required[2].



Yeast S.Cerevisiae Brewer's/Bakers yeast G10Z 30,000psi. Unlyzed, 1 pass, 5 passes, 10 passes

Table 2

	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	101.9	62	62
1 pass	73.2	65	61
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

Table 1

Disruption Equipment Used	Operating Pressure (psig)	Number of passes	Protein Concentration [Protein] (mg/ml)	Percent Lysis	Specific Catalase Activity (U/mg protein)	Total Product Catalase (U/mL)
HPH	10000	1	6.6	32%	160	1058
	12000	1	10.4	51%	108	1125
	15000	1	13.8	67%	103	1425
	10000	3	13.4	65%	119	1590
	12000	3	14.8	72%	85	1258
	15000	3	14.7	72%	77	1127
Microfluidizer	10000	1	10.2	49%	141	1444
	12000	1	12.6	61%	137	1729
	15000	1	14.7	72%	137	2019
	20000	1	18.1	88%	118	2122
	10000	3	17.2	84%	120	2066
	12000	3	16.1	79%	122	1963
	15000	3	17.4	85%	107	1385
	20000	3	20.1	98%	99	2007
	Control	100% Lysis	20.5			

+20% **+17%** **+40%**

Even excluding the 20,000psi result for the Microfluidizer, the results are impressively better than the HPH. The 20,000psi results for the Microfluidizer gives ***78% more Total Catalase than the best HPH data***

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Production:

High pressure homogenizers are the only alternative to a Microfluidizer for larger volumes. These are large scale versions of the lab units. This typically involves changes to the way the cells are ruptured to accommodate higher flow rates, resulting in inconsistency when scaling up. Multiple complex homogenizer valves may be required contributing to the downtime for these machines.

Why Microfluidics?

User friendly and easy to maintain

Customers that use our technology for cell disruption like the fact that Microfluidizers are very easy to use and clean. Multiple users in a lab can be comfortable with this technology because it does not require specialized skills or knowledge. Customers appreciate the fact that very little maintenance is required compared to HPH. Homogenizer valves have to be disassembled and cleaned manually; reinstalling requires specialist knowledge.

High Yield

The cooling is efficient and the protein and enzyme yields are therefore very good. During the cell disruption process, cooling is extremely important because the contents of the biological cells are typically temperature sensitive – in many cases they start to denature at temperatures above 4°C.

Within the Microfluidizer, the temperatures can certainly go above 4°C, but if the cooling devices are used well – with ice-water for example- the amount of time at elevated temperatures in the Microfluidizer is minimal.

Table 3

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

Table 3. 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.[1]

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[2] is much shorter than in an HPH. **The HPH heats the sample higher and longer—hence the increased denaturation that can be seen in the yield data.**

Wow that was quick!

The initial comment when we demo Microfluidizers is often “Wow, this is very fast”, because we process samples in a very short time compared to alternative methods. Dobrovetsky reports using 2 passes at 15,000 psi in a M110EH vs. 3 passes at 17,000psi in an Avestin Emulsiflex-C3[4]

Lower viscosity

The viscosity of the lysed cell suspension is important. If the viscosity is high it can make downstream handling e.g., filtration and accurate pipetting, difficult. 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 viscosity that is quite low already after one pass, and decreases even more on further passes. [1,2]

Improved Filtration

Cell disruption with the Microfluidizer gives an overall better separation of the cell disintegrates compared to the HPH. A Microfluidizer will break the cells efficiently but gently, resulting in large cell wall fragments. Particles produced by the Microfluidizer are 450nm *c.f.* 190nm for the HPH These large fragments are easier to separate from the cell contents, give shorter filtration times and better centrifugation separation than the material produced by other methods, in particular HPH[1,2,3,5]

Tips for using a Microfluidizer for cell disruption

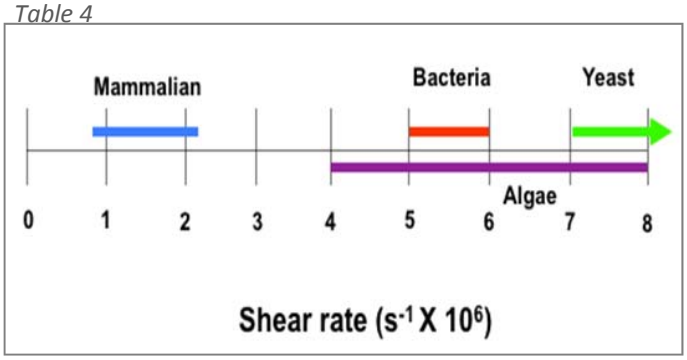
Do not over mix the pre-mix. Using a vortex mixer can entrap air in the cell suspension which will choke the Microfluidizer. This will stop the machine. The Microfluidizer is not plugged but the effect is the same. Gentle agitation is all that is required to keep the cells suspended.

Ensure the cooling bath is filled with **ice-water** during the process and refreshed as needed.

Process cells with **a 'Z' chamber with no APM**

Match the processing pressure to the type of cell. See tables 4 and 5. Bacterial cells vary markedly in their toughness. Gram negative cells like *E. coli* are the most commonly used and can be broken fairly easily. Gram positive cells are much tougher and should be treated like yeast or algae.

Table 4



Run the recommended process pressure and take samples at different numbers of passes. **Do not over-process.** Too many passes will result in a higher degree of rupture, but at the same time protein activities can be deteriorated by too much energy input/heat generation. Over processing may also make downstream filtration and pipetting more difficult.

Chamber blockages can happen when cells are re-suspended from frozen pellets if they are not all thawed, **ensure complete thawing.** Or when the cell concentration is too high (in that case dilute with more buffer if possible).

Avoid heating yeast cells to dryness before adding to a buffer suspension as this will make a tough cell wall even tougher

Cell Type	Pressure	Chamber
Mammalian	13.8-34.5 MPa 2,000-5,000 psi	L30Z (300µm)
Bacterial (E. coli)	82.7-124 MPa 12,000-18,000 psi	H10Z (100µm)or G10Z (87µm)
Yeast	138-207MPa 20,000-30,000 psi	H10Z (100µm) or G10Z (87µm)
Algae	69-207 MPa 10,000 -30,000 psi	H10Z (100µm) or G10Z (87µm)

Table 5

References

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Alfred Carlson, Mark Signs, Laura Liermann, Robert Boor, and K. Jim Jem3

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4. A robust purification strategy to accelerate membrane proteomics

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5. *Process Scale Disruption of Microorganisms*

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