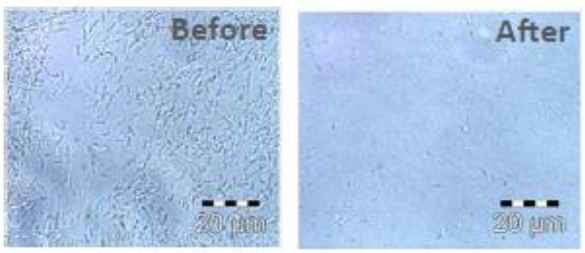
Microfluidics™

Application Note

Microfluidizer® Technology for Cell Disruption



Microfluidizer processors typically rupture >95% of E.coli cells in 1 pass

INTRODUCTION

This Application Note gives an overview of the techniques used for cell disruption, exploring the advantages that Microfluidics technology has over alternative cell disruption methods.

In the course of this paper we share tips on how best to achieve optimal cell processing with a Microfluidizer processor.

All cell disruption methods are not created equal. Results published in scientific literature shows 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]

In this paper we explore all of these important parameters and show why the Microfluidizer technology comes out tops.

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COMMONLY USED TECHNOLOGIES IN THE LAB

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. This is not scalable or repeatable and needs strength to close and open the valve. There are numerous hazards involved with using them and they are difficult and time consuming to clean, which has to be done for every sample. Although many manufacturers have discontinued production of the French Press they are still in use, available from small companies and second-hand.

High pressure homogenizers (HPH): these devices are the next best alternative to Microfluidizer processors for cell disruption. However, cooling, cleaning, wear of the 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 processor is the clear winner compared to the HPH. Table 1 highlights the increased yield from a Microfluidizer processor compared to an HPH.

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

Table 1

Ultrasonication: utilizes cavitational forces. An ultrasonic probe sonicates the cell suspension. This is often used for very small sample volumes. Whist the price of this technology is low, it has limitations on yield[2,4] due to the local high temperatures created near the probe and issues with scalability and noise.

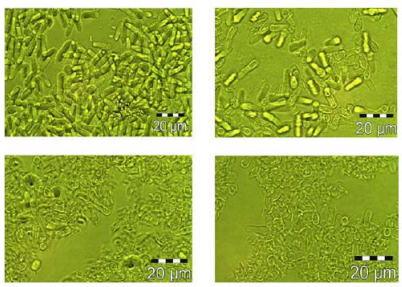
Freeze-thawing: subjecting the cell suspensions to variable temperatures results in rupture of the walls. This is not a very reproducible method so results will vary. It is only suitable for very small samples in the ml range.

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, so is therefore not scalable and not very repeatable. It is only suitable for small lab samples.

Media Milling: e.g. with DYNO®-MILLS or similar equipment. Contamination by the media and temperature control are hazards, other than that it 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 processor 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

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COMMONLY USED TECHNOLOGIES FOR PRODUCTION

High pressure homogenizers (HPH): the only alternative to a Microfluidizer processor for larger volumes. Creating higher flow rates typically involves changes to the way the cells are ruptured which causes inconsistency in scaling up. Multiple complex homogenizer valves may be required, which must be disassembled and cleaned manually, with reinstalling requiring specialist knowledge, which all contributes to the increased downtime for these machines.

WHY MICROFLUIDIZER TECHNOLOGY?

User-friendly and easy to maintain: customers that use our technology like the fact that Microfluidizer processors 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 also appreciate that very little maintenance is required.

High Yield: because the cooling process is efficient the protein and enzyme yields are therefore very good. The contents of the biological cells are temperature sensitive – often starting to denature at temperatures above 4°C.

Agerkvist & Enfors (Tables 2 & 3) higher temperatures reported processing with an HPH, the Microfluidizer processor gave a higher yield of ß galactosidase enzyme.[1]

Exit temperatures of 40-50ºC need not be unacceptable as heat denaturation of proteins depends on time as well as temperature. Residence time in the Microfluidizer processer 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.

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

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 3

That was quick! The initial comment when we demo our Microfluidizer processor is *"Wow, this is very fast"*, because we process samples in a shorter time than the alternatives. 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 difficult e.g., filtration and accurate pipetting. 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 processor 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 processor gives an overall better separation of the cell disintegrates compared to the HPH. A Microfluidizer processor will break the cells efficiently but gently, resulting in large cell wall fragments. Particles produced by the Microfluidizer processor 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 HPH.[1,2,3,5]

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TIPS FOR USING A MICROFLUIDIZER PROCESSOR IN CELL DISRUPTION

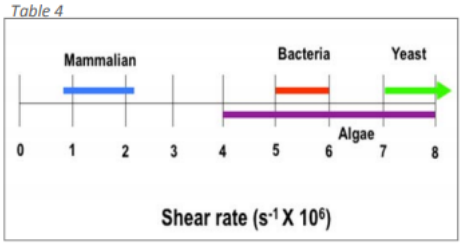
Do not over mix the pre-mix. Using a vortex mixer might entrap air in the cell suspension which, in turn, will choke the Microfluidizer processor and stop the machine. In fact, it is not actually plugged, but the effect is the same. Gentle agitation is all that is required to keep the cells suspended.

Use ice-water to fill the cooling bath and refresh as needed.

Process cells with a Z-type interaction chamber (IXC). An auxiliary processing model (APM) can be used and placed upstream to provide additional pre-dispersion of cell suspensions.

Match processing pressure to cell type. See tables 4 and 5. Bacterial cells vary markedly in their toughness due to differences between cell wall structures. Gram Negative cells like E. coli are the most commonly used and can be broken fairly easily. Whereas Gram Positive cells are much tougher due to their much thicker peptidoglycan layer presented in the cell membranes, therefore should be treated like yeast or certain tough algae cells with higher shear forces.

Table 4



Don't over-process: take samples at different numbers of passes and run at the recommended process pressure. Whilst many passes creates a higher degree of rupture it causes protein activities to be deteriorated by too much energy input/heat generation. Over-processing may also make downstream filtration and pipetting more difficult.

Ensure complete thawing: Chamber blockages can happen when cells are resuspended from frozen pellets if they are not all thawed. 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:

1. *I. Agerkvist and S.O. Enfors, Biotechnol Bioeng., 1990, 36(11): 1083-1089.*
2. J. Geciova, D. Bury and P. Jelen, Int. Dairy Journal, 2002, 12(6): 541-553.
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5. A.P. Middleberg, Biotechnol Adv., 1995, 13(3): 491-551.