# High Cell Disruption Rates and Protein Yield Utilizing a Microfluidizer® Processor

TB-CD-1

## Cell Disruption

From the gentle disruption of cultured mammalian cells for virus isolation to the challenging disruption of yeast and other fungi, Microfluidics offers technologies to meet variable and demanding needs for cell disruption. Microfluidizer processors enable extremely effective cell disruption (often >99% rupture for *E.coli* in only one pass), with high protein recovery. Protein integrity is maximized by the Microfluidizer processor's low temperature rise coupled with highly effective cooling provided by submerging the interaction chamber and placing a heat exchanger immediately downstream of the interaction chamber. These capabilities allow researchers to use the lowest pressures possible to reach target rupture rates while avoiding protein denaturation.

## Advantages of Microfluidizer Technology

- Highest protein integrity recovery
- Processes samples at a constant, controlled shear rate
- Guaranteed scalability of interaction chamber to production volumes
- No contamination - media-free and low-wear processing
- Sample volumes as small as 1 ml with the exclusive LV1

## Benefits of Continuous Processing Versus Batch

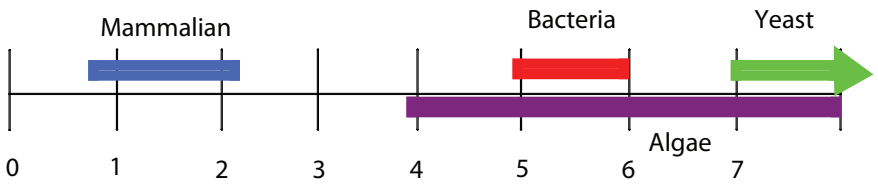
- Optimization of temperature control
- Residence time in high temperature zone is minimized (<1 second). Batch processes such as sonication and bead milling subject the cells to high localized energy which can not be easily removed.
- Continuous processing at constant pressure ensures that all cells will receive the same amount of energy input. With sonication, cells close to the probe will receive exponentially more energy than cells away from the probe.
- With batch systems, there is little control of the uniformity of the energy to each cell

## Representative Cell Disruption Applications:

| | |
|---|---|
| -Yeast | -Fungi |
| -E. coli | -Penicillium |
| -Mold | -Meningococcal cells |
| -Algae | -Bacteria |
| -Mammalian tissue | -Insect cells |

## Shear Rates

Shear rate ($s^{-1}$ X $10^6$)




Microfluidics

## Testimonials

*"...The specific enzymatic activity obtained was highest (on the Microfluidizer processor) probably due to the ease of cooling."*

J. Geciova, et al
Milchwissenschaft
57 (9/10) 2002

*"Originally we worked with a French press and Gaulin homogenizer, but they posed contamination and equipment cleaning problems. After a demonstration here at our lab, [the Microfluidizer processor's] performance and sterilization features convinced us to get one immediately."*

Stephen Cameron
Quality Assurance Manager
Microtek International
Victoria, British Columbia

*"The advantage of the Microfluidizer processor is that it allows us to break the E. coli fast, with minimal impact on the cell's internal compartments."*

Gabrielle Heilek-Snyder, Ph.D.
Tularik, Inc.
San Francisco, CA

*"98.18% Sporocyst recovery and 84.04% Oocysts cracked using 125 micron chamber at 4,000 psi."*

Methods of Releasing Sporocysts From Oocysts Using Controlled Shear Forces – J.E. Hutchins, et al, 2008

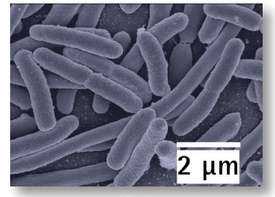
## Comparison of Mechanical Methods for Cell Disruption

| | Microfluidizer | Homogenizer | Sonicator | Bead Mill |
|---|---|---|---|---|
| Continuous | Yes | Yes | No | No |
| Scalable | Yes | Limited | No | Yes |
| Optimal Temp Control | Yes | Yes | No | No |
| Contamination Free | Yes | Yes | Yes | No |
| Minimum Volume | 1ml | 10 ml | 1ml | 1 ml |
| Constant Shear Rate | Yes | No | No | No |
| Shear Rate Potential | Highest | High | High | Medium |

## Results from the Technology Center

| Cell Disruption | | | |
|---|---|---|---|
| **Sample Description** | **Microfluidizer Processor Data** | | **Results and Comments** |
| | **Pressure (psi)** | **# Passes** | **Microns (µm)** |
| Cell wall material | 15,000 | 0<br>1<br>5<br>10<br>20<br>40 | 18.109<br>10.584<br>0.954<br>0.855<br>0.732<br>0.655 |
| E. coli cells | 23,000 | 0<br>1 | 0.978<br>0.538 |
| Meningicoccal cell past (0.4%) in buffer | 1,000 | 1 | Cooling used to maintain 10°C temperature. Complete rupture achieved in one pass. |
| Mammalian cells | 2,000 | 1 | Complete rupture of cells and easy separation of parasite within the cell achieved in 1 pass. |
| E. coli (10%) in buffer | 6,000<br><br>10,000<br>10,000 | 3<br><br>1<br>3 | 90% rupture achieved. Results scaled linearly from lab to production.<br>90% rupture achieve.<br>95% rupture achieved. |
| Penicillium urticae | 13,000 | 1 | 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 | 5,000<br><br>15,000 | 3<br><br>1 | Cooling used. 10 micron sphere throughout gelatin matrix shattered with no visible cellular fragments.<br>Same as above but required only 1 pass to continuous processing possible. |
| Baker's yeast | 2,000 | 10 | Cooling used. 95% rupture of yeast cells achieved. |
| Brewer's yeast (10%) in water | 10,000<br><br>20,000 | 1-10<br><br>1-10 | Cooling used to keep temperature below 5°C. 63% rupture at 10 passes.<br>98% rupture at 10 passes. Yield of enzyme significantly better than that achieved with other processing techniques including French press. |
| M. lysodeiktuis (1%) in deionized water | 25,000 | 25 | Cooling used. Approximately 50% rupture achieved; superior to other mechanical techniques. |

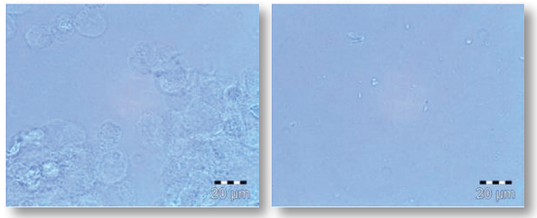
## Bacteria Cells




- Most commonly used cells for production of simple proteins
  - Very high growth rates
  - Little/no glycosilation ability
- Require medium to high shear rates
- Usually only require only 1 pass on a Microfluidizer processor to achieve >99% rupture efficiency

## Mamalian Cells

- Performed for NC State University Gene Therapy Center to release viral vectors from human embryonic kidney cells
  - Cells processed with the Microfluidizer processor for 1 pass yielded expected high amount of protein
- Process conditions: 1 pass 535 bar (5000 psi)
- Chamber: H30Z (200 microns)
- Shear rate: 1.40 X $10^6$ $s^{-1}$

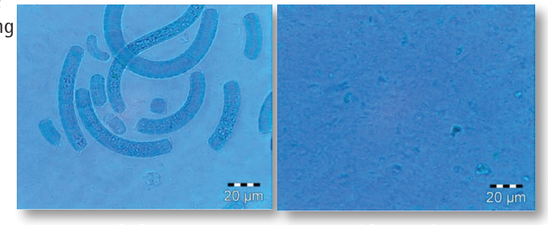



Before | After - 1 Pass

## Algae Cells

As the supply of fossil fuels diminishes, the need for renewable fuel sources will increase. Biofuels from algae cells are appealing because they grow quickly and can directly convert CO2 to longer chain oils which can be easily converted to biodiesel. The cells must be ruptured in order to gain access to oil, and because there is a wide variety of algal cells which all require different shear rates to rupture, Microfluidizer processors offer the flexibility and efficiency to get the job done.

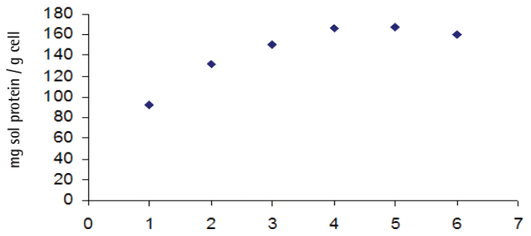
- Process conditions: 1 passes 690 bar (10,000 psi)
- Chamber: H10Z (100 microns)
- Shear rate: 4.14 X $10^6$ $s^{-1}$



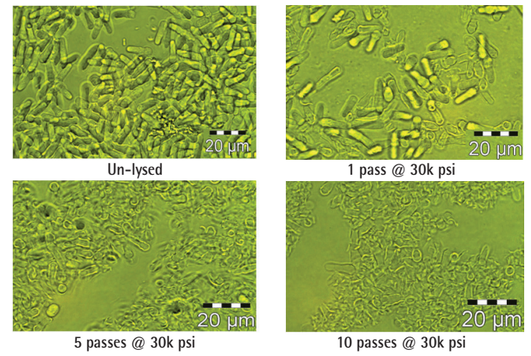

Before | After - 1 Pass

## Yeast Cells

- The maximum recovery of soluble protein is achieved at 5 passes
- Further passes appear to cause more protein to denature than are liberated by the lysis
- Process conditions: 2070 bar (30,000 psi)
- Chamber: G10Z (87 microns)
- Shear rate per pass: 6.94 X $10^6$ $s^{-1}$




*S. Pombe*

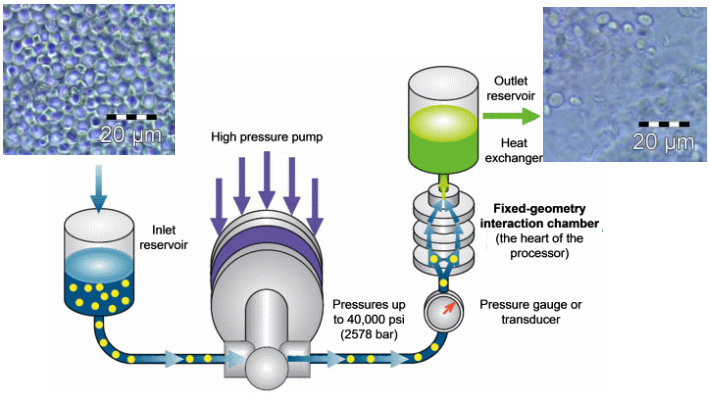



Un-lysed | 1 pass @ 30k psi

5 passes @ 30k psi | 10 passes @ 30k psi

# How Microfluidizer Processors Work

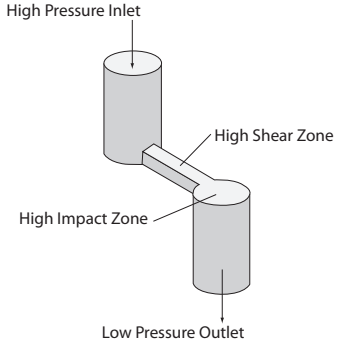
The intensifier pump drives the product through precisely defined fixed-geometry microchannels within the interaction chamber. As a result, the product stream accelerates to high velocities, creating shear rates within the product stream that are orders of magnitude greater than any other conventional means. All of the product experiences identical processing conditions, producing high yield cell disruption.



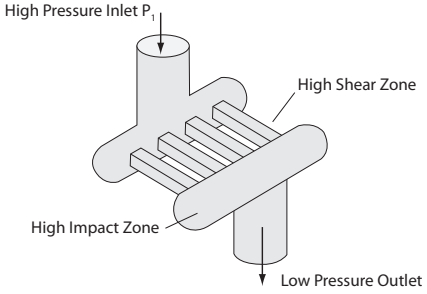

# Scaleup Guaranteed

Unlike other mechanical methods for cell disruption, Microfluidics guarantees scaleup by utilizing multi-slotted interaction chambers in conjunction with a constant pressure pumping system. This increases throughput from lab to production scale while ensuring the product stream is exposed to consistent shear and processing conditions.

**Single-Slotted "Z" Chamber**




**Multi-Slotted "Z" Chamber**




# Let Us Help You Improve Your Product

For more than twenty years, customers have partnered with expert engineers in the Microfluidics Technology Center to help determine the ideal processing conditions and interaction chamber configuration to achieve product goals. From *E. coli* to yeast, Microfluidizer high shear fluid processors provide scalable solutions for cell disruption to hundreds of biotechnology companies and universities around the world.

## Patent Examples

Applied For and/or Awarded

*5,721,120 - Method of disrupting cultured cells using an impinging jet device*

*6,455,287 - Mechanical disruption of bacterial cells for Plasmid Recovery*

*5,914,390 - Methods for Increasing Yields of Recombinant Proteins*

*6,120,732 - Microbial Inactivation by High-Pressure Throttling*

*0049829 - Concentration and Lysis of Adenovirus-infected cells in a Single Unit Operation*

*0191899 - Method for Disrupting Cells That Lack a Cell Wall*

*0233348 - Method for Isolating Proteins from Production Cells*

*0003602 - Method of Extracting Proteins from a Cell*

Microfluidics

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