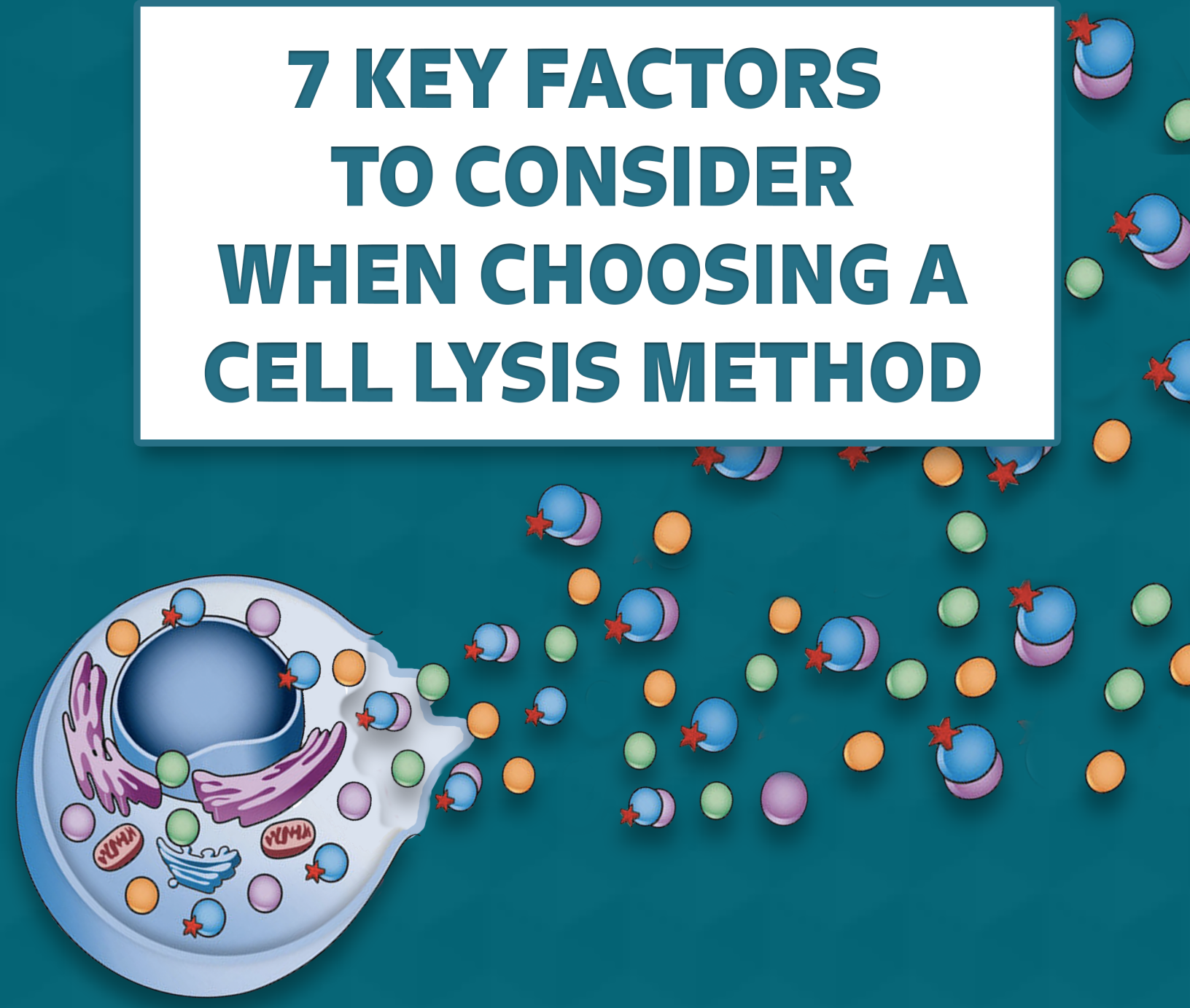
BEE INTERNATIONAL
NEXT GENERATION HOMOGENIZERS

7 KEY FACTORS TO CONSIDER WHEN CHOOSING A CELL LYSIS METHOD



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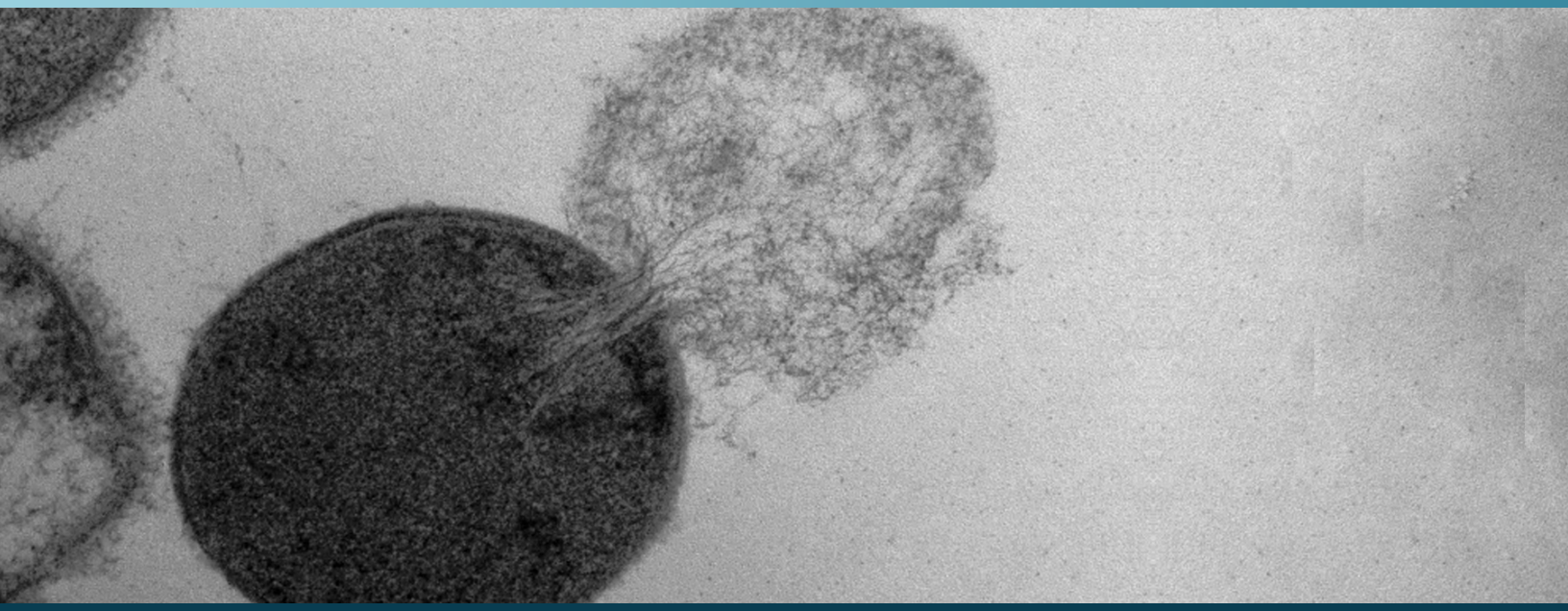
What is Cell Lysis?

Cell lysis is a process during which a cell is broken down due to an external condition. This process disrupts parts of the cell wall, extracting and separating the organelles, proteins, DNA, mRNA of microorganisms such as (but not limited to) E. coli, yeast, mammalian tissue, bacteria, algae and fungi.

Cell lysis can happen naturally, such as via viral infections, or it can be induced artificially for research in various industries, including biotech, pharmaceutical, food, cosmetic, chemical and food. Cell contents are also harvested for commercial manufacturing purposes.

For cell lysis to be useful for research purposes, it is vital to prevent the extracted materials from being inactivated, denatured or degraded by exposing them to an environment outside of the cell. For the food industry in particular, it is necessary to deactivate and destroy pathogens in foods, yet while retaining the nutritional properties.

To that end, several methods have been developed to prevent uncontrolled cell disruption, and ultimately achieve the best possible purity and yield.



Overview of Cell Lysis Methods

While there are several cell lysis methods available to researchers, there are five in particular that are the most common. These are highlighted in the following table:

Cell Lysis Method	Primary Forces at Work	Apparatus
High Shear Mixers and Traditional Homogenizers	Shear; Cavitation	Cell or tissue suspensions are sheared by forcing them through a narrow space.
Sonication	Cavitation	High frequency sound waves break cells apart.
Additives	Chemical	Cells can be treated with various agents to aid the disruption process. The agent must be removed after disruption.
Grinding	Impact; Shear	Blenders with rotating blades grind and disperse cells and tissues that have been frozen in liquid nitrogen
Chemical	Detergents (unique properties enable disruption of hydrophobic & hydrophilic interactions among molecules and used to lyse cells)	Detergents can be denaturing or nondenaturing. Examples of Denaturing anionic detergents are sodium dodecyl sulfate (SDS) or cationic such as ethyl trimethyl ammonium bromide. Non-denaturing detergents can be divided into nonionic detergents such as Triton* X-100, bile salts such as chocolate and zwitterionic detergents such as CHAPS.

Factors to Consider When Choosing the Best Cell Lysis Method

Ultimately, choosing the best cell lysis method should take into consideration the following seven criteria:

1. Accessibility of intracellular proteins

Extraction and, in particular, solubilization of proteins is considered by some to be the most difficult step in preparing protein samples proteomic studies.

As such, an effective cell lysis method must address this challenge by ensuring the accessibility of intracellular proteins.

2. Process Flexibility

It is not uncommon for research teams to shift their focus as a result of the iterative and exploratory nature of research itself.

For example, research teams attempting to produce biological bacteria and thus believe they require only a sonicator for cell lysis, may discover that the biological material cannot be fully active unless processed in a eukaryotic system.

An effective cell lysis method will have the flexibility to enable this shift efficiently and cost-effectively!

The ideal method provides gentle disruption of cultured cells for virus isolation to intense high pressures for rupture of yeast and other fungi, but without buying new equipment.

3. Easy to Use

Despite the intricate, meticulously detailed nature of cell lysis, the fact remains that a functional and practical cell lysis method must be easy to use. Otherwise, researchers risk spending an inordinate amount of time calibrating and, if necessary, re-configuring equipment.

4. High Yield in Less Time

In order to save money and control costs, an effective cell lysis method must provide the highest possible yield of ruptured cells in the shortest possible time. At the same time, a consistent yield of results is critical in order to develop a viable manufacturing protocol.

5. Reproducible and Scalable Results

An effective cell lysis method produces results that are reproducible and scalable. This is especially important if research teams propose clinical trials based on early experiences that show tremendous potential.

Scalability must also meet sanitary and manufacturing requirements, in addition to being cost effective.

6. Can Process Various Sample Sizes

For small samples, it is vital to minimize loss and maximize sample recovery. Other critical factors include avoiding contamination, ease of cleaning, and the ability to create repeatable results.

For large samples or for large capacity testing and manufacturing high yield, the critical factors include generating consistent results, sufficient throughput and capacity, and adhering to manufacturer sanitary and validation requirements.

7. Ability to Disrupt All Cell Types

An effective cell lysis method can disrupt all cell types and thus prevent researchers from purchasing and configuring new equipment when, for example, switching from spores (which are among the most difficult cells to disrupts) to E.Coli (which tend to be easier).

The BEE High Pressure Homogenizers Advantage

Earlier, this report highlighted five commonly-used cell lysis methods: High Shear Mixers and Traditional Homogenizers; Sonication; Additives; Grinding and Chemical. While all of them have benefits and drawbacks, as illustrated by the following table, none of them reflect all seven criteria that together comprise the "best" cell lysis method:

More details on the next page!

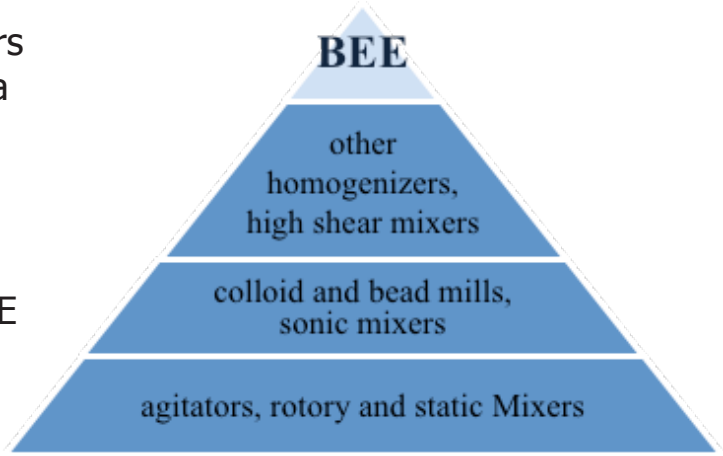
Cell Lysis Method	Accessibility	Flexibility	Ease	High Yield	Reproducible & Scalable	Various Sample Sizes	Disrupt all Cell Types
High Shear Mixers	⚠	⚠	✓	⚠	✗	✗	⚠
Traditional Homogenizers	⚠	✗	✓	⚠	⚠	✗	⚠
Sonication	⚠	✗	⚠	⚠	✗	⚠	⚠
Enzymes	✗	✗	✗	✗	✗	✗	✗
Grinding	✗	⚠	✓	✗	✗	✗	✗
Chemical	✗	⚠	⚠	✓	✗	⚠	⚠
BEE High Pressure Homogenizers	✓	✓	✓	✓	✓	✓	✓

Here is a more detailed look:

Cell Lysis Method	Accessibility	Flexibility	Ease	High Yield	Reproducible & Scalable	Various Sample Sizes	Disrupt all Cell Types
Shear Mixers	Low shear mixers require longer processing time, but can increase risk of inactivation & damage This improves slightly with high shear mixers	Requires more than 1 piece of equipment or expensive options	Good	Weak	Expensive maintenance costs; not all shear mixers are in-line	Not a wide range	May require different equipment for different types of cells
Traditional Homogenizers	Temperature Problems, May Not Work for Most Resistant Cells	Inflexible	Good	A less effective process means longer processing time to achieve a required yield	May require more process time because it is simpler & needs more passes	Not a wide range	May require different equipment for different types of cells
Sonication	Limited efficiency, works with less resistant cells, but not more resistant cells	Inflexible, process cannot be altered	May require short runs for larger samples	Not efficient, only one mixing force requires more time	Not an in-line process, equipment not available for large scale	Good for small samples, but not large batches	Works with less resistant cells
Enzymes	Long incubation time and reducibility problems	Inflexible	Complex	Not consistent for all products or from batch to batch	Must remove enzyme post-processing, expensive	Too expensive for large batches	Not consistent & not flexible
Grinding	Uneven process & incomplete lysis	Weak	Good	Low efficiency	Not widely used	Not a wide range	Inconsistent and incomplete lysis
Chemical	Can cause change in proteins	Weak	Costly for larger samples	Good	Expensive for large scale	Costly for larger samples	Depends on solution
BEE High Pressure Homogenizers	Temperature controlled, full capability for all products	Easily adaptable for all cells without buying new equipment	Simple to use, simple to adhust, easy to clean	Can fine tune process to reduce number of passes & increase yield with low time and low effort	Easy to scale from pilot testing to commercial production	Can run large or small samples in-line	Appropriate to create the optimal process for all types of cells.

There is one option that does indeed reflect all seven criteria, and leverages all primary mechanical forces – adjustable, turbulence pre-mix, cavitation, shear and impact – in order to deliver optimal results: BEE High Pressure Homogenizers.

BEE High Pressure Homogenizers use an in-line process to apply a variety of forces that can be easily adjusted to produce optimum results.



Researchers worldwide trust BEE High Pressure Homogenizers because they:

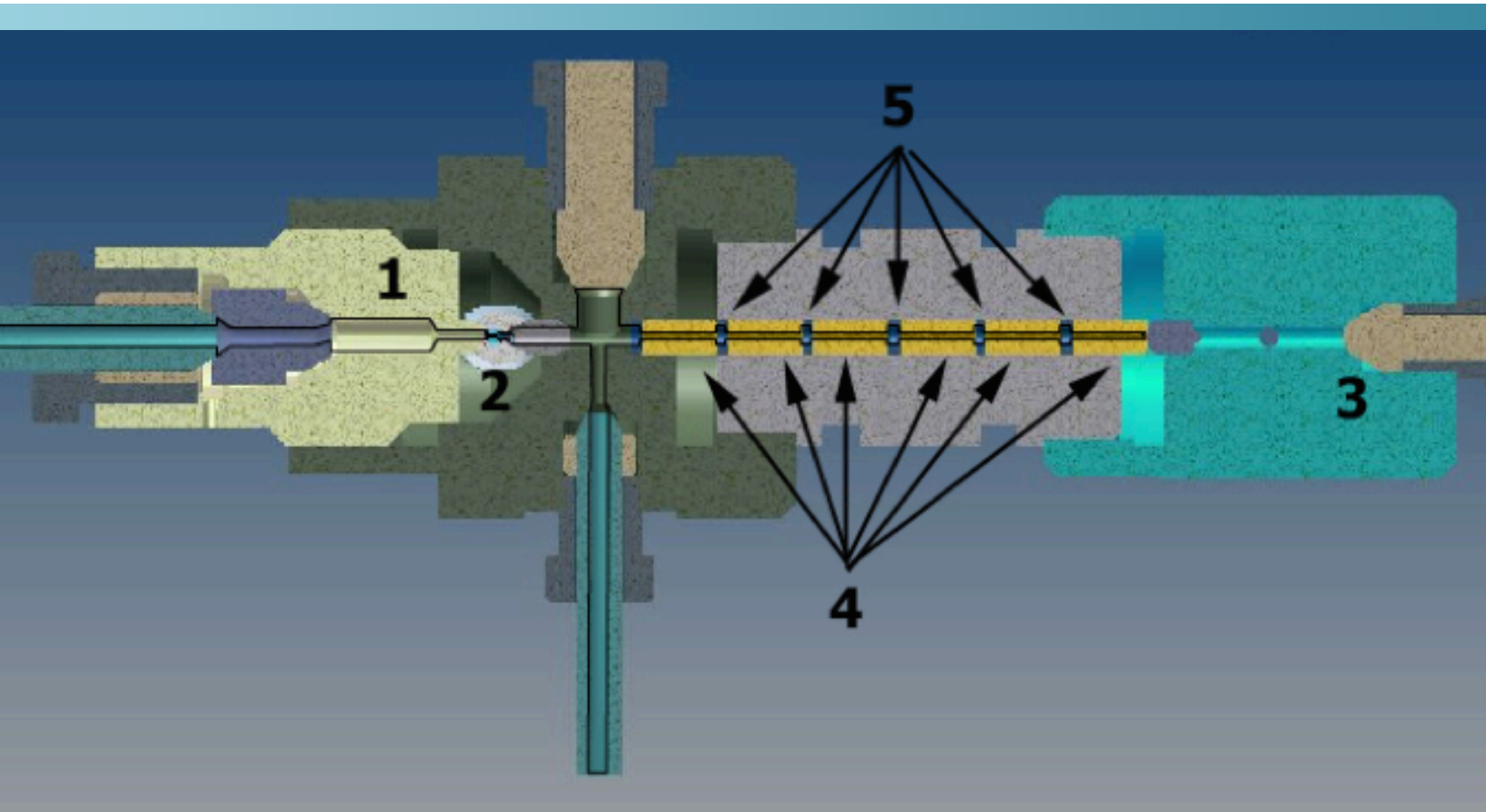
- Provide accessibility of intracellular proteins for extraction and solubilization.
- Offer process flexibility, and can be quickly and easily adjusted for different cell disruption strategies, such as gentle or harsher methods. They can also be adjusted to control the mixing process to deactivate cell contents, which is required in the food industry where it is necessary to deactivate and destroy pathogens in foods, but retain the nutritional properties.
- Are easy to use and require minimal training to get up and running. The sanitary design also allows for rapid reconfiguration, easy cleaning and low maintenance.
- Deliver high yields in less time, which lowers Total Cost of Operation (TCO).
- Are built with scalable technology, which allows research teams to elevate from laboratory scale to pilot scale, while achieving reproducible results.
- Support various sample sizes, and allows researchers to adjust the widest variety mixing forces.
- Disrupts all cell types, as researchers can change pressure, flow, cavitation, shear, impact and process time to get the best results regardless of what cells they are disrupting.

Proprietary Emulsifying Cell Design

BEE High Pressure Homogenizers are designed around a unique and proprietary Emulsifying Cell. This design replicates the product passing through the cell multiple times.

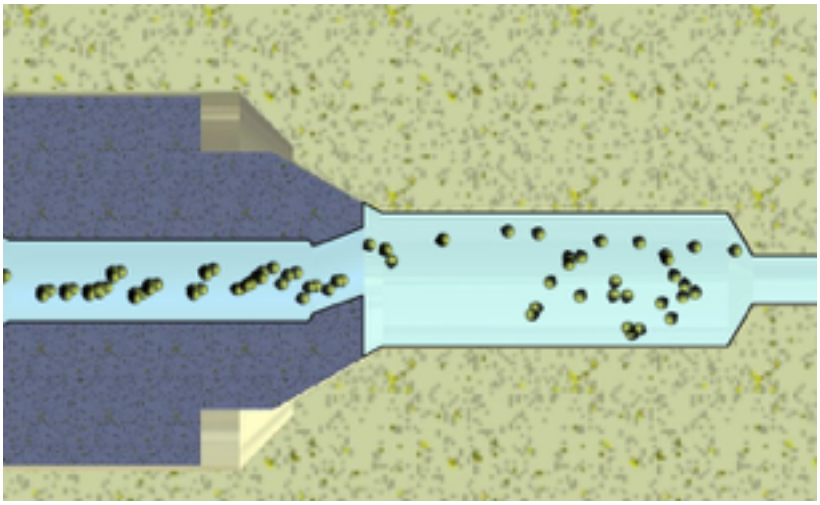
Below is a cross section of the Emulsifying Cell. While an overview of the particle size reduction process is also below, the details of where and how particle size reduction is achieved will be elaborated upon on the next few pages!

1. Turbulent Premixing
2. Passing Through the Nozzle
3. Impact
4. Shearing
5. Multiple Passes



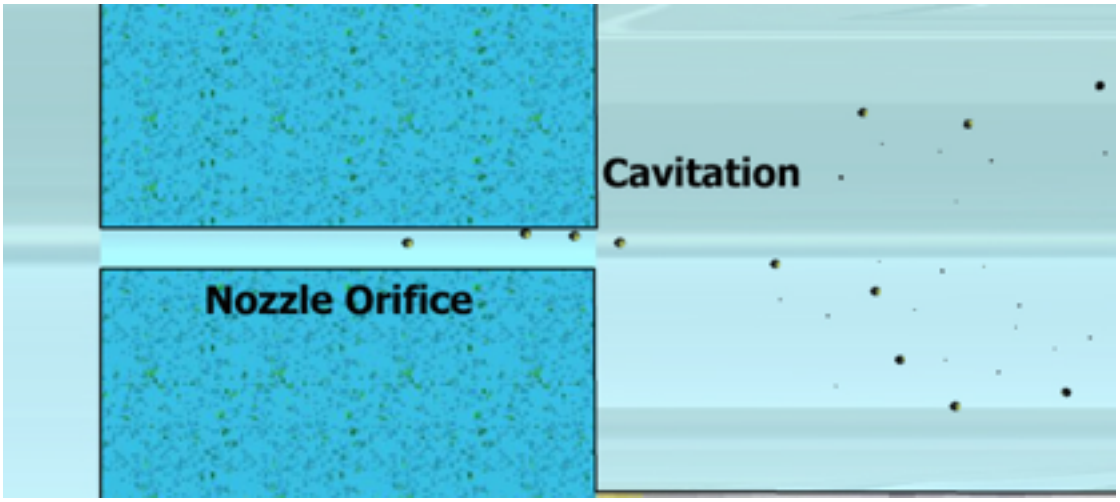
1. Turbulent Premixing (Similar to Stirrers or Agitators)

Before entering the nozzle a turbulent flow is created to "stir" the product. A laminar flow is also an option at this point.



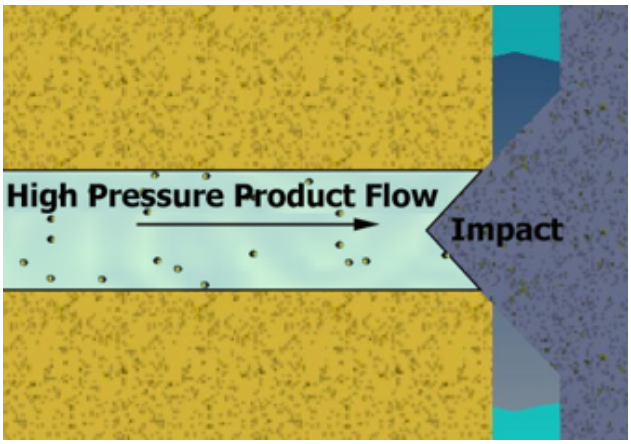
2. Passing Through the Nozzle (Cavitation like Sonic Mixers)

Upon exiting the nozzle, the force of cavitation contributes to breaking of cell walls. As the vapor bubbles move through higher pressure points they implode. More or less cavitation can be employed for gentler or harsher cell disruption by varying operating pressure. This is done simply with the turn of a dial.



3. Impact (Similar to Bead Mills)

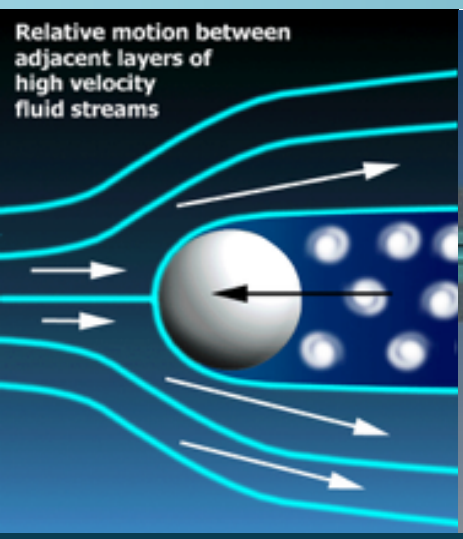
As product hits the end fixture in the Emulsifying Cell, the force of impact further breaks cells apart. Impact can be very useful for stronger cell walls (such as with spores), or it can be avoided altogether (such as with E. Coli) by using a Parallel Flow Setup.

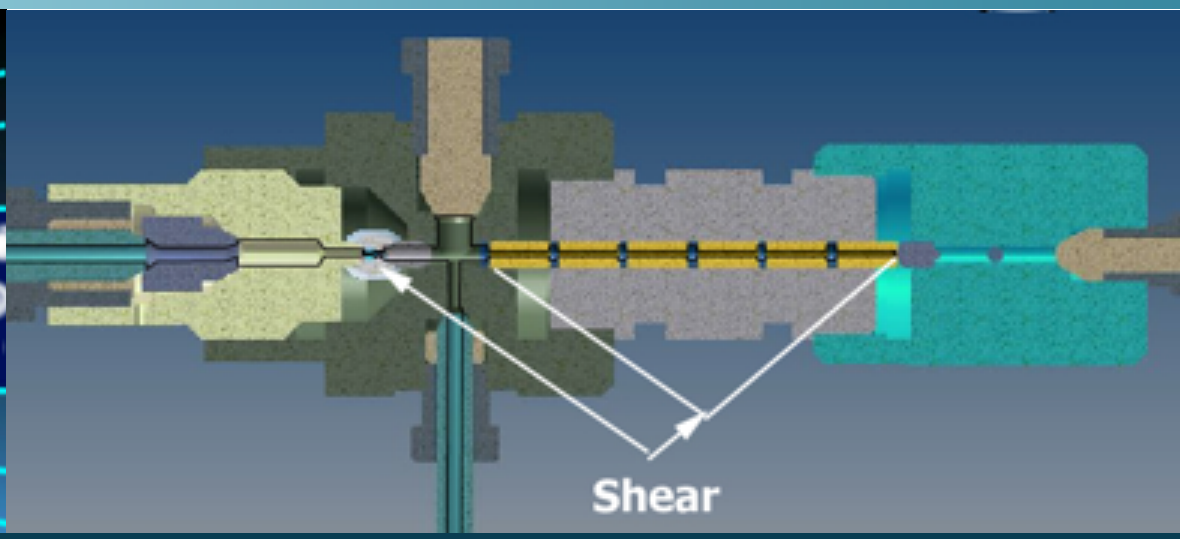


4. Shear (A Higher Shear Level than High Shear Mixers)

Shear tears particles apart reducing them in size. The rate of shear is for a flowing fluid is based on its velocity. Shear is employed throughout the Emulsifying Cell for continued particle size reduction. The force of the jet stream exiting the nozzle is powerful enough to cut metal.

Shear levels in the Emulsifying Cell are higher than that of high shear mixers. BEE technology is flexible, and the process duration can be shortened or extended, which allows for more or less shear.





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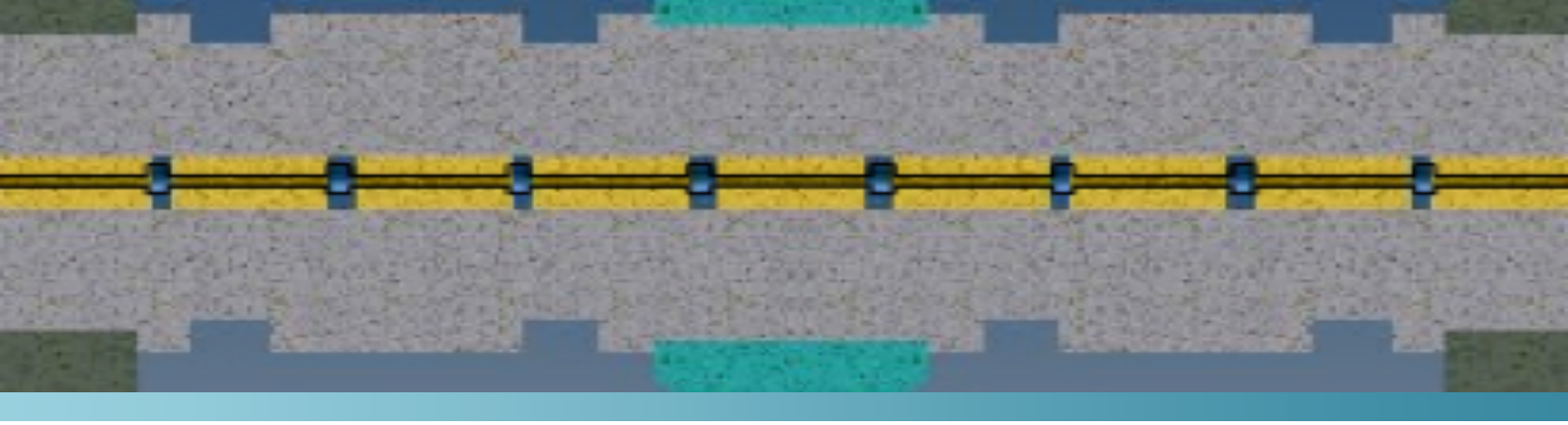

5. Two to Eleven Reactors to extend process duration (Multiple Passes)

The Reactors in the Emulsifying Cell induce absorption where fluid particles are diffused together, which creates a new blend of products and shortens or extends process duration. As particles exit the Emulsifying Cell, they are further reduced again and again.

This is why results are achieved with fewer passes on BEE systems. One pass through a BEE system has the same effect as multiple passes through other equipment.

Learn More about the BEE

BEE International is a worldwide supplier of high pressure homogenizers for the pharmaceutical, biotech, chemical, cosmetic and food industries. In the laboratory, our systems process fluids producing uniform particle size reduction to nanoparticles, and high yield cell disruption.

All our systems produce the same results. Scaling up to pilot and clinical trial settings, our high pressure homogenizers consistently produce the same results and have a reputation for reliability. These qualities continue into manufacturing, where the in-line process reduces costs by achieving better results in less time.

Contact us to learn more about our high pressure homogenization products and technology!

ABOUT BEE INTERNATIONAL

BEE International was established in Israel in 1994 for the sole purpose of developing and bringing to the market innovative homogenizing technology and equipment.

The company began in cooperation with Technion University Israel Institute of Technology which is among the top science and technology research universities in the world.

In 1998 BEE International opened a US operation. Today, BEE International has representation and equipment installations worldwide.

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