Cell Disruption
by Homogenization

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World Leader in Homogenization Technology

Cell Disruption

Recent developments in biotechnology and genetic engineering have produced many products from microorganisms. Most of these products (proteins and enzymes) are intracellular and need to be released from the interior of the cell. Cell disruption (CD) techniques for breaking cell walls have been studied for many years. Some of these methods are suitable only for small batches and can adversely affect enzymes and proteins.

One of the methods that can be used for small batches and for production batches is the cell disruption homogenizer. This report discusses the development of the CD homogenizer and other disruption techniques. Also, the effects of pressure, homogenizing valve design and multiple passes on cell disruption are discussed.

Introduction

In the downstream processing of fermentation products, the apparatus used for cell disruption has become very important to the efficiency of the overall process. Recent advances in recombinant DNA technology have brought about the development of new means for the production of useful enzymes and proteins[1]. Most fermentation and recovery operations require the breakage of the product microorganism to release these enzymes and proteins for separation and purification. Along with these biotechnological techniques has come the need to disrupt cells in large scale and in an efficient and economical manner.

Although CD techniques have been investigated for the past forty years, only recently has there been a need to apply these methods on a large scale. When considering the requirements of large-scale, efficient cell disruption, many methods fall short and only a few CD techniques can be applied to the rigorous demands of today's biotechnology operations. One device that fulfills these requirements is the CD homogenizer, and the purpose of this report is to describe the CD homogenizer and its use in downstream processing.

At this point, it is important to define the term "homogenizer". In recent years "homogenizer" has been applied to many different types of emulsification devices, but the original name was used by Auguste Gaulin in France to describe the machine he invented at the turn of the twentieth century for the processing of milk. Auguste Gaulin's machine consisted of a positive-displacement pump forcing product through a restricted orifice (homogenizing valve), which is adjustable so that pressure can be varied[2]. With a homogenizing valve designed to maximize cell disruption and with a pump designed for biotech applications, the machine is called a "CD homogenizer".

History

The archives of APV Products – Homogenizer, contain an interesting history of the development of the Gaulin CD homogenizer. The first mention of cell disruption in the archives relates to beer fermentation. A patent specification from 1932[3] proposed using high-pressure drop through an orifice for disrupting yeast cells used in the fermentation of beer. During the fermentation process, the yeast cells in the broth take up vitamins. When the yeast cells are removed from the broth, the vitamins are also removed. However, if the yeast cells are disrupted at the appropriate time in fermentation, then the cells would release the vitamins back into the beer, producing a vitamin-enriched beer. Investigation by Gaulin researchers at that time did not reveal any benefits from this process.

From 1951 to 1953, the Gaulin laboratory ran many tests on yeast to determine the effect different homogenizing valve configurations had on the efficiency of cell disruption. The evaluation tests were simple procedures involving centrifuging the disrupted samples and visually examining the type and amount of various layers formed in the centrifuge tube. The conclusion was that a knife-edge type valve was best for efficient disruption. At that time this valve was called a “high impact” valve.

In 1962, Gaulin researchers worked with a brewery to test the effect homogenization had on a brewer’s yeast and fermentation time. The idea was that an increase in fermentation rate would be accomplished by uniformly distributing the yeast in the broth by breaking up clusters of yeast cells or by releasing nutrients from disrupted yeast to furnish food for fresh yeast. However, these tests did not demonstrate any improvement in the fermentation process, despite some early claims that the fermentation time was reduced.

By 1963 Gaulin had a homogenizing valve available specifically for cell disruption. This special valve and seat with a knife-edge configuration on the seat is now designated a CR valve. The National Institute of Health used the Gaulin homogenizer and CR valve in 1967 for disruption of E. coli, Salmonella species, clostridium and bacillus species, fungus-Neurospora crassa and Aerobacter yeast.

By the early 1970s, work was being done by many research centers using the Gaulin homogenizer for cell disruption. Although there were a number of techniques for disrupting very small quantities of microorganisms, the homogenizer was a useful tool for disrupting large batches of slurry from one liter to several liters. The concern was not with the efficiency of cell disruption but rather with being able to process larger quantities of slurry than could be treated with other disrupting methods available at that time. The early sales of homogenizers were mostly to research laboratories and

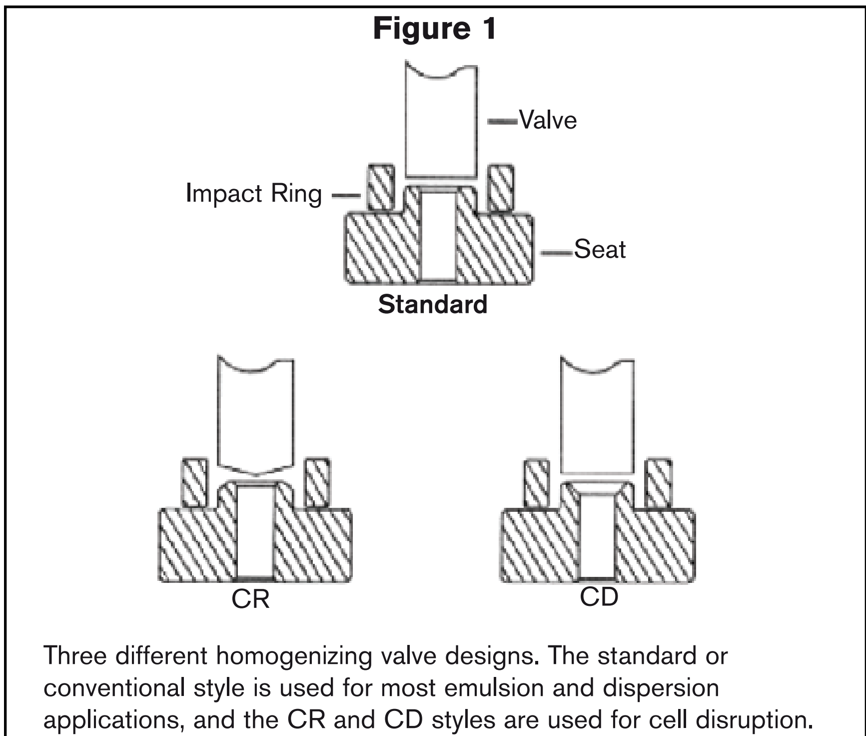
universities. By 1973, Gaulin had sold 66 homogenizers for cell disruption. Of these machines, 62 were laboratory units and only four were small pilot lab or production units. Of the 24 units sold in the United States, 18 were for universities.

The advent of recombinant DNA techniques in the 1980s brought many new products from microorganisms which now necessitated not only that larger quantities of cells be disrupted but that it be done continuously and efficiently in a processing system downstream from the fermenter. These demands initiated a re-examination at Gaulin of the CR valve and the homogenizer pump design for biotech applications. From this research, the CD valve replaced the old design CR valve, and a modified pump design was developed for special biotech applications (Figure 1). To date, Gaulin and Rannie have sold hundreds of homogenizers for cell disruption.

Cell Disruption

Single-cell organisms (microorganisms) consist of a semipermeable, tough, rigid, outer cell wall surrounding the protoplasmic (cytoplasmic) membrane and cytoplasm. The cytoplasm is made up of nucleic acids, proteins, carbohydrates, lipids, enzymes, inorganic ions, vitamins, pigments, inclusion bodies and about 80% water. In order to isolate and extract and of these

Figure 1



Three different homogenizing valve designs. The standard or conventional style is used for most emulsion and dispersion applications, and the CR and CD styles are used for cell disruption.

substances from inside the cell, it is necessary to break the cell wall and protoplasmic membrane. In some cases the cells may excrete the desired substance; but, in most cases, the cell wall must be disrupted to release these substances.

Methods for Cell Disruption

Over the years, many different techniques have been developed to disupt cells. One of the early references to cell disruption[4] describes the use of a pressure vessel with discharge through a needle valve. The slurry is placed in the vessel and a plunger in the vessel is used to bring the pressure up to 20,000 psi (137.9 MPa). The material in the vessel is released to atmosphere through the needle valve. The pressure change causes disruption of cells. This type of device was the precursor to the modern-day FRENCH® Laboratory Press (from a method developed by Dr. French). This apparatus is limited to small sample sizes but can reach high pressures. However, no special technology is involved in the design of the needle valve. One problem associated with this technique is the large temperature rise in the sample after passing through the valve. Duerre and Ribi[5] cooled the valve and removed heat from the product but still found degradation of protein when operated in the range of 25,000 to 55,000 psi (172.4 to 379.3 MPa). Wimpenny[6] found that Gram negative rod-shaped bacteria and Mycobacteria were the easiest cells to disrupt, and the Gram positive cocci and the alga chlorella were the hardest to break.

Garver and Epstein[7] used hand grinding to break cells. They scaled the process up by mixing glass beads with a cell slurry and processed the mix through a colloid mill. After 15 minutes, 99% of E. coli cells were ruptured; and, after 20 minutes, 99% of bakers' yeast was ruptured. Rodgers and Hughes[8] disrupted cells with glass beads also. In 1961 and 1962 Hughes[9,10] reported on the use of an ultrasonic probe, generating liquid cavitation at 20,000 Hz, for disrupting cells. The formation and collapse of cavitation bubbles can produce large temperature and pressure gradients in proximity to the collapsing bubbles. The theory was that the cells were disrupted due to the shearing forces from turbulent eddies produced by collapsing bubbles. The amount of disruption depended on the type of organism and the time of treatment. A one-hour treatment of E. coli produced a significant amount of disruption.

Tannenbaum and Miller[11] in 1967 made one of the first references to the use of the Gaulin homogenizer for cell disruption. The purpose of using the homogenizer for cell disruption was to release protein from cells that were being used in feeding experiments with rats. Compared to feedings with whole (unbroken) cells, the disrupted cells increased protein digestibility and net protein utilization and increased total body lipids. Other authors also described the benefits of using cell disruption to improve

the digestibility of cells from single cell protein (SCP) fermentation[12-15]. Although much attention was focused on SCP as a potential food source, activity in this project declined because of increased petroleum prices, lack of taste or visual appearance and lack of approval from health authorities for animal or human consumption of SCP[16].

Wiseman[17] and Zetelaki[18] in 1969 reviewed some of the different methods used for cell disruption and reported on the relative efficiencies of the various techniques. By 1971, Jakoby[19] indicated that the two devices used for large-scale cell disruption were the bead mill and the high-pressure homogenizer.

Follow, et al.[20] and Hetherington, et al.[21] reported on detailed studies using the Gaulin homogenizer for disruption of bakers' yeast. Follows used the homogenizer in a recycle mode to extract seven enzymes and protein from the cell slurries. Hetherington produced the most detailed study, up to that time, on the use of the high-pressure homogenizer for cell disruption. A first-order equation was derived from the data relating pressure and number of passes to yield. The other results of this test were:

1. Protein release is temperature dependent (higher is better).
2. Protein release is independent of yeast concentration.
3. Protein release is pressure dependent (higher is better).
4. A knife-edge seat is better than a standard seat.

Whitworth[22,23] also used the Gaulin homogenizer for cell disruption. In these tests, it was found that the extent of disruption was dependent on the operating pressure and the number of passes through the homogenizer. The homogenizer, operating at high pressure, did not denature protein.

In a report by Cunningham et al.[24], different techniques for cell disruption of SCP were discussed and evaluated. The conclusion was that the high-pressure homogenizer is the most feasible apparatus for scale-up of all the methods studied. Also, as mentioned earlier, cell disruption is a necessary step for the release and solubilization of intracellular proteins. If the whole cells are ingested, the tough cell wall may allow the cells to pass through the digestive tract intact with no utilization of the cell protein[25].

At this point it would be useful to review all of the methods used for cell disruption (Figure 2). As can be seen from the list, there are many non-mechanical methods for cell disruption. These techniques have been described in the literature, and they will not be reviewed here in detail[26, 27]. However, it can be said that some of the problems associated with these methods include high cost ingredients, denaturing of proteins, destruction of enzyme products, small-scale batches and lack of scale-up to continuous operation.

Some of the items listed as mechanical methods, such as sonification and the French press, have been covered here already. Freeze pressing involves pressurizing a chamber of frozen slurry until a phase change occurs, allowing the slurry to pass through a fixed orifice. Ice crystals in the mix may contribute to the grinding an disrupting of the cells[28]. Repeated cycles of freezing and thawing have been used to release cell components[29].

Decompression simply involves placing a cell slurry into a pressure vessel, charging with nitrogen gas to the desired pressure and then releasing the pressure while either retaining the slurry in the vessel or ejecting it through an orifice. Most of these mechanical methods, except for two, have

Figure 2

CELL DISRUPTION METHODS

Non-Mechanical Methods

CHEMICAL TREATMENT
- Acid
- Base
- Solvent
- Detergent

ENZYME LYSIS
- Lytic Enzymes
- Phage Infection
- Autolysis

PHYSICAL TREATMENT
- Freeze-Thaw
- Osmotic Shock
- Heating & Drying

Mechanical Methods

HIGH PRESSURE HOMOGENIZATION

WET MILLING

SONIFICATION

PRESSURE EXTRUSION
- French Press
- Freeze Pressing

DECOMPRESSION (PRESSURE CHAMBER)

TREATMENT WITH GRINDING PARTICLES

limitations with respect to batch size and scale-up. The only two that can accommodate large slurry batches are the high-speed bead mill and the homogenizer.

Wet milling methods include the use of high-speed mills. The slurry is pumped through a chamber containing beads and agitator discs[30]. The discs run at speeds of 1500 to 2000 rpm, and cell disruption is caused by grinding between the beads, collisions between the beads and the organisms, and shear forces due to velocity gradients caused by the beads' movement. The beads are loaded into the chamber at 80 to 85% of the free volume of the chamber. Glass beads at a diameter greater than 0.5 mm are best for yeast, and beads at a diameter less than 0.5 mm are best for bacteria. Figure 3 lists some of the parameters that affect the disruption efficiency of a bead mill[31]. Some of the problems associated with bead mills include large temperature rise, poor scale-up and contamination of the product by bead material.

Another technique for cell disruption that has been investigated involves the use of supercritical fluids (SCF) and explosive decompression. A supercritical fluid is a gas that is pressurized above its critical pressure, giving it unique properties of solvation and transport. The gas and cell slurry are introduced into a pressure cell. The pressure is held for a period and then the gas is released. The slurry is then analyzed for released proteins. One investigation reported the following results. At 25°C, with bakers' yeast and carbon dioxide gas, the maximum yield from CD occurred after 15 hours at 1000 psi, 12 hours at 3000 psi and 5 hours at 5000 psi[32]. This approach to cell disruption may be beneficial in preserving the released cell constituents because thermal and mechanical effects are minimized[33].

Of the different methods for cell disruption discussed here, the homogenizer satisfies most of the requirements for large-scale cell disruption. The homogenizer can be operated at high pressure; the

Figure 3

PROCESS VARIABLES OF A BEAD MILL

Agitator Speed

Proportion of Beads

Bead Size

Cell Suspension Concentration

Cell Suspension Flow Rate

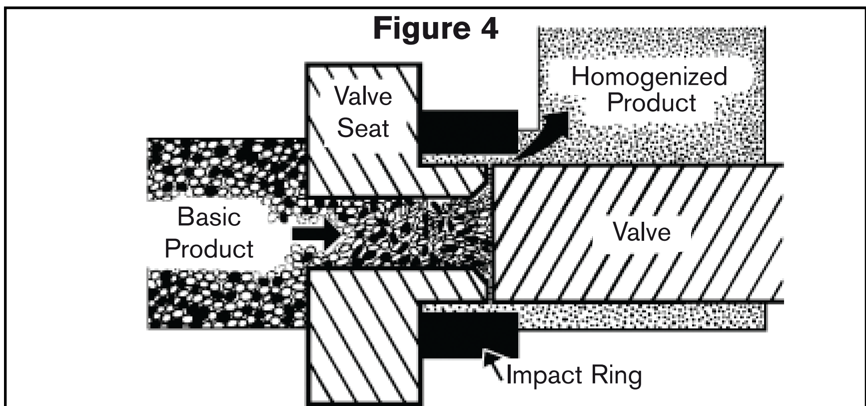
Agitator Disc Design

efficiency of disruption is good; it can be placed in a continuous system and special designs are available specifically for biotechnology applications[34].

Homogenizer

As previously mentioned the homogenizer consists of a positive-displacement pump and a homogenizing valve. The pump delivers a relatively constant flow of liquid, regardless of the pressure set on the homogenizing valve. Of course, there will be some pumping loss depending on the efficiency of the pump and the viscosity of the product. Also, the horsepower of the motor and the pressure rating of the pump determine the maximum operating pressure of a homogenizer.

Figure 4 shows flow through the homogenizing valve. In the jargon of the industry, the words “homogenizing valve” mean the combination of the valve, seat and impact ring. The pump forces liquid through the homogenizing valve assembly. The valve is pushed towards the seat by the action of a handwheel or hydraulic valve actuator, which reduces the flow area between the valve and the seat. Since the flow rate is constant, the pressure increases as the flow area is reduced. The liquid is under pressure from the discharge manifold of the pump up to the homogenizing valve. As the liquid passes through the homogenizing valve, the velocity increases and the pressure decreases rapidly (Bernoulli theorem). An example is: At 15,000 psi (103.4 MPa) the velocity of the liquid goes from about 6.1 m/s (or 762 miles per hour!) in a distance of about 0.254 mm in 7.5 microseconds. The pressure drops from 15,000 psi to atmospheric pressure also in this time. This intense energy transformation produces the effect called homogenization. For a mixture of two immiscible liquids and a surfactant, homogenization will produce an emulsion. For a dispersion, homogenization will break up solid agglomerates and disperse particles uniformly throughout a liquid. In cell disruption, the cell wall will be broken open.



In the past the actual mechanism of homogenization was not completely defined because of the difficulty of studying a phenomenon that occurs so rapidly and at high pressure. There were many theories proposed for the homogenization of emulsions including cavitation, turbulence and shear, but no one theory was absolutely proven. Research now suggests that intense turbulence at the exit from the gap between the valve and seat causes emulsification in liquids[2]. Some researchers have proposed mechanisms for cell disruption such as turbulence, cavitation and impingement[35-41]. Experimentation by this author and others strongly supports the mechanism of impingement as the cause for cell disruption. Therefore, cell disruption is governed by somewhat different hydrodynamic considerations than is emulsification. This type of information is useful in the proprietary design considerations of homogenizing valves used for cell disruption.

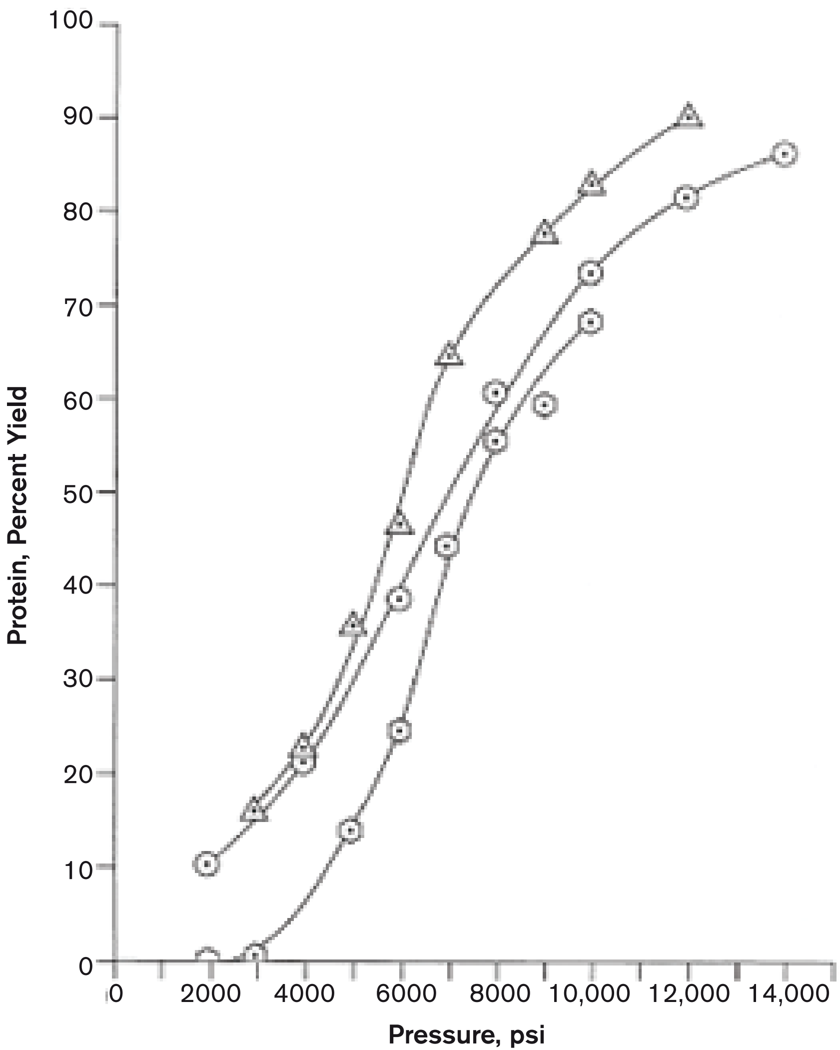
Obviously, homogenizing valve design for cell disruption is best studied by disrupting cell slurries with different valve configurations. The APV Products-Homogenizer laboratory uses bakers' yeast for the microorganism because it is inexpensive, easily obtained, nontoxic and has a relatively tough cell wall compared to an organism such as E. coli.

Test Methods

The test procedure used by this author involves the following. First, a slurry of 10% yeast (dry cell weight) in water is prepared[42]. The slurry is kept cold because homogenization will add heat to the broth. With water, the temperature rise through a homogenizer is 3.0°F (1.7°C) per 1000 psi (6.9 MPa) pressure. This temperature rise is inversely proportional to the heat capacity of the liquid and is independent of the type of homogenizer valve[43].

A batch of this slurry is run through the homogenizer, and samples are collected at different pressure settings. The collected samples are immediately cooled. Then the samples are diluted and centrifuged. Also, a portion of each original sample is diluted with 2N NaOH, heated to 90°C and then centrifuged. The samples treated with caustic are the controls for each test condition and contain 100% of the soluble protein. After centrifuging, the samples are again diluted, and the amount of soluble protein is determining using a protein assay reagent from Bio-Rad Laboratories (California). The amount of protein in the sample is divided by the amount of protein determined from the caustic treatment. This ratio gives the percent of soluble protein released from the cell for each condition. All assays are done in triplicate.

Because these analyses are performed using commercially obtained bakers' yeast, variations are encountered although the yeast is fresh and the operating conditions are the same. Figure 5 shows three curves



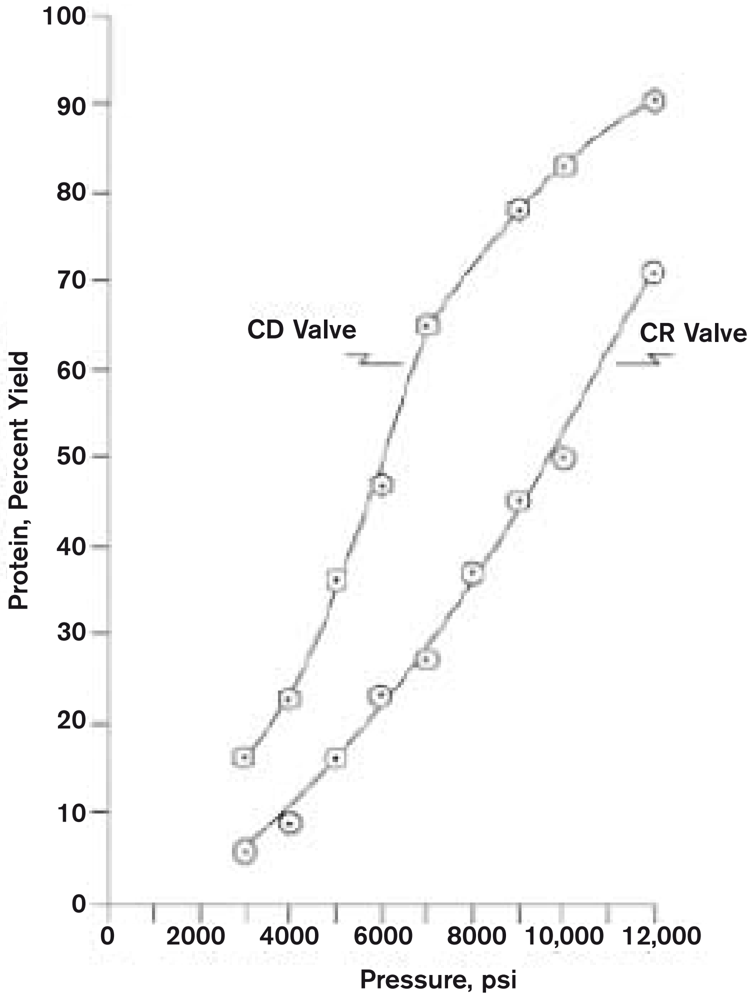
Pressure profile of soluble protein released from Saccharomyces cerevisiae (baker's yeast) from 3 different yeast batches processed in different years but using the same style CD valve and homogenizer.

indicating the percent of protein released at different homogenizing pressures. The homogenizer and the cell disruption valve were the same in each case, but the batches were processed in different years. Therefore, the variations are most likely due to the yeast. Kula[30] comments on the fact that the mechanical strength of a microorganism depends on the growth conditions and history of the biomass. This means that each test done on a batch of cell slurry must be judged independently of other batches. The only way to relate results from different batches would be to run a reference sample for each batch. The reference sample might be one run with the same homogenizer, valve and pressure. This reference point could be used to interconnect different batches. However, if the lab performing the CD test grows the organism and growth is rigidly controlled, then the batches would be more consistent than those made from commercially prepared organisms.

Valve Design and Operating Conditions

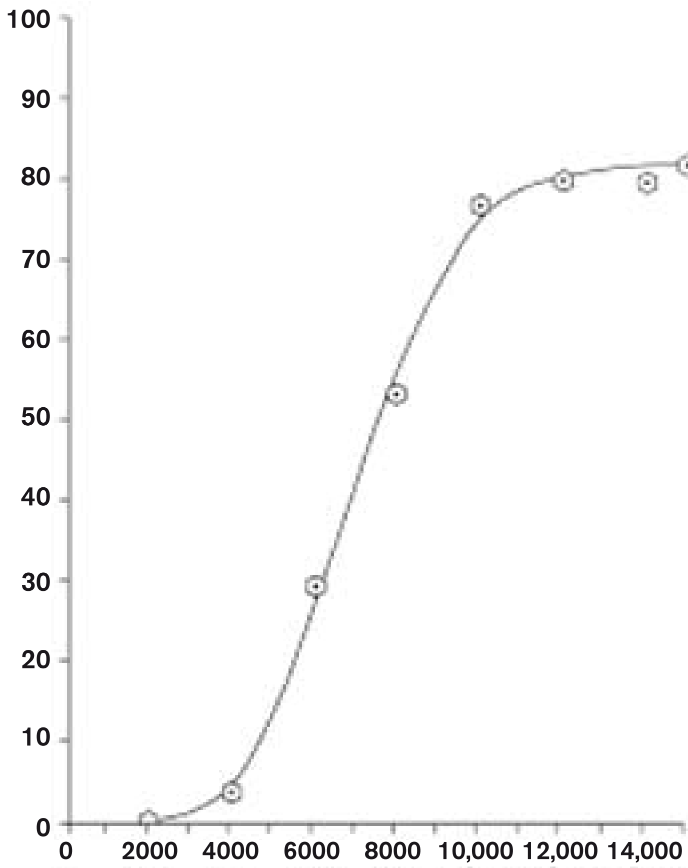
Using this method for protein analysis, this author has run many tests at flow rates from 5 gallons per hour (10 liters per hour) up to 500 gph (1892 Lph) evaluating many different designs and configurations of the cell disruption homogenizing valve. For example, Figure 6 shows the difference in efficiency between the CD design and the CR design. Obviously, these results show that the CD design is more efficient than the CR design. The results from the tests on various designs have allowed APV Products – Homogenizer to develop an efficient CD valve that can be scaled up to production flow rates.

Along with design considerations, the effect of pressure has also been evaluated. Pressure profile studies have shown that high-pressure, 10,000 to 15000 psi (69 to 103.4 MPa), is a benefit for cell disruption. Figure 7 shows the type of results obtained when going to high-pressure with bakers' yeast. It is apparent that the degree of disruption levels off above 12,000 psi (82.8 MPa). Middleberg reported a similar trend with the CD of E. coli at pressures up to 11,000 psi (75.8 MPa)[44]. Although this trend has been reported in the literature, no particular theory has been presented to explain it[38,42].
The use of high-pressure is an alternative to multiple passes. Many researchers have described the effect of multiple passes through the homogenizer[21]. The important consideration to make when analyzing these results is that multiple passing should be done in discrete steps. Recycling back to the original feed sample means that processed material is mixed into unprocessed material. Eventually portions of the slurry will have been homogenized several times, and other portions will not have been homogenized at all[45]. To be certain that all of the slurry sees at least one pass would require a much longer processing time than that for one discrete pass. Therefore, multiple-pass tests should be done without recycling.



Comparison of cell disruption for the CD and CR valve using baker's yeast.

Figure 7



Pressure profile for the 30CD showing a leveling off in yield about 12,000 psi (82.8 MPa).

An understanding of the effects of valve design, pressure and multiple passes can be put to use in processing cell slurries. It has been demonstrated here that the CD valve design is more efficient than the CR design, and high-pressure is more effective than low or moderate pressures. Therefore, the combination of these conditions can make cell disruption more efficient. Masucci[42] found that using the CD valve at high-pressure eliminated multiple passing at lower pressure with the CR valve. For example, one pass at 14,000 psi (96.6 MPa) with a CD valve released as much protein from Leuconostoc mesenteroides as did four passes at 8,000 psi (55.2 MPa) with the CR valve. A similar result can be seen in the work done by Middelberg on multiple passing for CD. He shows that one pass at 9572 psi (66 MPa) is equivalent to two passes at 6525 psi (45 MPa)[46].

In his thesis on cell disruption, Sanchez1 found that using the Gaulin 30CD homogenizer with a CD valve gave slightly better yield after one pass at 15,000 psi (103.4 MPa) than two passes at 8,000 psi (55.2 MPa) for E. coli. The percent of protein release was 79% for 15,000 psi (103.4 MPa) and 74% for two passes at 8,000 psi (55.2 MPa). One pass at 8,000 psi (55.2 MPa) released 61% protein.

Sanchez also found that, over the flow rates investigated, there were no significant differences in the amount of solids removed by centrifugation for slurries processed at 8,000 psi (103.4 MPa). This finding is surprising because high-pressure and multiple passing will increase cell debris and reduce particle size. The assumption is that this debris will make downstream separation more difficult. However, Sanchez did not find this to be the case.

Along with design changes in the homogenizing valve for efficient disruption, the configuration of the pump cylinder must be considered to accommodate high operating pressures and, in some cases, biotech containment[34, 47]. Biotech containment may include the use of double seals on the plunger to avoid releasing any product to the environment. The cooling water to the plungers can come from a closed system that collects any product passing by the plunger packing. Because plunger packing is a dynamic seal, there will always be some product that passes by the packing. Of course, if extremely hazardous pathogenic microorganisms are being processed, then the homogenizer should be contained in a secondary cabinet. Some other parameters to consider when selecting and installing a homogenizer are the flow rate, pressure requirements and the piping arrangement. The flow rate and maximum operating pressure will determine the size of the homogenizer, because these conditions determine motor power and the size of the pumping chamber including the plunger diameter.

The feed pressure of the Net Positive Suction Head (NPSH) available to the suction manifold of the homogenizer must satisfy the NPSH required by the homogenizer, so that it is not "starved". Inadequate feed pressure could result in damage to the pump chamber. Excessive entrained gases can also cause damage to the pump and result in poor operating of the homogenizer.

Cell disruption is usually a high-pressure application. Therefore, the homogenizer will incorporate any designs consistent with high-pressure operation. These design parameters may include the use of o-rings instead of gaskets for better sealing and special pump chambers to withstand the stresses associated with high-pressure pumping.

Cell Morphology

Researchers have reported that the type of microorganism affects cell disruption efficiency, the fermentation conditions used for culturing the cells, the structure and composition of the cell, and the pre-conditioning of the cell. For example, cells that contain protein inclusion bodies, which are dense, insoluble protein bodies formed inside the cells, are easier to disrupt than cells without inclusion bodies[44].

There is a decrease in disruption efficiency for E. coli cells that have been deactivated by heating to 60 to 70°C[48]. The E. coli may be heat treated to eliminate the possibility of environmental contamination during processing. Apparently, heat deactivation strengthened the cell wall and decreased the size of the cells.

Studies have shown that different batches of the same cell type can yield different cell wall strength[44]. Different cell strains can show differences in cell disruption efficiency. Also, it has been found that septated cells "are preferentially disrupted during homogenization[27]."

The type of cell, for example, bacteria versus yeast, will affect the degree of cell disruption. It has been reported that for some fermentations, 100% disruption of E. coli was achieved at a pressure of 9,000 psi[49]. For yeast cells, this degree of disruption may require more than one pass at pressures greater than 10,000 psi.

Viscosity

The viscosity of the slurry may influence the efficiency of disruption, but very little information on viscosity effects has been given in published literature. Considering that a high concentration of yeast does not affect efficiency of cell disruption and that the viscosity of the slurry most likely increases with the amount of yeast, it can be assumed that any effect of viscosity would be small. However, it is known that after the first pass through the CD

homogenizer, the release of nucleic acids from an E. coli slurry can increase the viscosity of the slurry. The slurry will now have a greater viscosity for the second pass through the homogenizer. It has been reported that the use of a second-stage valve on the homogenizer reduces the viscosity produced after the first pass.

Conclusions

Cell disruption techniques have been studied for several years; however, many of the methods cause loss of product and are not suitable for efficient processing of large batches. The CD homogenizer is suitable for efficient disruption of small or large batches of various microorganisms. The CD homogenizer consists of a pump designed for the biotech environment, usually with high-pressure capabilities, and a homogenizing valve developed to maximize cell disruption.

References

1. Sanchez-Ruiz, S.A., MS: *Studies on Cell Disruption and Cell Debris Removal in Downstream Bioprocessing*, 1989, Massachusetts Institute of Technology: Cambridge Massachusetts.
2. Pandolfe, W.D., *Homogenizers, in Wiley Encyclopedia of Food Science and Technology,* F.J. Francis, Editor. 1999, John Wiley & Sons, Inc.: New York. P. 1289-1294.
3. Lux, F., *Patent Specification* 367,063, 1932.
4. Milner, H.W., N.S. Lawrence, and C.S. French, *Colloidal Dispersion of Chloroplast Material Science*, 1950. **111**: p. 633.
5. Duerre, J.A. and E. Ribi, *Enzymes Released form Escherichia coli with the Aid of Servall Cell Fractionator*. Appl. Microbiol., 1963. **11**: p.467.
6. Wimpenny, J.W.T., *Breakage of Micro-organisms*. Process Biochemistry, 1967(7): p. 41.
7. Garver, J.C. and R.L. Epstein, *Method of Rupturing Large Quantities of Microorganisms.* Applied Microbiology, 1959. **7**(5): p. 318.
8. Rodgers, A. and D.E. Hughes, *The Disintegration of Microorganisms by Shaking with Glass Beads*. J. Biochem. Microbio. Tech. and Eng., 1960. **2**(1): p. 49.
9. Hughes, E.E., *The Disintegration of Bacteria and Other Microorganisms by the M.S.E.-Mullard Ultrasonic Disintegrator*. J. Biochem. Microbio. Tech. and Eng., **3**(4): p. 405.
10. Hughes, D.E., and W.L. Nyborg, *Cell Disruption by Ultrasound*. Science, 1962. **138**: p. 108.
11. Tannenbaum, S.R. and S.A. Miller, *Effect of Cell Fragmentation on Nutritive Value of Bacillus megaterium Protein*. Nature, 1967. **214**: p. 1261.
12. Batt, C.A. and A.J. Sinskey, *Use of Biotechnology in the Production of Single-Cell Protein*. Food Technology, 1984. **2**: p. 18-21.
13. Dunhill, P. and M.D. Lilly, *Protein Extraction and Recovery from Microbial Cells, in Single Cell Protein II*, S.R. Tannenbaum and D. Wang, Editors. 1975, MIT Press: Cambridge, MA. P. 179-207.
14. Hedenskog, G., H. Mogren, and L. Enebo, *A Method for Obtaining Protein Concentrates from Microorganisms*. Biotech. and Bioend., 1970. **XII**: p. 947.
15. Lawford, G.R., et al., *Production of High-Quality Edible Protein from Candida Yeast Grown in Continuous Culture*. Biotech. and Bioeng., 1979. **XXI**: p. 1163.

16. Zanetti, R.J., *Breathing new life into signle-cell protein.* Chemical Engineering, 1984. **2**: p. 18-21.
17. Wiseman, A., *Enzyme for Breakage of Micro-organisms*. Process Biochemistry, 1969(5): p. 63.
18. Zetelaki, K., *Disruption of Mycelia for Enzymes*. Process Biochemistry, 1969(12): p. 19.
19. Jakoby, W.B., *Enzyme Purification and Related Techniques, in Methods in Enzymology.* 1971, Academic Press: New York.
20. Follows, M., et al., *Release of Enzymes from Bakers' Yeast by Disruption in an Industrial Homogeniser*. Biotech. and Bioeng., 1971. **XIII**: p. 549.
21. Hetherington, P.J., et al., *Release of Protein from Baker's Yeast by Disruption in an Industrial Homogeniser*. Trans. Instn. Chem. Engrs., 1971. **49**: p. 142.
22. Whitworth, D.A., *Assessment of an Industrial Homogenizer For Protein and Enzyme Solubilization From Spent Brewery Yeast, in Comptes Rendus Des Travaux Du Laboratoire Carlsberg* (Report of Work at Carlsburg Laboratory). 1974, Danish Sci. Press Ltd. p. 19.
23. Whitworth, D.A., *Hydrocarbon Fermentation: Protein and Enzyme Soubilization from C. lipolytica Using an Industrial Homogenizer*. Biotech. and Bioeng., 1974. **XVI**: p. 1399.
24. Cunningham, S.D., et al., *Rupture and Protein Extraction of Petroleum-Grown Yeast*. J. of Food Sci., 1975. **40**: p. 732.
25. Lee, C., et al., *Disintegration of Dried Yeast Cells and Its Effect on Protein Extractability, Sedimentation Property, and Viscosity of the Cell Suspension.* Biotech. and Bioeng., 1979. **XXI**: p. 1-17.
26. Engler, C.R., *Disruption of Microbial Cells, in Comprehensive Biotechnology*, M. Moo-Young, Editor. 1985, Pegamon Press: Oxford, England.
27. Middelberg, A., *Process-Scale Disruption of Microorganisms.* Biotechnology Advances, 1995. **13**(3): p. 491-551.
28. Edebo, L., *Disintegration of Cells by Extrusion Under Pressure, in Enzyme Technology*, R.M. Lafferty, Editor. 1983, Springer Verlag: Berlin.
29. Johnson, B.H. and M.H. Hecht, *Recombinant Proteins Can be Isolated from E. coli Cells by Repeated Cycles of Freezing and Thawing*, in Bio/Technology 1994. 12. p. 1357.
30. Kula, M.-R. and H. Schutte, *Purifications of Proteins and the Disruption of Microbial Cells*. Biotechnology Process, 1987. **3**(1): p. 31.
31. Wiseman, A., D.J. King, and M.A. Winkler, *The isolation and purification of protein and peptide products, in Yeast Biotechnology*, D.R. Berry, I. Russell, and G.G. Stewart, Editors. 1987: London.
32. Lin, H.M., et al., *Disintegration of Yeast Cells by Pressurized Carbon Dioxide*. Biotechnology Progress, 1991. **7**: p. 201-204.
33. Castor, T.P. and G.T. Hong. *Presented Paper. in 199th ACS National Meeting*, Boston, April 1990.
34. Pandolfe, W.D. *The Cell Disruption Homogenizer. in Proceeding of the DTII/HSE/SCI Symposium on Large-Scale Bioprocessing Safety*. 1989. Waren Spring Lab, Stevenage, England.
35. Doulah, M.S., T.H. Hammond, and J.S.G. Brookman, *A Hydrodynamic Mechanism for the Disintegration of 'Saccharomyces cerevisiae' in an Industrial Homogenizer.* Biotech. and Bioeng., 1975. **XVII**: p. 845.
36. Engler, C.R. and C.W. Robinson, *New Method of Measuring Cell-Wall Rupture*. Biotech. and Bioeng., 1979. **XXI**: p. 1861.
37. Engler, C.R. and C.W. Robinson, *Disruption of Candida Utilis Cells in High*

Pressure Flow Devices. Biotech. and Bioeng., 1981. **XXIII:** p. 765.
38. Keshavarz, E., M. Hoare, and P. Dunhill, *Biochemical engineering aspects of cell disruption, in Separations for Biotechnology,* M.S. Verrall and M.J. Hudson, Editors. 1987, Ellis Horwood: Chichester, England. p. 62-79.
39. Keshavarz-Moore, E., M. Hoare, and P. Dunhill, *Disruption of baker's yeast in a high-pressure homogenizer: New evidence on mechanism.* Enzyme Microbiology Technology, 1990. **12**: p. 764-770.
40. Kleinig, A.R. and A.P.J. Middelberg, *On the mechanism of microbial cell disruption in high-pressure homogenization*. Chem. Eng. Sci., 1998. **53**(5): p. 891-898.
41. Shirgaonkar, I.Z., R.R. Lothe, and A.B. Pandit, *Comments on the Mechanism of Microbial Cell Disruption in High-Pressure and High Speed Devices.* Biotechnol. Prog., 1998. **14**(4): p. 657-660.
42. Masucci, S.F., *BS Thesis: Improving the Efficiency of High Pressure Homogenizers for Cell Disruption*, 1985, Massachusetts Institute of Technology: Cambridge, Massachusetts.
43. Mitten, H.R. and H.L. Preu, *Temperature Increase Due to Homogenization*. J. Dairy Sci., 1959. **42**: p. 1880.
44. Middelberg, A.P.J., et al. *A Novel Technique for the Measurement of Disruption in High-Pressure Homogenization: Studies on E. coli Containing Recombinant Inclusion Bodies*. Biotech. and Bioeng., 1991. **38**: p 363-370.
45. Leviton, A. and M.J. Pallansch, *Continuous Recycling in the Homogenization of Relatively Small Samples*. J. Dairy Sci., 1959. **42**: p. 20-27.
46. Middelberg, A.P.J., *Extension of the Wall-Strength Model for High-Pressure Homogenization to Muliple Passes*. Trans. IChemE, 1993. **71**(C): p. 215-219.
47. Wilkinson, B. and J.M. Bristol, *High Pressure Homogenizer Pump,* 1988: US. 4,773,833.
48. Collis, M.A., B.K. O'Neill, and A.P.J. Middelberg, *Thermal Deactivation Affects Disruption of Escherichia coli*. Biotechnology Techniques, 1995. **9**(2): p. 91-94.
49. Middelberg, A.P.J., et al., *A New Model For the Disruption of Escherichia Coli By High-Pressure Homogenization Part II: A Correlation For the Effective Cell Strength*. Trans. IChemE, 1992. **70**(C): p.213-218.

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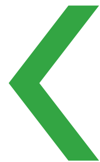

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