Yeast Cell Disruption by Scalable High-Intensity Ultrasound

Allied Innovative Systems

INDUSTRIAL SONOMECHANICS™

Cell disruption (lysis) is necessary for many industries, including pharmaceutical, cosmetic, biotechnology, energy, nutraceutical, food & beverage and chemical. It is, for example, an important step in recombinant protein production for medical applications.

Allied Innovative Systems (ALLIS) partnered with Industrial Sonomechanics (ISM) in order to determine the effectiveness of high-amplitude ultrasonic cell disruption and the feasibility of applying it on a large scale using Barbell Horn Ultrasonic Technology (BHUT).

Problem

- Chemical, enzymatic and conventional ultrasonic cell lysis methods are effective on laboratory scale but impractical for production.
- High-pressure homogenization (HPH) and bead milling (BM) methods are scalable but have many disadvantages (high cost, complexity, large footprint).

Goal

- Compare chemical and ultrasonic cell lysis methods.
- Transfer the ultrasonic cell lysis process from a laboratory to a pilot scale.
- Determine the productivity gain factor and confirm that the process is directly scalable with BHUT.

Results

- Ultrasonic lysis is far superior to chemical lysis.
- High ultrasonic amplitudes are necessary for efficient cell disruption and protein extraction.
- The ultrasonic cell lysis process can be directly scaled-up with BHUT.

ALLIS wanted to engage in a research and development project related to cell disruption of yeast cells. Since this is a very important process for many industries such as pharma, we wanted to see whether the process is feasible using ISM's ultrasonic solution. We not only showed that S. cerevisiae cells can be effectively disrupted by high-amplitude ultrasound, but also that the process can be done on a large scale by using ISM's ultrasonic equipment. ISM's ultrasonic processors were very straight-forward and easy to use so there was no special training or installation needed.

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BACKGROUND AND SUMMARY

Allied Innovative Systems (ALLIS) engaged in this project to evaluate the feasibility of using ISM ultrasonic equipment to intensify and scale up the yeast cell disruption process.

ABOUT Allied Innovative Systems

ALLIS has extensive experience in developing and implementing new biomedical technologies.

Dr. Simon Bystryak, the company's President, has developed, validated and marketed many new technologies in the field of life sciences. The company works with the government as well as with private organizations such as ISM.

ALLIS has assembled a group of highly skilled scientists and engineers with expertise in key scientific disciplines that are vital to the success of its development efforts. These disciplines include: biochemistry and organic chemistry, biomedical engineering and optics, medicine, computational methods, software development, developing new technologies and products as well as in their clinical validation.

Some of ALLIS' target customers include research labs, university labs and hospitals.

Company headquarters: Chatham, NJ

Industry: Pharma, biotechnology, healthcare, clinical diagnostics.

Website: http://www.allisystems.com/

Significance of the study

Among all available cell disruption (lysis) methods, sonication (ultrasonic processing) is the one most commonly used on the laboratory scale due to its effectiveness and simplicity. High pressure homogenization (HPH) and bead milling (BM) are, nevertheless, favored for the industrial scale because they are free from a well-known limitation of conventional ultrasonic technology – the inability to directly scale up.

HPH and BM are, however, very costly, complex, and involve harsh conditions, which can result in protein denaturation and product yield reduction. In contrast, ultrasonic cell disruption is inexpensive, fast, efficient, easy to use, and potentially offers advantages in terms of recovery of periplasmic, membrane-bound or insoluble recombinant proteins.

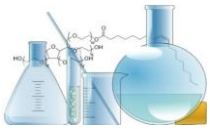
In this study, we compared the efficiency of cell disruption by sonication and chemical cell lysis. We also transferred the process for a laboratory to a pilot scale using BHUT, compared the results and determined the resulting productivity gain factors..

Why did we choose *S. cerevisiae*?

This yeast cell system was selected because of its significant industrial utility (e.g., for winemaking, baking and brewing) and because *S. cerevisiae* cell walls are notoriously resilient.

Yeasts are significantly harder to disrupt than bacteria (e.g., Escherichia coli). The structure of yeast cells also complicates the extraction of the desired intra-cellular proteins. Consequentially, the main difficulty related to the industrial utilization of *S. cerevisiae* is arriving at the right method for the disintegration of its cells for efficient protein release.

MATERIALS & METHODS



Chemical lysis

Yeast lysis reagent CelLytic-Y, TRIS buffered saline (TBS, pH8.0), Streptavidin-Alkaline Phosphatase from Streptomyces avidinii, and Bradford reagent were purchased from Sigma (St. Louis, MO). Bovine Serum Albumin (BSA) was purchased from Thermo Scientific (Rockford, IL) and used as a protein concentration reference standard. Ultrasonic amplitudes were confirmed by a high-speed photonic sensor (kd-300, MTI Instruments).

A 20% yeast cell suspension (w/v) was prepared by adding chilled CelLyticTM-Y Yeast Cell Lysis reagent to 1 g of dry yeast to a total volume of 5 mL (Sigma). During chemical lysis, the suspension was allowed to shake gently at room temperature, as recommended by Sigma protocol. Half-milliliter aliquots were withdrawn every 5 min for the total of 60 min of the cell lysis process.

Ultrasonic cell disruption

For the laboratory-scale ultrasonic experiments, a 20% yeast cell suspension (w/v) was prepared by adding chilled TBS (pH 8.0) to 9 g of dried yeast until the total volume was 45 mL. For the pilot-scale experiments, chilled TBS was added to 300 g of dried yeast until the total volume was 1500 mL. The suspensions were stirred until homogeneous pre-mixtures were obtained.

The experiments were conducted using two ultrasonic liquid processors obtained from Industrial Sonomechanics (ISM):

- LSP-500 – laboratory-scale processor configured in the batch mode (Fig. 1a).
- BSP-1200 – bench-scale processor configured in the flow-through mode (Fig. 1c).

Figure 1a. LSP-500 in batch mode using a conventional horn (CH).

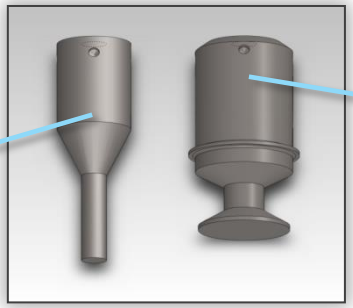


Figure 1b. Conventional horn (left) and Half-wave Barbell Horn (right).

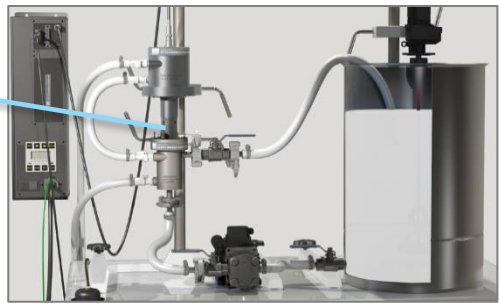


Figure 1c. BSP-1200 in flow-through mode using a Half-wave Barbell Horn (HBH).

Experiments performed with the LSP-500 were carried out in the batch mode (Fig. 1a), utilizing a beaker placed in an ice bath in order to maintain the temperature of the cell suspension at 18 ^{o}C. Sonication was conducted at the ultrasonic amplitudes of 75 and 20 microns (µpp).

Experiments performed with the BSP-1200 were carried out in the recirculating flow-through mode, utilizing a tank containing the pre-mixed cell suspension and a pump that recirculated the suspension through the bottom fitting of the tank, through the ultrasonic reactor chamber and back into the tank (Fig. 1c). The temperature of the suspension was maintained at 18 ^{o}C by passing cold water through the camber's temperature control jacket. Sonication was conducted at the ultrasonic amplitudes of 75 and 95 µpp.

RESULTS & CONCLUSIONS

The results are presented in Fig. 2 and described below. We arrived at the following conclusions:

1. **Ultrasonic cell disruption is far superior to chemical.**

   Protein extraction yields obtained with CelLytic-Y yeast cell lysis/extraction reagent were *four times* lower than those obtained with sonication at 75 µpp (4.2 mg/mL versus 16.0 mg/mL after 25 min).

2. **High ultrasonic amplitudes are needed for the efficient extraction of AP from *S. cerevisiae* cells.**

   Chemical lysis and sonication at 20 µpp were significantly less efficient for AP extraction than sonication at 75 µpp because high ultrasonic amplitudes provided higher shear forces for the S. cerevisiae cell disruption and the extraction of AP.

3. **The sonication process can be directly scaled up with BHUT.**

   For the resulting AP activity of 200 mIU/ml and the ultrasonic amplitude of 75 µpp, the productivity rates of the LSP-500 and BSP-1200 processors were ~ 4.5 ml/min and ~ 30 ml/min (scale-up factor of ~ 7). When the amplitude was raised to 95 µpp, the productivity rate of the BSP-1200 processor increased to ~ 90 ml/min. Further scale up can be achieved by using the ISP-3000 industrial-sale processor (the scale up factor between BSP-1200 and ISP-3000 is approximately 5).

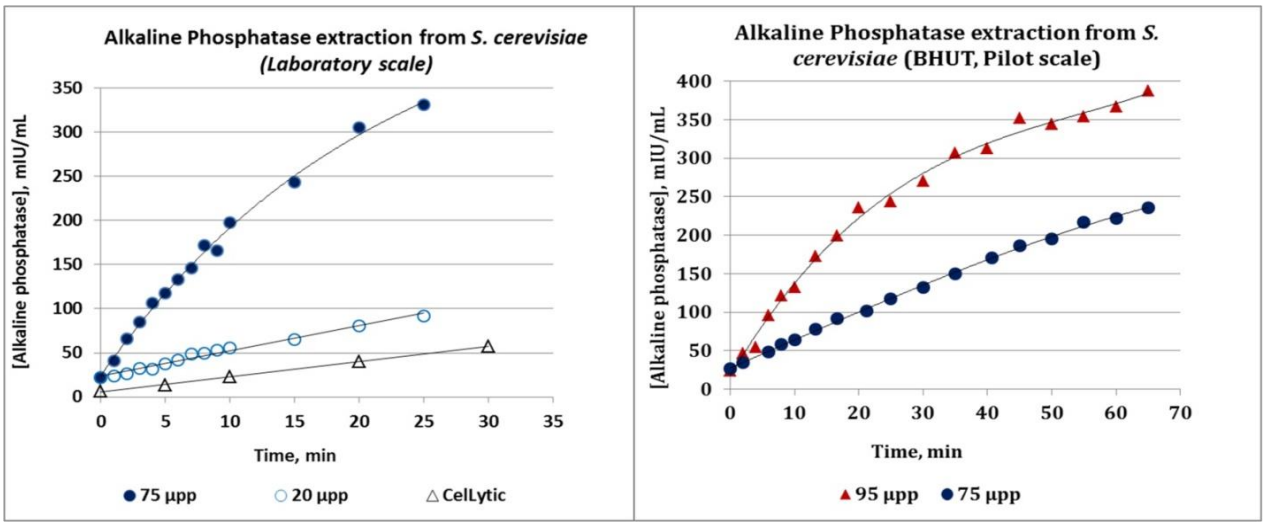


Figure 2. Results for the extraction of Alkaline Phosphatase protein (AP).

RESEARCH PAPER

Cell Disruption S. cerevisiae by scalable high-intensity ultrasound

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ABOUT INDUSTRIAL SONOMECHANICS

Industrial Sonomechanics (ISM) is a research & development, equipment design and process consulting firm, specializing in high-intensity ultrasonic technology for liquid treatment by acoustic cavitation. Our patented Barbell Horn Ultrasonic Technology (BHUT) allows generating extremely high ultrasonic amplitudes and cavitation intensities at any scale, making it possible to directly apply laboratory process optimization results in an industrial production environment.

ISM ultrasonic liquid processors (a.k.a. homogenizers, sonicators, mixers) are ideal for the production of nanoemulsions, nanocrystals and liposomes. Other common applications are cell disruption, plant extraction, degassing, dispersing, transesterification, desulphurization, and sterilization. Industries utilizing ISM technology include pharmaceutical, medical cannabis, cosmetic, nutraceutical, food & beverage, printer ink, paint, adhesive, pesticide, chemical and alternative fuel.

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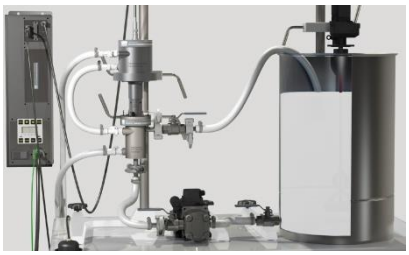
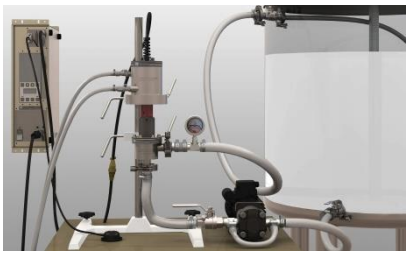
INDUSTRIAL SONOMECHANICS ULTRASONIC PROCESSORS

LSP-500
Laboratory-Scale Processor



BSP-1200
Bench-Scale Processor



ISP-3000
Industrial-Scale Processor

HAVE QUESTIONS?

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