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# The Performance of a Glass Bead Shaking Technique for the Disruption of *Escherichia coli* Cells

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**Abstract** The efficacy of a simple laboratory method for cell disruption based on the shaking of glass beads on a rotary shaker was assessed in this study, via measurements of the release of total protein and interferon-$\alpha$2b from *E. coli*. The optimum conditions for cell disruption were detected after 30 min of shaking in Tris-HCl buffer (pH 8) at 300 rpm with 1.5 g of glass beads (diameter: 0.5 mm) per mL of cell suspension volume. Three test runs were conducted under the above conditions and the maximum average protein release values were determined as 3.048, 3.564, and 3.015 mg/mL, respectively. The amount of protein release was comparable to the amount of protein release in ultrasonication and glass bead vortexing procedures. The amount of interferon-$\alpha$2b release in the ultrasonication, glass bead vortexing, and glass bead shaking trials were 240, 172, and 201 ng/mL, respectively. This method was shown to process between 1 and 10 mL of sample volume in a 50 mL Falcon tube without a great deal of deviation, and was able to handle in excess of 60 samples simultaneously. © KSBB

*Keywords: cell disruption, downstream processing,* E. coli*, glass beads, shaker, interferon-$\alpha$2b*

## INTRODUCTION

Cell disruption is considered to be an important process in downstream processing for the release of intracellular products. Extensive reviews of cell disruption processes have been previously published [1-5]. Methods of disruption are broadly classified into mechanical and non-mechanical techniques, and there has been some overlap in the subclassifications. For example, ultrasonication has been classed as a non-mechanical method by Chisti and Moo-Young [2], but was identified as a mechanical method by Harrison [4]. Detailed classifications are provided in the majority of the reviews mentioned above.

Preliminary studies of the selection of disruption technique would be conducted for the efficient extraction of products like DNA [6], RNA [7], proteins [8], and other substances [9-11]. Table 1 shows the comparison of disruption techniques conducted by some researchers for the selection of a technique for product recovery. It should also be noted that the selection of techniques is dependent on the product of interest and the ease of operation of the technique.

Among the available methods, enzymatic digestion, freeze-thawing, French press, ultrasonication, and disruption using glass beads are all being utilized at laboratory scale. On the industrial scale, mechanical methods including bead milling and high pressure homogenization are in general use [4,5,9,12-14], although in some cases, gentler methods such as enzymatic digestion are considered [1,5]. Disruption using glass beads can be compared with bead milling for the purpose of scaling up from laboratory to industrial scale.

French press and ultrasonication techniques both require particular equipment, but enzymatic digestion, freeze-thawing, and glass bead disruption techniques require no special equipment. The action of enzymatic digestion depends on a number of factors, including the pH and temperature of the medium, and normally require a pretreatment or combined chemical treatment for efficient disruption [1,15]. This also

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**Table 1.** Selection of disruption technique by comparative study

| S. No. | Methods | Organisms | Product analysis | Claims | Comments | Ref. |
|---|---|---|---|---|---|---|
| 1 | Ultrasonication, grinding, stirring, and vortexing with glass beads | *E. coli* | Total protein and fructosyltransferase | Glass beads stirring released higher activity of enzyme though it released lower amount of total protein. | The effect of higher release was due to lesser shear force applied per unit time. | [27] |
| 2 | NaOH-SDS solubilization, French press, ultrasonication, and enzyme digestion | *Clostridium perfringens* | Total protein | Mechanical methods were giving comparable results and better than other methods. | | [34] |
| 3 | Ultrasonication, high pressure homogenization, and hydrodynamic cavitation | Baker's yeast | Total protein and Invertase | Ultrasonication yielded highest amount of total protein but very less amount of specific protein. High pressure homogenization yielded higher amount of total protein and also with respect to specific protein. Hydrodynamic cavitation yielded very low total protein and also with respect to specific protein. But it gave high selectivity. | Though hydrodynamic cavitation gave higher selectivity, it has released only 6.1% of maximum specific protein. | [32] |
| 4 | Ultrasonication, high pressure homogenization, and bead millling | *Lactobacillus bulgaricus* | β-Galactosidase | Bead milling and high pressure homogenization gave comparable and better release than ultrasonication. Ultrasonication gave only around 30% of target enzyme than other two methods. | | [9] |
| 5 | Bead vortexing, freeze-thaw, French press, and ultrasonication | *E. coli* | Total protein and few enzymes | Mechanical methods gave comparable results than freeze-thaw. Bead vortexing was the easiest method of all. | | [16] |
| 6 | Ultrasonication and bead beating | *Mycobacteria* | Proteomic analysis and protein | Both processes gave comparable result in proteomic analysis. | Risk of aerosol formation was avoided in bead beating and so considered to be better than ultrasonication. | [35] |
| 7 | Enzyme digestion and high pressure homogenization | *Pseudomonas putida* | Polyhydroxyalkanoate and inclusion body | Enzyme digestion gave the better release of inclusion bodies and also the amount of target protein. | | [11] |
| 8 | Hydrodynamic cavitation, high pressure homogenization, French press, osmotic shock, and EDTA treatment | *E. coli* | Total protein, acid phosphatase, and β-galactosidase | At cavitation no 0.17, hydrodynamic cavitation gave higher recovery and selectivity of enzymes. | Osmotic shock had released acid phosphatase better than hydrodynamic cavitation. | [36] |

(continued)