# HIGH PRESSURE CELL DISRUPTION OF SIX COMMON YEAST SPECIES

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High pressure disruption is widely used to release cellular contents. The cellular contents are usually of pharmaceutical value necessitating an efficient process of disruption under highly contained conditions. Using a Constant Systems cell disrupter a survey has been conducted on the disruption of six common yeast species, (*Saccharomyces cerevisiae*, Brewer's yeast, *Pichia pastoris*, *Hansenula polymorpha*, *Candida utilis* and *Kluyveromyces fragilis*). It was found that more than 70% soluble protein release, could be achieved with a single pass at pressures above 2000 Kgf/cm$^2$ for each yeast. Apart from Baker's yeast which gave a hyperbolic pressure protein release curve all the other yeast species produced sigmodial pressure protein release curves. Analysis of the data revealed that the model presented by Hetherington *et al* could not fit data as well as a new model developed in this study.

**KEYWORDS:** Cell disruption, Yeast, Disrupter, Model, Parameters

## INTRODUCTION

Micro-organisms are an important source of cellular products. With the advent of genetic engineering, cells can be manipulated to produce proteins and enzymes normally associated with other species of micro-organisms or even humans. Some cellular products are naturally secreted by organisms and can be harvested directly from the fermentation for pharmaceutical uses. When the product is not naturally secreted or cannot artificially be induced to, the micro-organism must be disrupted to release the useful product. Yeasts' are of particular interest for the production of therapeutic products, especially baker's yeast (*Saccharomyces cerevisiae*). *S. cerevisiae* has a reasonably well known genetic system and like other yeasts' are able to perform simple glycosylation which enables human proteins to be produced in much the same way as it would be in the original host. Several other yeasts' have been considered as expression vectors, these include *Pichia pastoris*. It has been noted form previous studies that some yeasts' are relatively difficult to disrupt using low pressure homogenisers. Being able to perform efficient disruption of yeasts' is therefore particularly desirable.

The Constant Systems disrupter is a relatively new piece of equipment and compared with bead mills and traditional homogenisers, it has been designed to give high levels of containment and can be cleaned in place. It works by forcing the sample through a small orifice at very high pressure, which is achieved by an hydraulic piston pump.

The purpose of this study is to investigate the disruption of various yeast species using the Constant Systems disrupter operated at a range of pressures. These results are presented as disruption profiles of the percentage of protein released from the cells versus the pressure at which the disrupter is operated.

## MATERIALS AND METHODS

The Six yeasts' investigated were baker's yeast, (*Saccharomyces cerevisiae*), brewer's yeast (NCYC 1681), *Pichia pastoris* (NCYC 175), *Hansenula polymorpha.(A16 Leu-)*, *Candida utilis* (NCYC 707) and *Kluyveromyces fragilis* (NCYC 851). Each species was grown batch wise on a standard YPD medium (Yeast extract 1%, Mycological peptone 2%, Glucose 2%, pH 5.5) except brewer's yeast which was grown on MYGP media[1].

Each batch was grown in a 10 litre airlift fermenter at 27$^0$C for 48 hours. The culture was then harvested and washed by micro-filtration, the cells being suspended in 25 mM phosphate buffer, (pH 7.0). The purpose of the washing was to remove the majority of peptides present from the media. The concentrated cell suspensions (15-20 g/l dry weight) were then disrupted.

The disruption was conducted over a range of pressures from 0-275 MPa ( 0-2812 Kgf/cm2). The disrupter was operated using an orifice of 0.18 mm. The cell suspensions were at 4$^0$C and aliquots of this suspension (100ml) were pumped through the disrupter. The disrupted material after a single pass was at a temperature of 14-20$^0$C depending on the pressure employed. 100 ml of cell suspension was passed through the disrupter at each selected pressure. The first 50 ml of disrupted cell suspension was discarded to avoid contamination from previous runs or dilution from washing cycles with distilled water. The second 50 ml was collected in a bottle and immediately placed in ice.

To measure protein release resulting from disruption, protein assays were conducted using the Folin-Lowry method[2]. A calibration was performed using Bovine serum albumin of known concentration. A sample of undisrupted culture suspension was treated with 1N NaOH at 90$^0$C, to provide total soluble protein release. 1 ml of each disrupted sample was taken and centrifuged for 2.5 minutes in a micro-centrifuge (10,000xg). The supernatant was carefully withdrawn and protein assay conducted on the supernatant. The alkali treated sample was also centrifuged before conducting a protein assay. To obtain a background protein measurement a protein assay was performed on centrifuged undisrupted sample. To calculate the percentage protein released by the disrupter at each pressure the following equation (1) was used,

$$((P_D-P_B) / (P_T-P_B)) \times 100 \qquad (1)$$

Where, $P_D$ = Protein released by disrupter, $P_B$ = Background protein, $P_T$ = Protein released by NaOH.

## RESULTS

### DISRUPTION OF YEAST'S

The results of the disruption experiments for the six yeasts' are shown in Figure 1A-1B. The percentage protein release was calculated according to the methods section and percentage protein release plotted versus pressure. Significant disruption, greater than 70% protein release, was obtained with a single pass and pressures above 2000 Kgf/cm$^2$ (~195 MPa), for all the yeasts'. However, in a number of cases 100% protein release was not obtained even at the maximum pressure possible.

The disruption profiles for the yeast species were also different. Baker's yeast disruption gave a hyperbolic curve while other yeasts' gave sigmodial shaped curves. To compare the ease of disruption of each yeast, the pressure required to produce 50% protein release was noted. Baker's yeast is the easiest to disrupt with 50% protein release achieved at a pressure of approximately 600 Kgf/cm$^2$. *Pichia pastoris* appears to be much harder to disrupt requiring approximately 1800 Kgf/cm$^2$ to release 50% protein, Table (1).

### MODELLING

With the data obtained from the disruption studies, it was decided to attempt to model the disruption as a function of operating pressure. Hetherington *et al* [3] performed work on the disruption of *Saccharomyces cerevisiae* using a Manton Gaulin-APV homogeniser. This type of homogeniser works in a similar way to the Constant Systems disrupter using high pressure to force the cell suspension through a valve/orifice. Hetherington *et al* produced a model, equation (2), describing the release of protein from the cells using a Manton Gaulin homogeniser as a function of operating pressure.

$$\text{Log}\,[R_m / (R_m-R)] = KNP^a \qquad (2)$$

Where, $R_m$ = maximum protein available for release, R = protein released, N = number of passes, K = function of temperature, P = pressure, a = exponent (2.9).

Subsequent studies have revealed that the two parameters a and K are very much dependent upon the type of organism and its growth phase. Middelberg *et al* [4] demonstrated this with work conducted on *E .coli*.

Using a computer data analysis and graphics program, "Grafit" by (Erithacus Software Ltd.), the experimental data obtained for each yeast species were entered and using equation (2) the program performed iterations to produce a line of best fit and gave the relevant parameters used to produce this line. The program also gave chi squared values to assess how well the line of best fit formed from the model, matches the experimental data. The units of pressure used in calculations were Kgf/cm2, this enabled a direct comparison to be made between calculated values and those reported by Hetherington *et al* [3]. The parameters calculated are shown in Table (1).

Lines of best fit were produced using the Hetherington *et al* [2] model for each yeast disruption profile. It was discovered that the model worked well for baker's yeast, Figure 3, fitting the data almost perfectly. However, for the yeast's with sigmodial profiles, the model did not produce an acceptable prediction as seen in Figure 2. The model parameters and chi squared values, indicating the degree of fit are shown in table 1. The inability of the Hetherington model to produce acceptable predictions of the disruption profiles lead to the production of a new equation, equation (3).

$$R_p = \exp( b \times (1- \exp(-K \times P^a)) \qquad (3)$$

Where, $R_p$ = fractional protein release(percentage protein release/100), b = fitted parameter which represents ln(max percentage protein release), K = constant, a = exponent, P = pressure (Kgf/cm$^2$).

This equation (Coss model) can be used to predict the percentage protein release possible at any pressure for each yeast. The equation produced much better lines of fit than the Hetherington model for each yeast, indicated by the lower chi squared values, Table (1). Figure 2. shows that the equation can accurately fit the experimental data obtained from the disruption of *Candida utilis* and using parameters specific for each yeast Table (1), also fit the data for Baker's yeast, Figure 3.

Approx
7KPSI
13KPSI
22KPSI
18KPSI
10KPSI
15KPSI

| Yeast | Pressure for 50% Protein Release (Kgf/cm$^2$) | Hetherington Model | | | Coss Model | | | |
|---|---|---|---|---|---|---|---|---|
| | | K | a | Chi squared | b | K | a | Chi squared |
| Baker's | 500 | 0.0006442 | 1.0209 | 14.70 | 4.633 | 0.0755 | 0.5331 | 17.69 |
| Brewer's | 850 | 0.0000080 | 1.5645 | 4.345 | 4.606 | 0.0053 | 0.8738 | 3.57 |
| *P.pastoris* | 1500 | 0.0000008 | 1.7607 | 9.591 | 4.529 | 0.00087 | 1.0626 | 8.017 |
| *H.polymorpha* | 1230 | 0.0000037 | 1.5762 | 9.606 | 4.472 | 0.00067 | 1.1273 | 4.495 |
| *C.utilis* | 705 | 0.0002269 | 1.0779 | 62.15 | 4.406 | 0.00046 | 1.2865 | 3.892 |
| *K.fragilis* | 1000 | 0.0003890 | 0.9498 | 20.63 | 4.323 | 0.00597 | 0.8657 | 2.772 |

**Table 1. Model parameters for the fitting of the experimental data from the disruption of various species of yeast.**

## CONCLUSIONS

The Constant Systems cell disrupter was able to disrupt all the yeasts' tested, with over 70% release of soluble protein with a single pass. However, there was a degree of variability between baker's yeast, which was broken relatively easily and *Pichia pastoris* where far higher pressures were required to release 50% soluble protein, (500 and 1570 Kgf/cm2) respectively, as shown in Table 1.

Another interesting feature was the shape of the protein release profiles, which apart from baker's yeast, were sigmodial in shape. We are currently investigating the possible causes of this phenomenon using *Pichia pastoris*. We are focusing on organism related factors, for example the possibility that the yeast cell populations vary within various stages of batch culture, rather than factors associated with the disrupter itself.

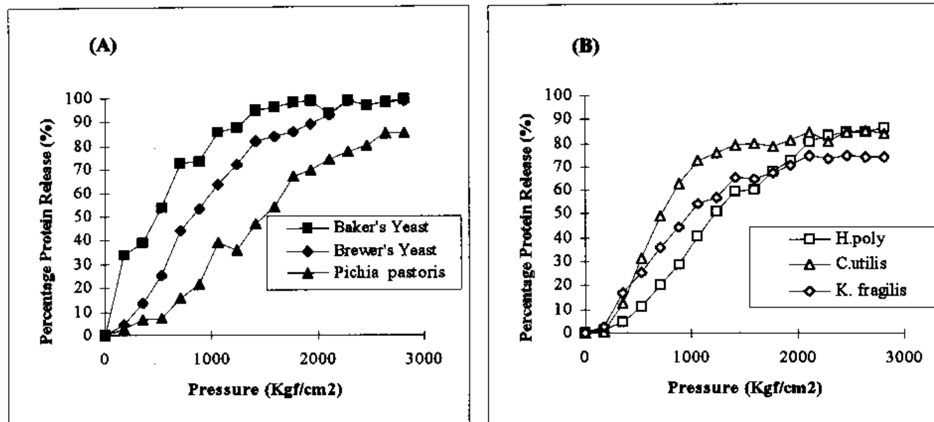


**Figure 1: (A) and (B) Disruption of several species of yeast. Percentage protein released verses the disrupter pressure**

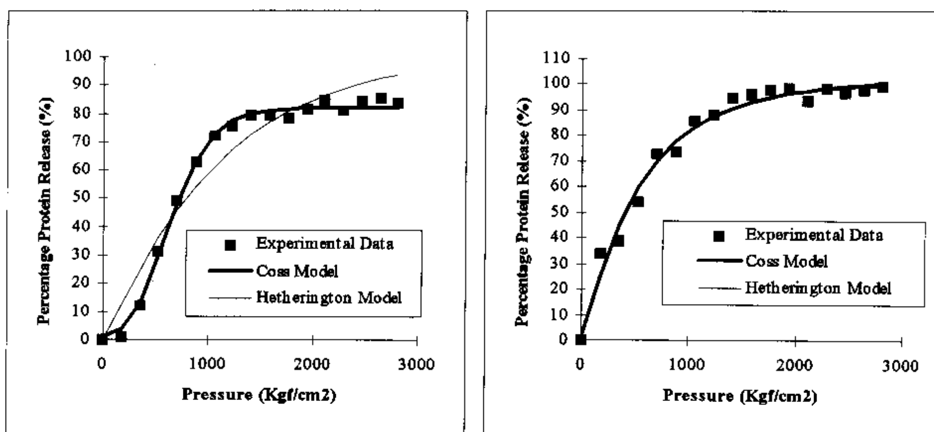


**Figure 2: Modelling the disruption of *C. utilis* using the Constant Systems disrupter.**

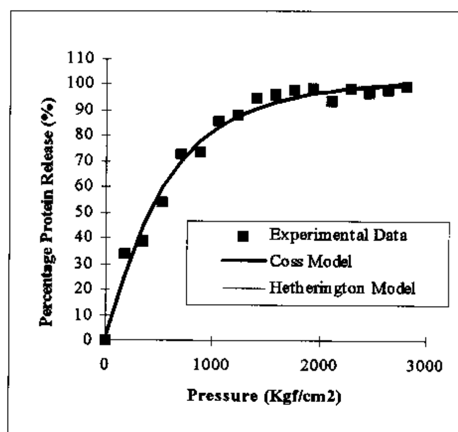


**Figure 3: Modelling the disruption of Baker's yeast using the Constant Systems disrupter.**

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