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The Disruption of *Saccharomyces cerevisiae* Cells and Release of Glucose 6-Phosphate Dehydrogenase (G6PDH) in a Horizontal Dyno Bead Mill Operated in Continuous Recycling Mode

Chow Yen Mei[1], Tey Beng Ti[2], Mohammad Nordin Ibrahim[1], Arbakariya Ariff[3], and Ling Tau Chuan[1]*

[1] Department of Process and Food Engineering, Faculty of Engineering, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[2] Department of Chemical and Environmental Engineering, Faculty of Engineering, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[3] Department of Bioprocess Technology, Faculty of Biotechnology and Molecular Science, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract Baker's yeast was disrupted in a 1.4-L stainless steel horizontal bead mill under a continuous recycle mode using 0.3 mm diameter zirconia beads as abrasive. A single pass in continuous mode bead mill operation liberates half of the maximally released protein. The maximum total protein release can only be achieved after passaging the cells 5 times through the disruption chamber. The degree of cell disruption was increased with the increase in feeding rate, but the total protein release was highest at the middle range of feeding rate (45 L/h). The total protein release was increased with an increase in biomass concentration from 10 to 50% (w/v). However, higher heat dissipation as a result of high viscosity of concentrated biomass led to the denaturation of labile protein such as glucose 6-phosphate dehydrogenase (G6PDH). As a result the highest specific activity of G6PDH was achieved at biomass concentration of 20% (ww/v). Generally, the degree of cell disruption and total protein released were increased with an increase in impeller tip speed, but the specific activity of G6PDH was decreased substantially at higher impeller tip speed (14 m/s). Both the degree of cell disruption and total protein release increased, as the bead loading increased from 75 to 85% (v/v). Hence, in order to obtain a higher yield of labile protein such as G6PDH, the yeast cell should not be disrupted at biomass concentration and impeller tip speed higher than 20% (w/v) and 10 m/s, respectively.

Keywords: cell disruption, yeast cells, glucose 6-phosphate dehydrogenase, Dyno bead mill, Zirconia beads

The yeast, *Saccharomyces cerevisiae (S. cerevisiae)* has simple glycosylation capability; high growth rate, low protease levels and a reasonably well understood genetic system. Furthermore, it has been traditionally used for the production of food related fermentation products such as alcoholic beverage and as a starter culture in the bakery industry. Therefore, yeast is preferred when a GRAS (Generally Regarded as Safe) organism is required for the production of simple glycosylated and food related protein. Unfortunately, the protein secretory mechanism of yeast cells does not function to release most of the intracellular proteins into the medium [1]. Consequently, cell disruption for the release of intracellular product is required following yeast fermentation.

Cell disruption may be achieved in a number of ways. Generally, the cell disruption methods can be classified as mechanical and non-mechanical. The non-mechanical methods such as chemical and enzymatic methods are considered as restricted and expensive for large scale extraction of intracellular proteins. Mechanical method, include techniques based on liquid shear and solid shear forces and they are preferred for large scale operation. High pressure homogenization is the most widely used liquid shear method for the recovery of intracellular products. However, the major drawback of high pressure homogenization is the relatively high power consumption during large scale operation.

Another commonly used mechanical method using solid shear force technique is the agitation of a cell suspension with abrasives. Various designs of bead mills had been used, such as the vertical and horizontal mills. Horizontal mills are preferred if higher bead loading and smaller bead size are desired. Bead mill is more suitable for large scale operation since the rate of power consumption is relatively low. Numerous physical process parameters like, design and speed of the agitator [2,3],

***Corresponding author**
Tel: +60-3-8946-6366 Fax: +60-3-8656-7123
e-mail: ltc555@eng.upm.edu.my

bead loading [3], bead size [3], biomass concentration [3], and temperature [2] have been investigated for their influence on the efficiency of cell disruption in bead mills. Beside these physical process parameters, the flow rate of feed-stock and the number of passages in the continuous recycle mode of operation will also affect the efficiency of cell disruption.

In the present study, the effect of impeller tip speed, initial biomass concentration and bead loading on the liberation of intracellular proteins have been investigated. As for the demonstration of principle, the cytoplasmic enzyme glucose 6-phosphate dehydrogenase (G6PDH) was used to discuss the generic applicability of such an approach for the release of labile intracellular products of yeast. Moreover, till date there are no reports available for obtaining G6PDH by continuous recycle disruption of *S. cerevisiae* through a Dyno bead mill.

Commercial compressed bakers' yeast (*S. cerevisiae*) was used as a source of G6PDH enzyme. A 1.4-L stainless steel chamber of the Dyno horizontal bead mill (Willy A. Bachofen AG Maschinefabrik CH-4005 Basel, Switzerland) was used in the present study. The disruptor had a horizontal centre shaft with centric disk arrangement. About 75 and 85% (v/v) of 0.3 mm diameter zirconia beads were filled into the chamber. Cell disruption was performed under a continuous recycle mode at the agitation speed of 1939, 2387, and 3342 rpm with 8, 10 and 14 m/s of impeller tip speed, respectively. Two liter of cell suspension [10, 20, 30, 40 and 50% (w/v)] in Tris-HCl buffer (pH 7.5) was fed into the chamber at the flow rates of 28, 45, and 98 L/h, respectively. During operation, the disruption chamber was cooled by outer cooling jacket by circulating cold water. The temperature of the cell suspension was maintained at 22~24°C.

The intact cell density was determined spectrophotometerically at a wave length of 660 nm in a visible spectrophotometer (Cecil CE2502, 2000 series, Cecil Instruments Ltd, Cambridge, England). The concentration of soluble protein was determined by Bradford's method [4] by using bovine serum albumin as standard. G6PDH activity was determined in the supernatant by the spectrophotometric reduction of nicotinanide adenine dinucleotide phosphate (NADP) at 30°C, according to Bergmeyer *et al.* [5]. Degree of cell disruption was determined by light microscopic examination. The degree of cell disruption was defined as the ratio between the number of intact cells after and before disruption.

In a continuous mode bead mill operation, a single pass is usually not sufficient to liberate intracellular products from the yeast cells. The yield of liberated protein can however be alleviated by recycling the cell suspension. The total protein released and the degree of cell disruption as a function of the number of passages is shown in Fig. 1. After a single pass, the degree of cell disruption was about 80%; however, the total protein release was only half of the maximum attainable protein release. This suggests that, the yeast cell was not completely disrupted. If the cell wall was well disrupted, the cytoplasmic enzyme such as G6PDH would not have been released. Hence, the partially disrupted cell suspension was recirculated in

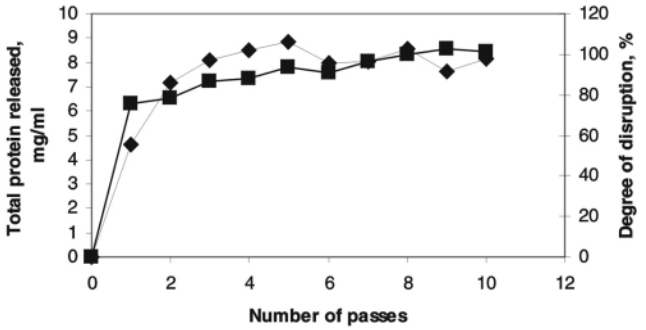

Fig. 1. Release of soluble protein (diamond) and degree of cell disruption (square) after disruption of *Saccharomyces cerevisiae* with the Dyno-mill as a function of the number of passages. Disruption operation was conducted with biomass concentration of 20% (w/v), bead loading of 75% (v/v), impeller tip speed of 10 m/s and feeding rate of 45 L/h.

order to achieve a higher degree of cell disruption for the complete release of intracellular products. After 5 passages, the degree of cell disruption was about 95%, and the total protein release was maximum (9 mg/mL). The light microscopic image of yeast cells before and after 5 disruption passages is shown in Fig. 2. After continuous passages for 5 times, majority of the yeast cells lost their cell wall integrity, and thereby remained unstained by methylene blue. A 100% of cell disruption was achieved only after when the cell suspension was subjected to 9 passages in the bead mill, however, there was no further increase in the release of total protein. Hence, in the subsequent experiment the cell suspension was subjected to only 5 passages.

Table 1 shows the influence of various operating parameters on the degree of cell disruption and release of total protein of bakers' yeast disrupted in a horizontal bead mill. Generally, the degree of cell disruption was decreased with an increase in the feeding rate, which was agreed with the observation reported by other researchers [3]. The increase in feeding rate would result in a decrease in the residence time of the cell suspension inside the bead mill and since cell disruption is a first order kinetic, the yield should decline with increasing feeding rate [1,6]. Beside the reduction in the degree of cell disruption, the operating pressure is another limiting factor for the higher feeding rate operation. A 0.7 bar of operating pressure was recorded at the feeding rate of 98 L/h compared to only 0.4 bar at 28.0 L/h. Nevertheless, our current result showed that the total protein release was maximum at a feeding rate of 45 L/h but not at 28 L/h. The decrease in total protein level at lower feeding rate was possibly due to its prolonged residence time. Longer residence time means more exposure of the released protein to heat and shear denaturation.

As the increase in biomass concentration is known to result in increase in the viscosity of cell suspension, there must be some influence on the degree of cell disruption too. The effect of initial biomass concentration on the cell disruption and total protein release at feeding rate of 45

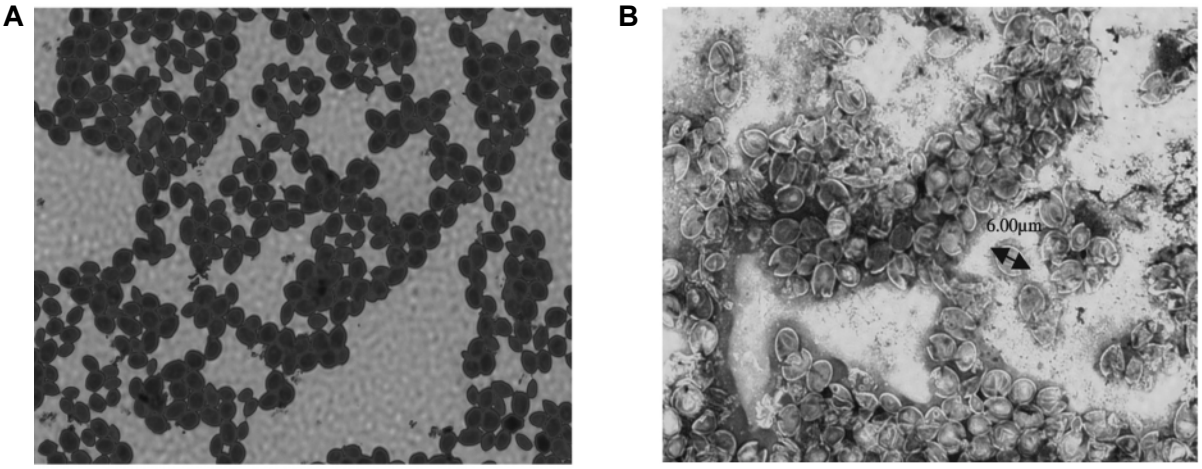

Fig. 2. Morphology of yeast cells A, Intact cells before passage, B, Disrupted cells after passaging 5 times through Dyno-mill (Magnificent, ×100).

Table 1. The influence of feeding rate, initial biomass concentration, impeller tip speed and bead loading on total protein release and degree of cell disruption of *Saccharomyces cerevisiae* through Dyno-mill. The feeding rate, initial biomass concentration, impeller tip speed, and bead loading are 45 L/h, 30% (w/v), 10 m/s and 75% (v/v) respectively unless otherwise stated

		Degree of cell disruption, %	Total protein released, mg/mL
Feeding rate (L/h)	28	99.6	15.35
	45	94.8	15.56
	98	90.5	14.77
Initial biomass concentration [% (w/v)]	10	92.9	4.51
	20	93.6	9.00
	30	94.8	15.56
	40	94.7	22.06
	50	91.5	37.30
Impeller tip speed (m/s)	8	89.9	14.89
	10	94.8	15.56
	14	97.6	18.53
Bead loading [% (v/v)]	75	94.8	15.56
	85	97.0	18.33

L/h, impeller tip speed of 10 m/s and bead loading of 75% (v/v) is shown in Table 1. The degree of cell disruption was increased as the biomass concentration increased from 10 to 30% (w/v). However, a further increase in biomass concentration from 30 to 50% (w/v) led to a decrease in degree of cell disruption. A biomass concentration of 40% has been reported to be optimal for the cell disruption with an impeller tip speed of 5.1 m/s [3]. In our present result, the difference in the degree of cell disruption between 30 and 40% (w/v) was less than 0.2%. Therefore, the optimal biomass concentration for yeast cell disruption was between 30 to 40% (w/v). In controversy to our present results, many studies have demonstrated that degree of cell disruption was either decreased [7] or not affected [8] with an increase in the biomass concentration.

As shown in Table 1, the total protein was increased with an increase in the biomass concentration from 10 to 50% (w/v). Our recent result is in accord with Ricci-Silva *et al.* [7], who also reported a correlation between protein release and the biomass concentration. Higher protein release at higher biomass loading suggested the possibility of operating the bead milling of *S. cerevisiae* at biomass loading as high as 50% (w/v). By doing so, the power consumption per unit weight of biomass was decreased. However, this advantage is shadowed by the higher heat dissipation as a result of higher viscosity, which was indicated by the higher temperature recorded at higher biomass concentration (Table 2). As such, the specific activity of G6PDH was decreased at the biomass concentration of 30% (w/v). Higher viscosity also requires more intense centrifugation forces for the separation of soluble compounds from the nonsoluble particles during the subsequent step of downstream processing. Hence, the optimal biomass concentration for the bead milling of *S. cerevisiae* was 20% for the release of labile protein such as G6PDH.

The tip speed of the impeller has great influence on the frequency of bead-cell collisions and on the intensity of shear generated by the impeller disc. The rate constant of cell disruption was proposed to be proportional to the tip speed of the impeller [9,10]. However, this linear relationship does not hold good at very low impeller tip speed. If the impeller tip speed is below than the so called critical velocity, no cell disruption would occur [11]. The critical velocity of a particular bead mill is in turn influenced by the bead diameter and bead loading. The critical velocity increased with a decrease in bead diameter and an increase in bead loading [1]. The impeller tip speed of the Dyno bead mill used in our current study can be ad-

Table 2. The influence of impeller tip speed and initial biomass concentration on total protein release and glucose 6-phosphate dehydrogenase (G6PDH) specific activity of *Saccharomyces cerevisiae* disrupted through a Dyno-mill. The feeding rate and bead loading were 45 L/h and 75% (v/v), respectively.

Impeller tip speed (m/s)	Biomass concentration [% (w/v)]	Temperature (°C)	Total protein (mg/mL)	Specific enzyme activity (U/mg protein)
10	10	22	5.64	1.38
	20	24	10.34	2.12
	30	28	17.28	1.52
14	10	25	5.65	0.94
	20	27	10.00	1.32
	30	32	17.25	1.22

justed to a range of 8 to 14 m/s. The influence of impeller tip speed on the degree of cell disruption and total protein release for bakers' yeast at feeding rate of 45 L/h, bead loading of 75% (v/v) and biomass concentration of 30% (w/v) is shown in Table 1. Generally, the degree of cell disruption and total protein released were increased by increasing the impeller tip speed. Schutte *et al.* [3] have also reported that higher impeller tip speed (in the range of 5.1 to 10.5 m/s) leads to the higher cell disintegration for several organisms such as *S. cerevisiae, S. carlsbergensis* and *Candida boidinii*. However, when the bead loading was increased from 75 to 85% (v/v), the increase of impeller tip speed from 10 to 14 m/s no longer showed any obvious increase in total protein release. Nevertheless, the specific activity of G6PDH was decreased substantially (Table 2). Obviously, the decreased specific enzyme activity was due to the heat denaturation of heat-labile protein, G6PDH as a result of high heat generation at higher impeller tip speed and bead loading. The frequency of bead-bead and bead-cell collisions was higher at higher bead loading if the impeller tip speed was increased. The higher collision rate of bead resulted in a higher heat generation. As has been reported by Mario Canales *et al.* [12] that 33% of total heat generated was dissipated into cell suspension, and hence the rate of heat dissipated into the cell suspension would increase as the impeller tip speed and bead loading increased. There was an increase in temperature to about 4°C as the impeller tip speed was increased from 10 to 14 m/s. The temperature as high as 32°C has been recorded at the impeller tip speed of 14 m/s and biomass concentration of 30% (w/v) suggesting that indeed a big quantity of G6PDH was heat denatured (Table 2).

The influence of bead loading was examined at a flow rate of 45 L/h, biomass concentration of 30% (w/v) and impeller tip speed of 10 m/s. As shown in Table 1, both degree of cell disruption and total protein release were increased as the bead loading increased from 75 to 85% (v/v). However, the power consumption and heat generation may be excessive when bead loading is more than or equal to 90% [3]. Higher bead loading and cell concentration also will create high operating pressure and will require higher power consumption. In the present study, 1.2 bar of pressure was recorded in the disruption chamber at the bead loading of 85% (v/v). As a result, an upper limit of 90% (v/v) bead loading has been proposed for most of the bead milling operation [9].

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