Protein Releasing Kinetics of Bakers' Yeast Cells by Ultrasound

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The protein releasing kinetics of *Saccharomyces cerevisiae* cells were investigated by using the ultrasonication method. The effects of acoustic power, duty cycle % of a sonicator, medium pH and cell concentration on protein release were examined. An attempt was also made to enhance cell disruption further by adding glass beads to the solution. An increase with protein release was observed with increasing acoustic power, duty cycle and glass beads loading %. The protein release was found almost independent of cell concentration and optimum pH was obtained as 7. The relationship between protein release and processing time at various process conditions were studied; and the data were fit to a first order kinetic expression. By using the kinetic data, energy calculations with respect to protein release % were made; and found that using high acoustic power is not feasible for disruption process. By increasing the duty cycle %, no significant energy alteration was observed to achieve the same protein release %. However, disruption time decreased considerably. The effect of ultrasonic energy which is a function of both acoustic power and duty cycle % on the kinetic coefficients was also investigated using an exponential expression based on Arrhenius equation; and the activation energy of protein release was found as 0.44 kJ L^{-1}.

Key words:
Cell disruption, kinetic coefficients, protein release, yeast, sonication process

Introduction

Microbial cells as the source of valuable enzymes, proteins and other bioproducts, have been exploited very intensively in the last 30 years. Microorganisms produce two basic types of biological compounds as extracellular and intracellular.[1] Isolation and recovery of intracellular biological compounds follows a complicated sequence of operations. The downstream processing involves the cell disruption, primary separation, isolation and purification. Cell disruption is a necessary unit operation for the isolation of intracellular biological compounds that are not secreted by the cell.[2]

The methods used for cell disruption can roughly be classified as mechanical (employing shear force) and non-mechanical (including physical, chemical and enzymatic treatments).[3,4] Among the mechanical methods, ultrasonication is one of the most widely used laboratory disruption methods.[1,5] Ultrasonic disintegrators generally operate at frequencies of 15 to 25 kHz. The ultrasonic converter, equipped with an ultrasound oscillating system, transforms the electrical energy delivered by the generator into mechanical oscillations of the same frequency. The disruption that occurs when the cells are irradiated with ultrasonic energy is due to the cavitation phenomenon. Doulah[6] has suggested that cell disintegration is caused by shear stresses developed by viscous eddies arising from shock waves produced by imploding cavitation bubbles.

Disruption of yeasts is generally easier because of their larger size and different cell wall structure. The basic structural components of the yeast cell wall have been identified as glucans, mannans and proteins. Glucans are moderately branched molecules composed of glucose residues, primarily in β-(1–3) and β-(1–6) linkages. Mannans are characterized by a backbone of mannose residues in (1–6) linkage having short oligosaccharide side chains. Many of the proteins found in yeast cell walls are enzymes rather than structural components.[1]

In this work, the ultrasonication method was used to disrupt and investigate the protein releasing kinetics of bakers' yeast cell. In addition, an attempt was made to enhance cell disruption further by adding glass beads to the processing solution. The effects of sonication parameters (acoustic power and duty cycle %), the effects of medium pH, cell concentration and glass beads loading % on the protein releasing kinetics were examined, and the effect of ultrasonic energy (a function of both acoustic power and duty cycle) on kinetic coefficients was determined.

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Materials and methods

Microorganism

S. cerevisiae commonly referred to as bakers' yeast, was used as a source of protein and obtained from the local supplier in pressed form of wet mass. All experimental results were given in dry mass basis. For this purpose, cell dry mass was determined from the average of ten samples in a pressed form of the wet mass dried at 45 °C until reaching a constant mass. After drying, it was found that the samples contained approximately 70 % moisture.

Measurement of protein

The concentration of protein (background protein, total protein and protein released after processing) was determined by the Lowry method[7] using bovine serum albumin as standard. The total protein in the yeast cell suspension was measured after alkaline hydrolysis, achieved by incubating the cells in c = 0.05 mol L^{-1} sodium hydroxide at 90 °C for 10 min.[8] Three measurements were made for each sample and the data given are an average of these results. The deviation of the measurements was in the range of ±2 %. In dried basis, the total protein content of the yeast was found as approximately 47 %.

Ultrasonication process

The disruption experiments were performed by using Bandelin Sonopuls HD 2200 sonicator at various acoustic powers (from 20 to 140 W) and duty cycles (from 10 to 80 %) at 10 ± 1 °C in 30 mL of yeast solution that was suspended in a c = 25 mmol L^{-1} phosphate buffer. The tip of the sonicator horn (TT13 Titanium tapered tip of 12.7 mm diameter) was immersed about h = 1 cm into the solution that processed in a 70 mL cylindrical glass vessel with a diameter of d = 4 cm. The temperature of the yeast solution was intermittently checked and kept constant by the use of a cooling bath containing ethylene glycol-water mixture. The experiments were carried out in duplicate and the reproducibility was within the range of ±5 %.

Computational work

The software package MATLAB 5.0 was used for numerical calculations. The parameters were evaluated by the nonlinear least squares method of Marquardt-Levenberg until minimal error was achieved between experimental and calculated values.

Results and discussion

The effect of acoustic power on protein releasing was investigated, and the results were determined as protein release % against the disruption time shown in Fig. 1a. Protein release was increased with increasing acoustic power as the increase in acoustic power caused a higher disruption rate which was also reported for different microorganisms by Kapucu *et al*.,[5] Wang *et al*.,[9] James *et al*.[10] and Feliu *et al*.[11] On the other hand, processing time decreased by increasing the acoustic power. To determine the optimal acoustic power for disruption, the total energy added to the solution for protein release % were determined for each acoustic power used, and the results were given in Fig. 1b. The results show that high acoustic power is not feasible for disruption because the energy increases much more rapidly, which was not proportional to

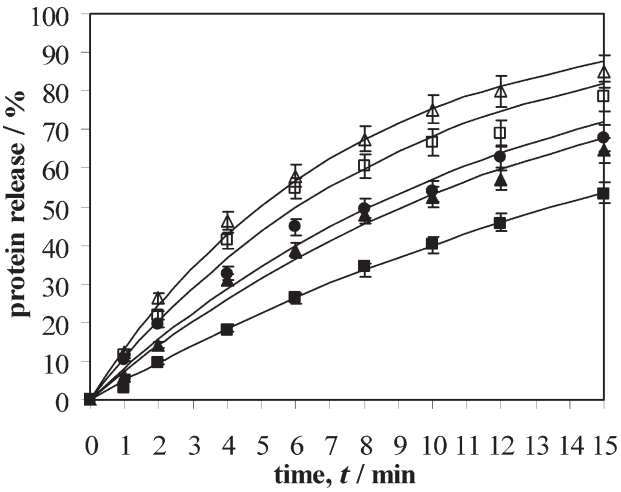

Fig. 1a – *Effect of acoustic power on protein releasing at 50 % duty cycle, 10 ^{0}C temperature, pH 7 and 90 g L^{-1} yeast; ■ 20 W, ▲ 40 W, ● 60 W, □ 100 W, Δ 140 W, – kinetic models*

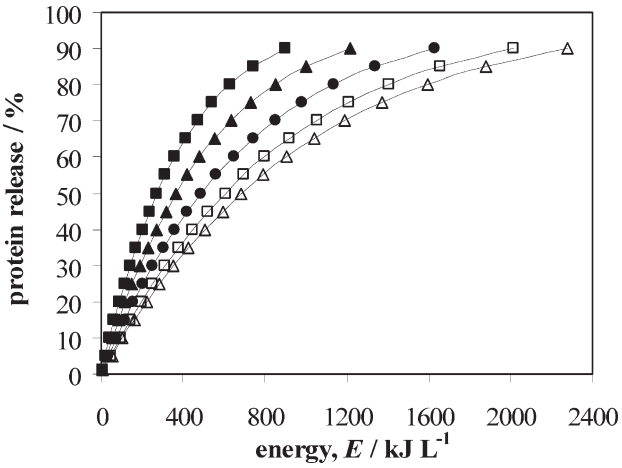

Fig. 1b – *Protein releasing % with respect to total energy added at various acoustic powers; ■ 20 W, ▲ 40 W, ● 60 W, □ 100 W, Δ 140 W*

the time saved. For example, by taking the 20 % protein release basis for evaluation, it can be seen from Figs. 1a and 1b, that, as the acoustic power is increased from 20 to 40 W, the energy also increased from 87.40 to 117.67 kJ L^{-1} (increased 34.63 %), and required time decreased from 4.37 to 2.94 min (decreased 32.72 %). On the other hand, as acoustic power increased from 20 to 140 W, the energy increased from 87.40 to 220.92 kJ L^{-1} (increased 152.77 %), and required time decreased from 4.37 to 1.59 min (decreased 63.62 %). As a result, from the economical point of view, the acoustic power should be kept as low as possible.

The cell disruption studies under ultrasonic treatment presented in the literature[5, 9-11] so far dealt with the relationship between protein release and acoustic power depending on processing time. Therefore, the effect of duty cycle % was investigated in the range of 10 – 80 %. The duty cycle indicates pulsed mode. In pulsed mode, ultrasonic vibrations are transmitted to a solution at a rate of one pulse per second, e.g. for 10 % duty cycle the active interval is 0.1 s. With increasing the duty cycle %, an increase in the protein release was observed, as the duration of applied power increased (Fig. 2a). To see the effect of duty cycle % on disruption clearly, and to determine the optimal duty cycle %, the total energy added to the solution for protein release % was determined for each duty cycle % used, and the results given in Fig. 2b. Figs. 2a and 2b show that no significant energy alteration was observed by increasing the duty cycle %. However, the time savings increased considerably by applying higher duty cycle % instead of lower duty cycle % to achieve the same protein release %. For example, to achieve 20 % protein release with 10 % duty cycle, the required energy and time are 155 kJ L^{-1} and 12.92 min, respectively. On the other hand, the required energy and time to achieve 20 % protein release with 80 % duty cycle are 148 kJ L^{-1} and 1.53 min, respectively.

Comparison of these results with the results obtained from acoustic power experiments show that duty cycle is a more effective parameter on the disruption rate rather than the acoustic power, and the same disruption efficiency with the higher acoustic power with moderate duty cycle rate can be attained using lower acoustic power with higher duty cycle rate. For example, to produce 50 % protein release with the operating conditions of 140 W–50 % of duty cycle and 20 W–80 % of duty cycle, the required energies are 686.14 kJ L^{-1} (required time is 4.95 min) and 457.25 kJ L^{-1} (required time is 4.76 min), respectively. Therefore, the energy consumption of the disruption process as well as the overall processing cost may be reduced.

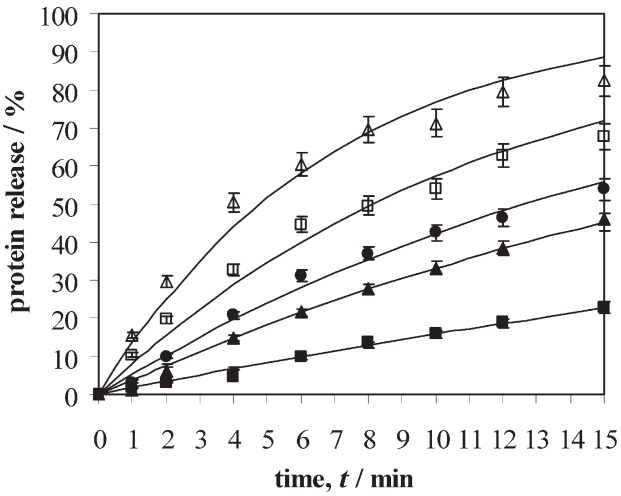

Fig. 2a – *Effect of duty cycle % on protein releasing at 60 W acoustic power, 10 °C temperature, pH 7 and 90 g L^{-1} yeast; ■ 10 % DC, ▲ 20 % DC, ● 30 % DC, □ 50 % DC, Δ 80 % DC, – kinetic models*

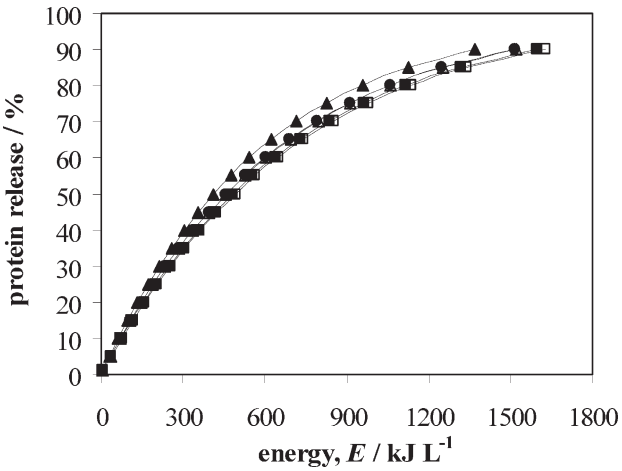

Fig. 2b – *Protein releasing % with respect to total energy added at various duty cycle %; ■ 10 % DC, ▲ 20 % DC, ● 30 % DC, □ 50 % DC, Δ 80 % DC*

To study the effect of pH on protein release, the 90 g L^{-1} suspensions of bakers' yeast were prepared in 25 mmol L^{-1} phosphate buffer solutions which were prepared with pH values of 5, 7 and 9. Experiments were performed at 60 W and 50 % duty cycle rate and results were given in Fig. 3. As the pH of sonicated medium was increased from 5 to 7, the protein release increased from 48 to 68 % (approx. 1.4 times) in 15 min. At pH 9, the results were found almost the same as obtained with pH 7 depending on the processing time. This result is not in agreement with the result found by Agrawal *et al.*,[2] who reported that protein release decreased sharply at pH 9 by disrupting wet compressed bakers' yeast (w = 2%, mass basis), under the following operating conditions; acoustic power 11.62 W and 5 : 5 s on:off basis.

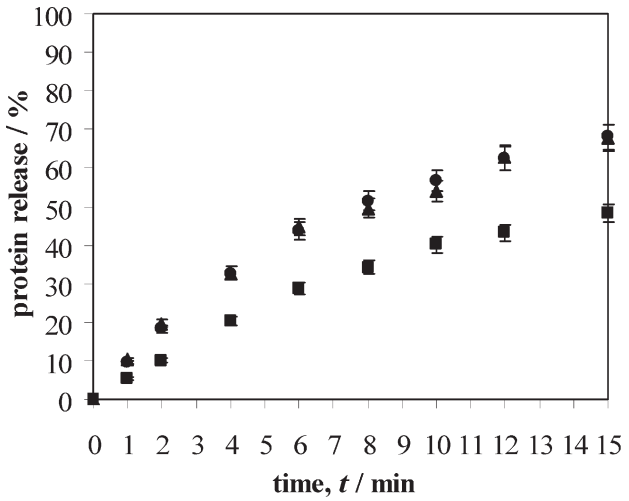

Fig. 3 – *Effect of medium pH on protein releasing at 50 % duty cycle, 60 W acoustic power, 10 ^{0}C temperature and 90 g L^{-1} yeast; ■ pH 5, ▲ pH 7, ● pH 9*

The effect of yeast mass concentration was investigated in the range of γ = 30–150 g L^{-1} yeast and the results were given in Fig. 4. As seen in this figure, the protein release is almost independent of cell concentration. For brewers' yeast, Wang *et al.*[9] also reported that the release of total protein was independent of cell concentration up to 600 g L^{-1} (wet mass).

To enhance cell disruption further, *S. cerevisiae* cells were disrupted with the addition of glass beads in diameter of 0.5 mm into the yeast suspensions and the results were given in Fig. 5a. It was found that the presence of glass beads lead to improved rates of protein release as the addition of glass beads increased the shear effects. Using glass bead loadings in the range of 13–33 %, protein release increased by 12–27 % with respect to the experiment performed without glass beads at the end of 15 min. Total energy added to the solution for protein release % was determined for each glass bead loading % used and results were given in Fig. 5b. Figs. 5a and 5b show that the required energy and time for disruption could be decreased considerably by using glass beads. In other words, the same disruption efficiency with higher acoustic power and duty cycle rates can be attained using glass beads with lower acoustic power and duty cycle rates. Operating conditions of 140 W–50 % of duty cycle (required energy 2100 kJ L^{-1}) and 60 W–50 % of duty cycle with glass bead loading of 33 % (required energy 900 kJ L^{-1}) produced the same protein release of 85 % in 15 min. Therefore, the energy consumption of the disruption process and as well as the overall processing cost could be reduced by using glass beads.

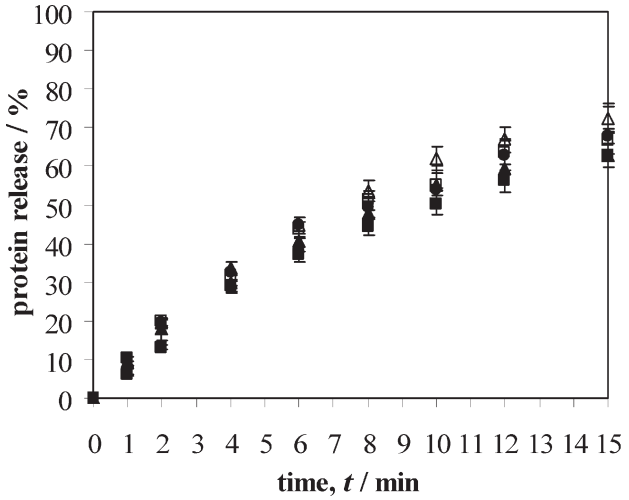

Fig. 4 – *Effect of yeast mass concentration on protein releasing at 50 % duty cycle, 60 W acoustic power, 10 ^{0}C temperature and pH 7; ■ 30 g L^{-1}, ▲ 60 g L^{-1}, ● 90 g L^{-1}, □ 120 g L^{-1}, Δ 150 g L^{-1}*

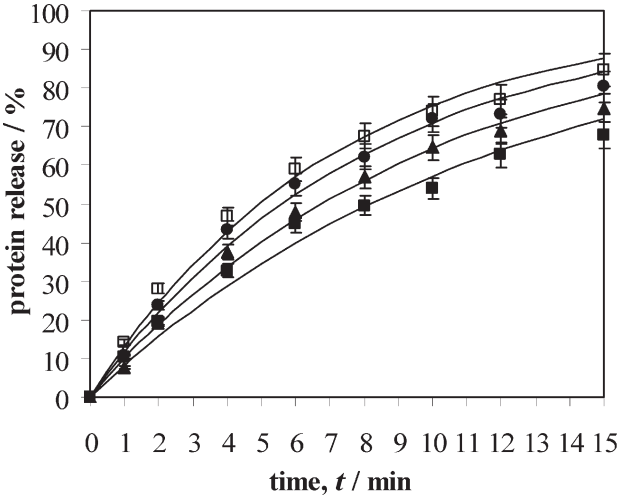

Fig. 5a – *Effect of glass bead loading % on protein releasing at 50 % duty cycle, 60 W acoustic power, 10 ^{0}C temperature, pH 7 and 90 g L^{-1} yeast; ■ without glass beads, ▲ 13 %, ● 26 %, □ 33 %, – kinetic models*

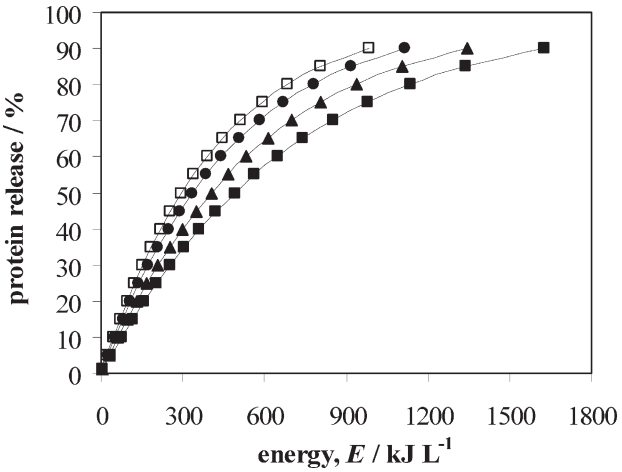

Fig. 5b – *Protein releasing % with respect to total energy added at various glass bead loadings; ■ without glass beads, ▲ 13 %, ● 26 %, □ 33 %*

Protein releasing kinetics

The kinetic studies appearing in the literature so far have dealt with the effect of acoustic power on protein releasing. No consideration has been made to study the effect of duty cycle % and glass bead loadings on protein releasing to determine the kinetic parameters. Therefore, the data of protein releasing experiments were evaluated for conditions mentioned above. These data were fit to the first order kinetic expression (eq. (1)) used by Currie *et al.*[12] and Marffy and Kuala[13] for cell disruption in high-speed mills and by Doulah[6] and Kapucu *et al.*[5] for cell disruption by ultrasonication. r_t values in eq. (1) were calculated by using eq. (2).

$$r_t/r_{max} = 1 - \exp(-k \cdot t) \quad (1)$$

$$r_t = 100 \cdot (\gamma_t - \gamma_b)/(\gamma_{tot} - \gamma_b) \quad (2)$$

r_{max} is the maximum protein released (equals to 100 for this study as the results were given in terms of % values), r_t is the released protein in sonication time t (percentage value calculated from eq. (2)) and k is the kinetic coefficient of the protein release. In eq. (2), γ_t is soluble protein mass concentration at sonication time t, γ_{tot} is total protein mass concentration and γ_b is background protein mass concentration. The kinetic coefficients and statistical values for eq. (1) were estimated for each experimental run and were given in Tables 1–3.

After evaluation of the data given in Tables 1–3, it was estimated that the relationship between k and acoustic power, other variables being at constant, is $k \propto (P)^{\alpha}$. By fitting the data, the value of α was estimated as 0.52 with the regression coefficient and standard deviation of 0.998 and 0.003. On the other hand, the relationship between k and duty cycle (DC %), was estimated as $k \propto (\text{DC \%})^{\beta}$. The value of β was determined as 1.0 with the regression coefficient and standard deviation of 0.998 and 0.003. The exponential relationship between k and the loading % of glass beads was determined as $k \propto (\text{Loading \%})^{\varphi}$, with $\varphi = 0.33$, the regression coefficient of 0.986 and the standard deviation of 0.004.

The exponent ($\beta = 1.0$) representing the dependence of k on the duty cycle is higher than those obtained for acoustic power ($\alpha = 0.52$) and glass bead loading ($\varphi = 0.33$) under the experimental conditions employed. Therefore, the duty cycle is the more effective parameter for the disruption rate than the others.

On the other hand, to predict the effect of ultrasound energy which is a function of both acoustic power (W) and duty cycle rate (s) on the kinetic coefficients of protein release, an exponential expression was used in the following form based on Arrhenius equation,

Table 1 – *Estimated constants and statistical values at various acoustic powers at duty cycle rate of 50 %*

Acoustic power, P / W	k min^{-1}	Standard error σ	R^2 statistical
20	0.051	0.746	0.999
40	0.076	2.532	0.994
60	0.085	3.358	0.990
100	0.114	3.469	0.992
140	0.140	2.500	0.997

Table 2 – *Estimated constants and statistical values at various duty cycle rates at acoustic power 60 W*

Duty cycle DC / %	k min^{-1}	Standard error σ	R^2 statistical
10	0.017	0.784	0.995
20	0.040	1.218	0.997
30	0.055	1.863	0.995
50	0.085	3.358	0.990
80	0.146	4.317	0.989

Table 3 – *Estimated constants and statistical values at various glass bead loadings %*

Glass bead loading %	k min^{-1}	Standard error σ	R^2 statistical
13	0.103	2.277	0.997
26	0.124	2.820	0.995
33	0.141	2.848	0.995

$$k = k_0 \cdot \exp(-E_u/(P \cdot \text{DC})) \quad (3)$$

Where k (min^{-1}) is the kinetic coefficient of the protein release, k_0 is the pre-exponential constant (min^{-1}), E_u (kJ L^{-1}) is the activation energy of protein release, $P \cdot \text{DC}$ (kJ L^{-1}) is the ultrasonic energy applied per sample volume. As seen in Fig. 6, the data accurately fit to eq. (3). The values of k_0 and E_u were estimated as 0.15 min^{-1} and 0.44 kJ L^{-1}; and the standard error (σ) and R^2 statistic values were obtained as 0.197 and 0.963, respectively.

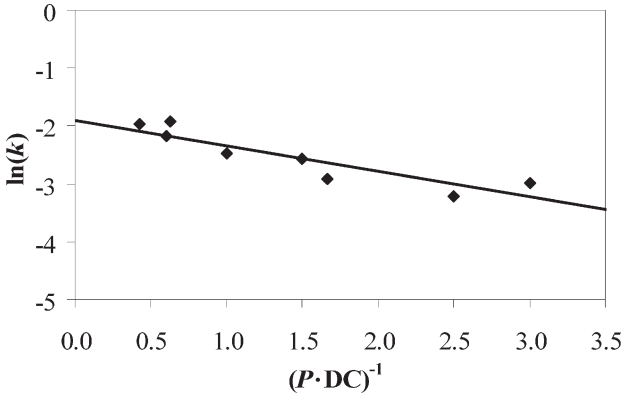

Fig. 6 – *Effect of ultrasound energy on kinetic coefficient of protein release*

Conclusions

The percentage of protein release of *S. cerevisiae* cells increased and disruption time decreased with increasing the acoustic power. However, the results show that the increase in energy consumption is much higher than the increase in protein release % and decrease in processing time. This result indicates that the use of high acoustic power is not feasible for the disruption process. On the other hand, to achieve the same protein release %, no significant energy alteration was observed by increasing the duty cycle %. However, the disruption time decreased considerably.

The results show that protein release was found almost independent of cell concentration and optimum pH for protein release was obtained as 7. On the other hand, the energy consumption and processing time of disruption process could be reduced considerably by the addition of glass beads.

A first order expression was used to explain the protein releasing kinetics by ultrasonication. The activation energy of protein release was found as 0.44 kJ L^{-1}. As a result, for yeast cell disruption in laboratory conditions, the ultrasonication process is an efficient method and could be enhanced substantially by optimizing the process conditions together with the addition of glass beads that provides higher protein releasing in a short processing time with lower energy consumption.

List of symbols

DC % – duty cycle, %

DC – duty cycle rate (active interval of duty cycle), s

c – concentration, mol L^{-1}

d – diameter, cm

E – acoustic energy, kJ L^{-1}

E_u – activation energy of protein release, kJ L^{-1}

h – depth, cm

k – kinetic rate coefficient, min^{-1}

k_0 – pre-exponential constant, min^{-1}

m – mass, g

P – acoustic power, W

r_t – released protein in sonication time t, %

r_{max} – maximum protein release, %

R^2 – statistical coefficient

t – sonication time, min

T – temperature, °C

V – volume, mL, L

w – mass fraction, %

α, β, φ – exponents

γ_b – background protein mass concentration, g L^{-1}

γ_t – soluble protein mass concentration at sonication time t, g L^{-1}

γ_{tot} – total protein mass concentration, g L^{-1}

σ – standard error

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