Microbial cell disruption: role of cavitation

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Abstract

A novel technique of using hydrodynamic cavitation for the large-scale disruption of yeast cells is described. Baker's yeast and brewer's yeast cells in a pressed yeast form were used. Cell disruption was monitored in the form of increase in soluble protein content. Disruption by hydrodynamic cavitation is compared with that obtained by established techniques such as blade blender and acoustic cavitation (ultrasonication).

The effect of cell concentration, time of treatment and number of passes in the flow loop system on the extent of cell disruption is reported. The energy efficiency of the hydrodynamic cavitation setup is compared with that of established techniques. Hydrodynamic cavitation was found to be at least an order of magnitude more energy efficient than established techniques such as ultrasonication or blade blender (mixer).

1. Introduction

A key factor in economical production of industrially important microbial components is an efficient large-scale cell disruption process. For the large scale disruption of microorganisms, mechanical disintegrators such as high-speed agitator bead mills [1], high pressure industrial homogenizers [2], or ultrasonic generators [3], are commonly employed. With currently available methods, disruption is a high-cost, energy-intensive unit operation. The typical energy efficiencies of the current methods are in the range 5%–10%, 90%–95% of the dissipated energy is lost in the form of heat. Analysis of the equipments indicates that the cavitating conditions must prevail to obtain efficient disruption of the cells. The work presented here describes a simple setup which generates cavitating flow. Harrison and Pandit [4] used such cavitating devices to rupture the microbial cells for the first time. The hydrodynamic cavitation setup described has been shown to use only 5%–10% of the energy used otherwise by the established techniques.

2. Experimental details

The microbial systems used were (i) baker's yeast and (ii) brewer's yeast.

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2.1. Established processes

To compare the efficiency of the hydrodynamic cavitation setup, two different established techniques were also studied, namely mixer (blade blender) and an ultrasonic generator horn.

The mixer used was a Remi domestic, having three speed ranges. An ultrasonic generator (Fig. 1(a)), "Dakshin" make, was operated at a frequency of 22 KHz and an amplitude of about 4 microns.

The experimental procedure was as follows. A known concentration (0.01% to 10% by weight) of yeast cells was suspended in distilled water and subjected to treatment (either ultrasonic irradiation or mixer blender) for different time intervals. Treated samples were then analyzed by various techniques, described in Section 3, to assess the degree of disruption. A strict control over the temperature was maintained, either by the use of ice or by circulating cooling water.

2.2. Hydrodynamic cavitation setup

This is a novel cavitation setup being used for cell breakage (Fig. 1(b)). It consists of an overhead tank, in which the cell broth can be pumped at different discharge pressures. The tank can process up to 200 l of broth. When the liquid is allowed to pass through the throttled valve, the static pressure drops to some critical value, which is generally lower than the vapour pressure of the liquid. Some very small bubbles or cavities are formed (cavitation inception). With increasing liquid velocity, the pres-

0923-0467/94/$07.00
SSDI 0923-0467(94)06062-9

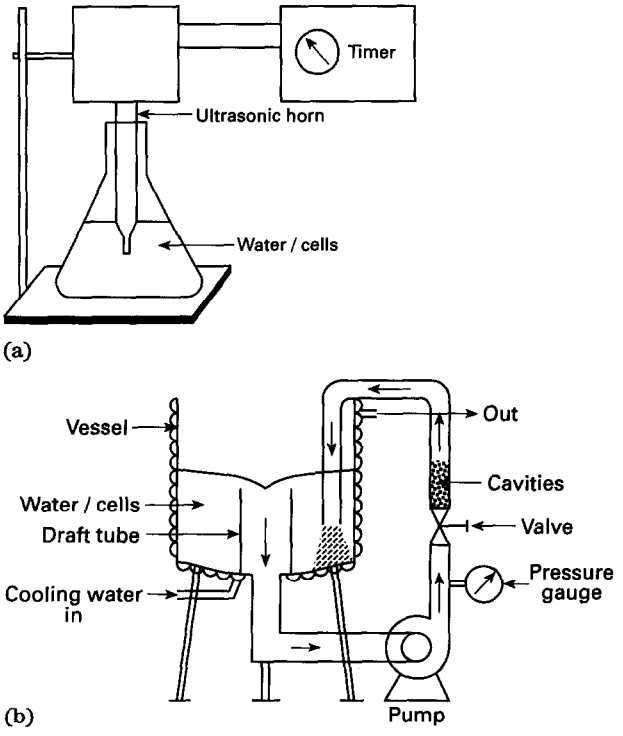

Fig. 1. Experiments with (a) ultrasonic horn and (b) cavitating valve.

sure change across the orifice increases, resulting in a further reduction in static pressure at the *vena contracta*, generating larger cavities or a larger number of them. This phenomenon is accompanied by intense noise and vibration. As the pressure recovers further downstream of the valve, the vapour cavities condense violently (collapse of the cavity). During the collapse, a pressure impulse is generated. If the gas content inside the cavity is small enough, the pressure impulse could be as high as several hundred bars, causing serious damage or rupture of the cell wall.

A known concentration of suspended yeast cells (0.01% to 2.5%) was treated at different discharge pressure levels (different velocities) by varying the number of passes of the cell suspension through the cavitating zone. Samples were withdrawn and analyzed.

3. Analysis

The extent of disruption was monitored using two different methods.

3.1. Direct observations under microscope

Smears were made from the samples on clear, grease-free slides. They were heat fixed and stained with 1% crystal violet or 1% methylene blue for 3 min, washed, dried and observed under oil immersion microscope (1000×) for change in morphology.

In order to quantify the extent of disruption, it was essential to count the number of cells. A direct count of suitably diluted samples was taken using a graduated chamber (haemocytometer) and observed under 400× magnification.

3.2. Indirect method

When the cells are broken, the protoplasm contents emerge, consisting mainly of proteins, nucleic acids *etc.* Monitoring the increase in protein content of the treated sample is also an established method to quantify the extent of cell disruption.

The samples were centrifuged up to 3000 rev min^{-1} for 30 min or at 7000 rev min^{-1} for 10 min. The intact cells as well as debris settled at the bottom giving a comparatively clearer supernatant whose absorbance was read at 280 nm against distilled water using a Perkin Elmer λ-38 UV/visible spectrophotometer.

To obtain clearer samples, the centrifuged samples were filtered through a sintered glass filter with a Whatman GF/C glass microfibre pre-filter.

4. Results and discussion

4.1. Disruption by mixer–blender

Tables 1(a) and 1(b) indicate the extent of protein released, measured by UV absorption for two different concentrations of pressed baker's yeast cells. It can be seen from the tables that the time of

TABLE 1(a). Effect of speed and time of operation on cell disruption

Mixer operation speed	Time (min)	Absorbance of supernatant at 280 nm
0	0	0.013
Low	15	0.012
Medium	5	0.056
Medium	15	0.094
High	40	0.080

Baker's yeast concentration 0.01% wt wt^{-1}.

TABLE 1(b). Effect of mixer speed on cell disruption

Mixer operation speed	Time (min)	Absorbance of supernatant at 280 nm
0	0	0.140
Low	15	0.140
High	15	0.281
High	20	0.363

Baker's yeast concentration 0.1% wt wt^{-1}.

treatment and the speed of the mixer were the two critical parameters responsible for cell disruption. At medium and high speeds, cavitation could be detected audibly, accompanied by a substantial rise in the absorbance indicating increased cell disruption.

To confirm the above results, microscopic observations were made of the untreated and mixer-treated samples having 0.01% concentration (wt wt^{-1}) of baker's yeast cells. Untreated samples indicated cells in the form of larger colonies and round or oval in shape. Samples treated for 40 min in the mixer–blender showed about 50%–60% of the cells in ruptured form (either a small nick or total mutilation was observed). No segregated cell colonies were observed, indicating that mixer–blender is a good dispersing method to distribute the cells uniformly.

4.2. Disruption by ultrasonic irradiation

Tables 2(a) and 2(b) show the results for the pressed baker's yeast cells of 2.5% and 10% concentration. It can be seen from these tables that the time of exposure to ultrasound is the critical parameter responsible for cell disruption. Since the oscillating frequency and the amplitude of oscillation are fixed, the rate of energy input is fixed. The tables show a steady increase in absorbance with time of exposure. Table 2(c) gives the results of brewer's yeast cells, 2% by weight concentration was used. It could be observed by comparing Tables 2(a) and 2(c) that, although almost equal concentrations of baker's yeast and brewer's yeast were used, the extent of rupture and the level of protein release is also dependent on the type of cell used.

Observations of the treated samples under microscope again indicated 52%–65% of the cells being ruptured in 40 and 60 min of irradiation respectively.

4.3. Cell disruption by hydrodynamic cavitation

The valve downstream of the pump was partially closed to adjust the discharge pressure of the pump. The onset of cavitation at the throttled valve was detected at a pump discharge pressure of 20 psig or more. Tables 3(a) and 3(b) show the results for 0.01%–0.1% (wt wt^{-1}) concentration of baker's yeast. As observed in the experiments with mixer–blender or ultrasonic irradiation, a steady increase in absorbance was detected, indicating a gradual increase in the extent of cell disruption. Since the pump flow rate at any discharge pressure is known, the second column in these tables indicates the time required for the entire contents of the tank (200 l) to pass through the throttled cavitating valve

TABLE 2(a). Effect of time of ultrasonic irradiation on cell disruption

Time taken for ultrasonication (min)	Absorbance at 280 nm	
	With distilled water as blank	Original 0 time sample as blank
0	0.532	–
5	0.662	0.126
10	0.691	0.157
20	0.727	0.191
30	0.889	0.350
40	1.032	0.494

Baker's yeast concentration 2.5% wt wt^{-1}.

TABLE 2(b). Effect of time of ultrasonic irradiation on cell disruption

Time taken for ultrasonication (min)	Absorbance of supernatant at 280 nm
10	0.605
20	0.735
30	0.910

Baker's yeast concentration 10% wt wt^{-1}.

TABLE 2(c). Effect of time of ultrasonic irradiation on cell disruption

Time taken for ultrasonication (min)	Absorbance at 280 nm	
	With distilled water as blank	Original 0 time sample as blank
0	0.274	–
5	0.307	0.031
10	0.334	0.062
15	0.332	0.063

Brewer's yeast concentration 2% wt wt^{-1}.

TABLE 3(a). Effect of discharge pressure and number of passes on cell disruption

Discharge pressure (psig)	Number of passes	Time (min)	Absorbance of supernatant at 280 nm
20	0	0	0.014
	1	0.5	0.054
	5	2.56	0.064
	15	9.23	0.069
	25	12.82	0.070
30	1	0.5	0.056
	5	2.8	0.077
	15	8.0	0.064
	25	14.0	0.070

Baker's yeast concentration 0.01% wt wt^{-1}.

TABLE 3(b). Effect of discharge pressure and number of passes on cell disruption

Discharge pressure (psig)	Number of passes	Time (min)	Absorbance of supernatant at 280 nm
20	1	0.5	0.096
	5	2.5	0.103
	15	9.0	0.085
	25	13.0	0.082
30	1	0.5	0.080
	5	2.5	0.092
	15	9.0	0.091
	25	13.0	0.073

Baker's yeast concentration 0.1% wt wt^{-1}.

TABLE 3(c). Effect of number of passes on cell disruption

Discharge pressure (psig)	Number of passes	Time (min)	Absorbance at 280 nm
35	1	0.5	0.330
	5	2.5	0.385
	15	9.0	0.385
	25	12.8	0.494

Baker's yeast concentration 1.0% wt wt^{-1}.

TABLE 3(d). Effect of number of passes on cell disruption

Discharge pressure (psi)	Time (min)	Absorbance at 280 nm	
		Water as blank	Original untreated sample as blank
0	0	0.317	–
40	3	0.349	0.073
	7	0.342	0.032
	12	0.387	0.081
	17	0.515	0.198
45	4	0.449	0.142
Sample left overnight	–	0.315	–
45	3	0.403	0.090
	15	0.588	0.282

Brewer's yeast concentration 1.0% wt wt^{-1}, from red wine manufacturer.

once (one pass) or more. Here again the key parameters appear to be the discharge pressure of the pump (representative of the severity of the cavitation) and the number of passes. Increasing either the pump discharge pressure or the number of passes results in an increase in the extent of cell disruption (increase in the absorbance).

Table 3(c) shows the results for 1% by weight suspension of baker's yeast cells. The extent of increase in the absorbance is only seven times, as against 100 times, increase in the concentration, indicating that increase in the cell concentration reduces the extent of disruption.

TABLE 3(e). Effect of discharge pressure and number of passes on cell disruption

Discharge pressure (psi)	Time (min)	Absorbance at 280 nm	
		Water as blank	Original untreated sample as blank
0	0	0.287	–
40	5	0.291	0.014
	17	0.288	0.007
	40	0.310	0.020
45	15	0.327	0.034
Sample left overnight	–	0.501	–
50	10	0.553	0.052

Brewer's yeast concentration 1.0% wt wt^{-1}, from beer manufacturer.

TABLE 3(f). Effect of discharge pressure and number of passes on cell disruption

Sample kept pressure (psi)	Time (min)	Absorbance at 280 nm	
		Centrifuged sample with original sample as blank	Filtered sample with distilled water as blank
0	0	–	0.496
40	11	0.290	0.436
	23	1.415	0.813
	30	0.754	0.492
	40	0.387	0.395
45	20	0.423	0.405
Sample kept overnight	–	0.931	0.922
50	15	0.923	0.531
	30	0.963	0.475

Baker's yeast concentration 2.5% wt wt^{-1}.

1% concentration of brewer's yeast also showed a steady rise in the absorbance (Tables 3(d) and 3(e)).

Table 3(f) gives the results of 2.5% baker's yeast suspension. The temperature of cell broth was reduced by adding ice. The decrease in temperature led to intense cavitation due to decrease in vapour pressure and it increased the extent of cell disruption, as can be observed from the absorbance levels.

Stained slides did show disrupted cells, especially single cells. Cells which were clumped together showed changes in morphology.

5. Energy efficiencies of various disruption processes

Actual electrical power inputs have been considered. Motor–pump or ultrasonic horn efficiencies have been neglected.

5.1. Mixer blender vs. pump setup

Comparing Tables 1(a) and 3(b), treatment with mixer–blender for 15 min is equivalent to 15 min of treatment on a pump setup at a same cell concentration level. The power consumptions of mixer–blender and pump motor are 500 W h^{-1} and 3000 W h^{-1} respectively. Similarly, the mixer–blender treats 500 ml of the yeast suspension compared with 200 l in a pump setup.

Hence, the energy requirement per millilitre of suspension to observe the same level of protein release is calculated as

$$\text{mixer–blender } \frac{500 \text{ J s}^{-1} \times (15 \times 60) \text{ s}}{500 \text{ ml}}$$

$$= 900 \text{ J ml}^{-1}$$

$$\text{pump setup } \frac{3000 \text{ J s}^{-1} \times (15 \times 60) \text{ s}}{200 \times 1000 \text{ ml}}$$

$$= 13.5 \text{ J ml}^{-1}$$

As seen from the above calculations, hydrodynamic cavitation by throttling a valve is far more energy efficient than the operation of mixer–blender for cell disruption.

5.2. Ultrasonic horn vs. pump setup

Comparing Tables 2(a) and 3(f), 30 min of ultrasonic irradiation is equivalent to 23 min of treatment on the pump setup at the same concentration level.

The power consumption of the ultrasonic horn is 230 W h^{-1} where, as for the pump setup, it is 3000 W h^{-1}. The quantity of the suspension treated was 300 ml and 200 l respectively.

Thus, the energy utilization per ml of yeast suspension to observe the same level of protein release is calculated as

$$\text{ultrasonic horn } \frac{250 \text{ J s}^{-1} \times (30 \times 60) \text{ s}}{300 \text{ ml}}$$

$$= 1500 \text{ J ml}^{-1}$$

$$\text{pump setup } \frac{3000 \text{ J s}^{-1} \times (23 \times 60) \text{ s}}{200 \times 1000 \text{ ml}}$$

$$= 20.7 \text{ J ml}^{-1}$$

As seen from the above calculations, again the hydrodynamic cavitation setup uses an amount of energy about two orders of magnitude less than that used by the ultrasonic horn.

6. Comments

It was observed that, for the pump setup, the concentration of cells in the suspension influenced the disruption process significantly. This concentration effect was not as strong for the case of either the mixer–blender or the ultrasonic horn. An increase in concentration beyond 5% by weight is expected to reduce the quantity of energy advantage of the pump setup, though no quantification has been done at this stage. The method described here also needs to be optimised with respect to pump discharge pressure (related to the strength of the cell wall) and the concentration of the yeast cells.

The growth stage of the yeast cells is another parameter which is likely to affect the energy efficiencies, though it would affect them for all the disruption processes. Preliminary experiments with fresh fermentation broth indicated that the cells in an exponential growth phase are far more susceptible to the disruption compared with those which are either stored or frozen.

The operating temperature seems to have a two-way effect on the cell disruption. As observed during these experiments, on lowering the temperature the severity of the cavitation was increased due to the more violent collapse of the vapour cavity, which led to an increase in the level of cell disruption. However, with an increase in temperature, though the severity of the cavitation decreases, the cell wall becomes weak and is more susceptible to breakage.

7. Conclusions

Comparing the slides and absorbance readings for the established cell disruption techniques with that for the hydrodynamic cavitation setup, one can say that cell breakage is indeed taking place in the hydrodynamic cavitation setup. Comparison of energy input levels shows that the hydrodynamic cavitation setup is a much simpler, cheaper and more energy-efficient way of cell breakage than the established techniques. It also opens up the possi-

bilities of treating large quantities of broth, as required industrially, in a continuous manner.

Acknowledgment

ABP would like to acknowledge with gratitude the generous supply of brewer's yeast by Vittal Malya Research Institute, Bangalore and Nova Creations, Taloja. ABP also wishes to acknowledge the Ministry of Human Resource Development (MHRD) and the Department of Science and Technology (DST) for financial support.

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