Comments on the Mechanism of Microbial Cell Disruption in High-Pressure and High-Speed Devices

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The dominant mechanism for microbial cell disruption in a high-pressure homogenizer and a high-speed homogenizer used in this study has been identified. It was found that the cavity collapse and the pressure pulse resulting from such a collapse have a significant influence on the rates of cell disruption. The similarities among the operating conditions for the decomposition of the aqueous KI solution to liberate iodine, the reaction occurring only under cavitating conditions, and that required for the substantial disruption of microbial cells have been pointed out. The liberation of iodine by the aqueous KI decomposition is treated as evidence of cavitation, and hence microbial cell disruption occurring at an identical discharge pressure confirms the mechanism of cell disruption as cavitation, in the high-pressure homogenizer valve. In the case of the high-speed homogenizer, shear and cavitation both play a significant role in cell disruption.

Introduction

The process of microbial cell disruption for the extraction of intracellular enzymes has been carried out on a laboratory as well as an industrial scale using a high-pressure homogenizer. A lot of controversy exists in the literature as regards the exact cause of the cell disruption in the high-pressure homogenizer (HPH). Save et al. (1994) have proposed that cavitation and the shock waves/pressure impulses produced as a result of cavity collapse are responsible for cell disruption whereas Keshavaraj Moore et al. (1990) have proposed the impingement and impact are the main causes for the cell disruption. Middelberg (1995) has reviewed the proposed physical processes responsible for the disruption of fat globules and microorganisms during homogenization. For microorganisms, proposed processes include the rate and magnitude of pressure drop (Brookman, 1975; Kelemen and Sharpe, 1979) and turbulence (Doulah et al., 1975). As such it is quite possible that all the fluid flow related disruption mechanisms, i.e., pressure differential, fluid shear, and impact or cavitation (i.e., collapse of the cavity results in all the previous three effects), are playing some role in cell disruption.

Acoustic cavitation or ultrasonication on a laboratory scale has been used for the microbial cell disruption. It is a known and well-established fact that the chemical (sonochemistry) and physical transformations (emulsification, stirring, dispersion, cell breakage) occurring in the medium as a result of ultrasonic irradiation are due to acoustic cavitating conditions created in the medium.

In hydrodynamic devices, the onset of cavitation is predicted by the cavitation inception number defined as follows:

$$C_v = \frac{P_2 - P_v}{{}^1/_2 \rho V_{\text{th}}{}^2}$$

where P_2 is the downstream pressure, P_v is the vapor pressure of the liquid, ρ is the density of the liquid, and V_{th} is the maximum liquid velocity through the constriction. Under ideal conditions cavitation occurs, when $C_v \leq 1.0$. In reality, experiments have shown that the cavitation inception number can be higher than 1. This has been either attributed to the presence of dissolved gases, providing nuclei, or shown to be a function of the geometry (Yan and Thorpe, 1990). Thus, the prediction of cavitation inception cannot be correlated with certainty with the cavitation number for all the cavitating geometries.

In the case of the high-pressure homogenizer, the accurate estimation of V_{th} is difficult as, in addition to the liquid flow rate, correct estimation of working gaps in the valve are required. Even if one estimates these accurately using the literature information, one can get only a possibility of cavitation occurring, which needs to be confirmed by experiments as described later in this work. The relation of cavitational activity and cavitation number (beyond inception) has been elucidated by Senthil Kumar et al. (1998), but such a discussion is beyond the scope of this work.

Harrison and Pandit (1992) have shown that maximum cell disruption occurs when the cavitation number is below the cavitation inception number, indicating cavitating conditions must exist for efficient cell disruption. Cavitating conditions can be generated hydrodynamically for large-scale application since the amount of liquid in contact with the cavities is more compared to the acoustic cavitation. Equipment based on pressure and velocity such as the high-pressure homogenizer and high-speed homogenizer can be used as a source of generating cavities. In the high-pressure homogenizer cavitation occurs as a result of fluid flow through an orifice. The presence of free radicals as a result of cavitation has been confirmed (Botha, 1993), while in the high-speed homogenizer it occurs at the impeller tip due to high impeller speed. Because of the high velocity generated by the rotating blade, a pressure reduction occurs at the tip of

S8756-7938(98)00052-6 CCC: $15.00
Published on Web 07/17/1998

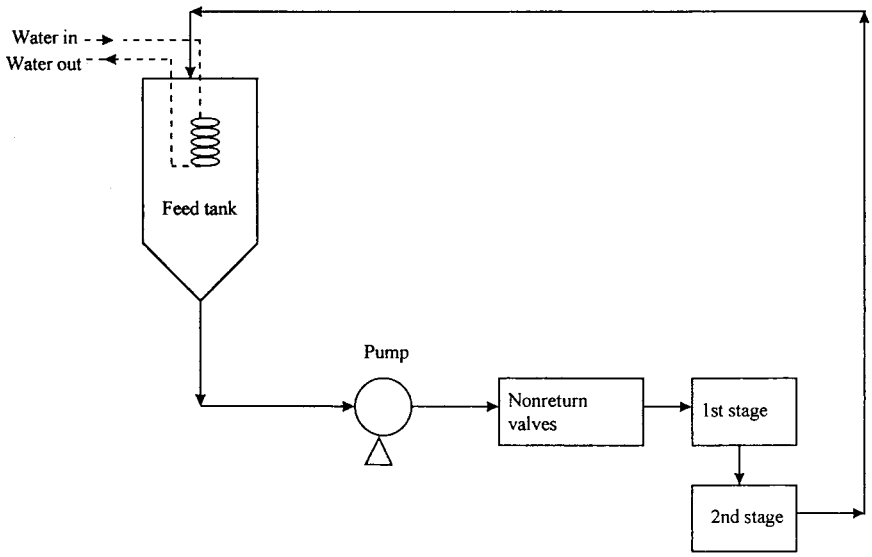

Figure 1. Schematic representation of the experimental setup of the high-pressure homogenizer.

the blade. Once the pressure at the tip of the blade falls below the threshold pressure for cavitation, cavities are generated.

Moholkar and Pandit (1997) have presented an analogy between acoustic cavitation and hydrodynamic cavitation. They have shown that the hydrodynamic cavitation is dependent on the (i) initial cavity radius, (ii) time of pressure recovery, (iii) final discharge pressure, and (iv) noncondensable gas fraction.

To further elucidate the dominant mechanism of cell disruption in two main pieces of equipment used on the laboratory or industrial scale, it was thought desirable to conduct a chemical reaction in the high-pressure homogenizer which occurs only under cavitating conditions and compare these operating conditions in terms of the degree of cell disruption.

For this we have taken oxidation of potassium iodide to iodine as a model reaction which has been widely used for elucidating the parameters affecting acoustic cavitation (Weissler et al., 1950; Naidu et al., 1994). Decomposition of aqueous KI solution has been attributed to the generation of strongly oxidizing OH radicals as a result of cavitation. Thus, KI decomposition cannot occur by fluid shear, impact, or pressure variation (unless this pressure variation is accompanied by cavitation), and thus decomposition of aqueous KI is a sure indication of the presence of cavitating conditions (iodine liberation from aqueous KI solutions is synonymous to cavitation). Thus, there is no need to define the cavitation inception number as it depends on the gap width in the HPH valve, which varies and hence cannot be estimated with certainty.

Experimental Section

High-Pressure Homogenizer. The high-pressure homogenizer used in the study was an APV Gualin GmbH model (Type-DF 1 CHBA, Ser. No. 2429) consisting of basically a high-pressure positive displacement pump. The HPH consists of a feed tank and two throttling valves designated as first stage and second stage. The liquid from the feed tank is driven by a pump to the first-stage valve. Pressure up to 1000 psi can be attained by throttling of this valve. Further increase in the pressure is achieved by using the second-stage valve. Pressure up to 10 000 psi can be obtained by this valve. From the second-stage valve the liquid is recirculated back to the feed tank. With an increase in the throttling pressure, there is a rise in the temperature of the liquid. To maintain the temperature at ambient conditions, a coil immersed in the feed tank was used through which cooling water was circulated.

The schematic representation of the experimental setup of the high-pressure homogenizer is shown in Figure 1. Prior to the experiments the feed tank was washed with distilled water. The discharge pressure and flow rate relationship has been established and reported in Table 1. A 2 L solution of 5% potassium iodide was used. A 25 mL portion of carbon tetrachloride was added. The presence of CCl_4 is known to enhance the rate of KI decomposition as a result of interfacial cavitation (Naidu et al., 1994). The liquid, after addition of freshly prepared starch solution, was then circulated through the pressure valves and subjected to a known operating pressure for 2 min. Since a rise in temperature of the liquid was observed, the HPH feed tank was continuously cooled to room temperature by circulating water. The experiments were carried out in the discharge pressure range of 1000−5000 psi. The samples withdrawn periodically were analyzed for iodine content at 354 nm on a UV−vis spectrophotometer.

For cell disruption studies, 1% Baker's yeast (*Saccharomyces cerevisae*) suspension was prepared and kept overnight in a refrigerator at 4 °C. This has been found to be an optimum concentration to get maximum cell disruption in the two setups used in the study (Save et al., 1994). Industrially, the concentrations are much higher, and indeed this could influence the degree and possibly the dominant mechanism of disruption. A 2 L portion of this suspension was subjected to high-pressure homogenization for 15 passes (1 pass is equivalent to 100 s). In this case the temperature of the feed was also maintained at ambient conditions. Samples were withdrawn after each pass, filtered, and analyzed for the protein released on a UV−vis spectrophotometer at 280 nm. The protein content released was estimated from an absorption calibration plot of BSA. The other details can be found from Gopalkrishnan (1996). The microscopic examination of the cells indicate either a "nick" or total mutilation as described in our earlier publication (Save et al., 1994).

High-Speed Homogenizer. The high-speed homogenizer consists of an impeller (rotor) and a stator, both

Table 1. Flow Rate in the High-Pressure Homogenizer

discharge pressure (psi)	flow rate (mL/s)	discharge pressure (psi)	flow rate (mL/s)
0	27.17	3000	25.10
1000	26.47	4000	23.80
2000	25.87	5000	23.30

of which are made up of stainless steel. The impeller is driven by a high-voltage motor. The impeller blades (9 in number) are 6 mm apart, whereas stator blades (13 in number) are 6 mm apart. The distance between the o.d. of the rotor blade and the i.d. of stator blades is 2 mm. This distance can be varied by using different rotors and stators. A plate with holes attached to the stator has been provided which can be used for inserting baffles so as to avoid vortex formation and gas induction. The rpm of the homogenizer can be varied by changing the applied voltage. The rotor speed was measured with the help of a stroboscope.

A schematic representation of the experimental setup is shown in Figure 2. An 800 mL portion of 5% KI solution was added to a 1 L beaker. To this were added 5 mL of CCl_4 and 10 mL of freshly prepared starch solution. The beaker was placed on a screw-jack. Two glass rods were used as baffles to prevent vortex formation and eliminate gas induction. Initially the impeller was rotated for a few seconds at low speed (<6000 rpm) to remove any entrapped gas. The impeller speed was varied in the range 5000−12000 rpm. Since a rise in temperature was observed at this speed, the temperature of the solution was maintained using an ice-cooled water bath. Samples were withdrawn at 15 min intervals. The samples were analyzed for liberated iodine on a UV−vis spectrophotometer at 354 nm.

For the cell disruption studies, 800 mL of the 1% Baker's yeast suspension was subjected to high-speed homogenization for 15 min. The samples withdrawn at 3 min intervals were centrifuged at 7000 rpm and microfiltered, and the filtrate was then analyzed for the protein released on a UV−vis spectrophotometer at 280 nm. In this case the temperature of the solution was also maintained at ambient conditions.

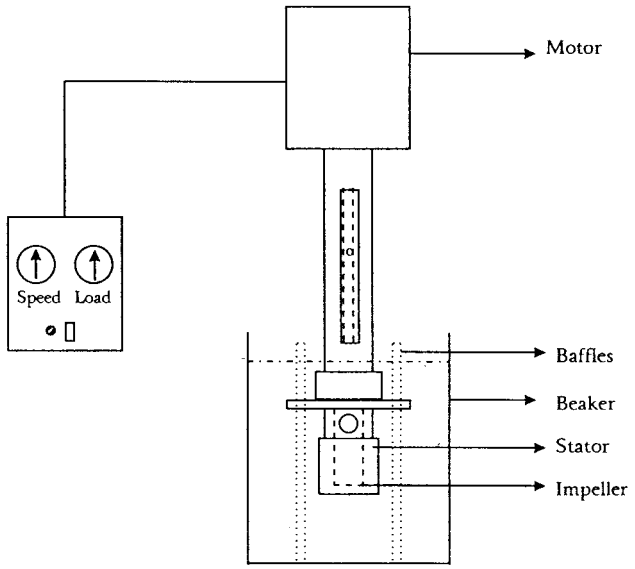

Figure 2. Schematic representation of the experimental setup of the high-speed homogenizer.

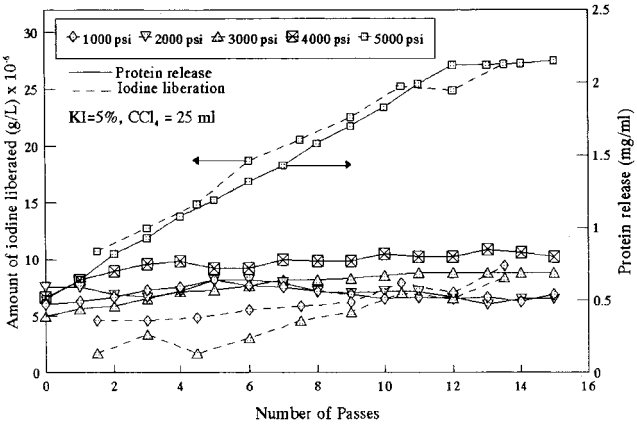

Figure 3. Iodine liberation and protein release on the high-pressure homogenizer.

Results and Discussion

From the previous discussion it can be conclusively said that any iodine liberated from the aqueous KI solution would directly indicate the phenomenon of cavitation. The amount of iodine liberated with the high-pressure homogenizer is shown in Figure 3 with the time of treatment. From the figure it is seen that at low operating pressures, i.e., up to 4000 psi, the amount of iodine liberated is very low and does not vary significantly with either the change in discharge pressure or number of passes. However, at higher operating pressure, i.e., 5000 psi, there is a considerable increase in the amount of liberated iodine, indicating the occurrence of cavitation.

The results of cell disruption studies carried out on the HPH are also plotted in Figure 3. The total protein released in the cell suspension (after clarification) is a result of cell disruption. The details of this indirect cell disruption measurement technique can be found elsewhere (Save et al., 1994).

From Figure 3 it can be seen that HPH discharge pressures up to 4000 psi are not very effective either in disrupting yeast cells or in iodine liberation, whereas at a discharge pressure of 5000 psi substantial cell disruption and iodine liberation occur. The similarity/identical nature of operating conditions to disrupt the cells or to liberate iodine is not coincidental, and hence it can be concluded that at least for the yeast cells under this study, the presence of cavitating conditions for efficient cell disruption is essential. At this stage, the experimental results of identical operating pressures for substantial cell disruption and the release of iodine can be treated as evidence of the presence of cavitating conditions in the high-pressure homogenizer and its effect on microbial cell disruption. Practically no variation in the liquid flow rate as reported in Table 1 has been observed. It also indicates the changing valve aperture and hence the cavitation number with the operating pressures. Save et al. (1997) have also shown that different types of hydrodynamic cavitating devices can result in cell disruption effectively and at different cavitation numbers.

As the cavitating conditions generated in the high-speed homogenizer are different from those of the high-pressure homogenizer and the fluid shear in the former is likely to be significant, it was thought desirable to check the iodine liberation and cell disruption trends in the high-speed homogenizer in an identical fashion.

The results of iodine liberation by the HSH are plotted in Figure 4. From the figure it is seen that there is negligible iodine liberation at an impeller speed up to 7000 rpm whereas it increases abruptly at 8000 rpm.

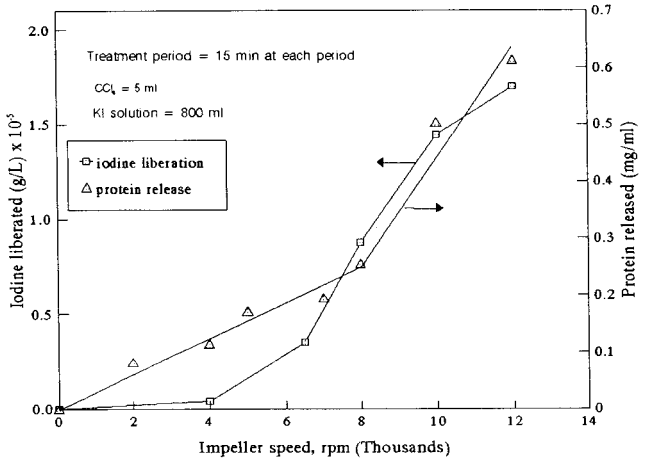

Figure 4. Iodine liberation and protein release on the high-speed homogenizer.

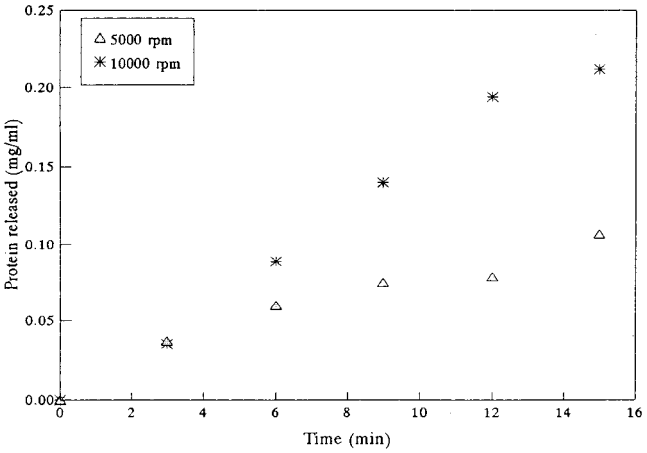

Figure 5. Cell disruption with respect to time on the high-speed homogenizer.

This indicates the existence of cavitation phenomena beyond the impeller speed of 8000 rpm. An independent hydrodynamic analysis (Senthil Kumar and Pandit, 1998) in terms of cavitation numbers confirms the occurrence of cavitation above this speed. The cell disruption studies carried out on the high-speed homogenizer show trends similar to those shown in Figure 4. Comparison of the protein released at 15 min of treatment at speeds below 8000 rpm and above 8000 rpm and the trends in iodine liberation again confirm the observation made in the HPH. The cell disruption with respect to time at two different impeller speeds is shown in Figure 5, which is equivalent to Figure 3 for the HPH. The different rates of protein released indicate that at 10 000 rpm both the mechanical forces and the cavitating conditions are responsible for the cell disruption while only the mechanical forces influence the cell disruption at 5000 rpm.

Conclusion

The extent of protein released under cavitating conditions (above 5000 psi for the high-pressure homogenizer and above 8000 rpm for the high-speed homogenizer) is 3−4-fold higher than that released under noncavitating conditions in the same treatment period. Nondetection of iodine below 5000 psi for the high-pressure homogenizer and below 8000 rpm for the high-speed homogenizer has been taken into consideration while cavitating and noncavitating conditions in both pieces of equipment have been determined. Therefore, for effective cell disruption cavitating conditions can be exploited successfully. Since some amount of protein release is observed below 8000 rpm in the case of the high-speed homogenizer, the shear forces could also be responsible for the cell disruption. The possible use of these hydrodynamically generated cavitating conditions could be a viable alternative to the acoustic cavitation technique used for microbial cell disruption.

Acknowledgment

A.B.P. acknowledges the funding of the Department of Science and Technology (DST), Government of India, for the project.

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Accepted June 8, 1998.

BP980052G