Review

Application of Cavitational reactors for cell disruption for recovery of intracellular enzymes

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Abstract: Cavitational reactors are a novel and promising form of multiphase reactors, based on the principle of release of a large amount of energy owing to the violent collapse of cavities. This paper presents an overview of cavitational reactors in the specific area of cell disruption for the recovery of intracellular enzymes, in terms of the basic aspects, different reactor configurations including recommendations for optimum operating parameters and review of earlier literature reports. It has been observed that under optimized conditions, cavitational reactors can reduce the energy requirement for the release of intracellular enzymes by an order of magnitude compared with conventional cell disruption techniques used on an industrial scale. However, problems associated with efficient scale-up and operation at conditions required for industrial scale, hamper the successful utilization of cavitational reactors at this time. Some recommendations have been made for the future work required to realize the dream of harnessing the spectacular effects of cavitation phenomena.

Keywords: cavitational reactors; cell disruption; process intensification; intracellular enzymes

INTRODUCTION

A key factor in economical production of industrially important intracellular enzymes is an efficient large-scale cell disruption process. The intracellular nature of many recombinant products and the potential use of the bacterial storage product PHB as a commodity thermoplastic have developed interest in improvements in this unit operation.[1,2] For the large-scale disruption of microorganisms, mechanical disintegrators such as high-speed agitator bead mills and high pressure homogenizers[3] are commonly employed but typical energy efficiencies of these methods are in the range 5–10%. The rest of the energy is dissipated in the form of heat, which needs to be removed efficiently to retain the integrity of these delicate bio-products. Much research work has been reported in the literature using the above methods,[3–5] however, there is no integrated approach to cell disruption. With the aim of improving the efficacy of the cell disruption process, keen interest has developed over the last decade in newer techniques, including acoustic and hydrodynamic cavitation. Harrison and Pandit[6] were the first to report the use of cavitational reactors for the cell disruption process. After this pioneering work, concentrated efforts have been made by Pandit's group in India and by Harrison's group in South Africa to harness the spectacular effects of cavitational reactors for improvement and intensification of cell disruption operations. The present work critically reviews the existing knowledge in this field, covering the basics, designs of cavitational reactors with recommendations for optimum parameters, intensification of cell disruption operation for maximizing the release of the desired intracellular enzymes, and suggests a path forward for successful exploitation of cavitational reactors on an industrial scale.

BASIC ASPECTS OF CAVITATION PHENOMENA

Cavitation is defined as the phenomena of the formation, growth and subsequent collapse of microbubbles or cavities occurring in extremely small time intervals (micro to milliseconds) releasing large amounts of energy into a very small volume. Very high energy densities (energy released per unit volume) are obtained locally, resulting in high pressures (of the order of 100–5000 bar) and temperatures (in the range 1000–10 000 K) and these effects are observed at multiple locations in the reactor.[7] Cavitation also results in the formation of highly reactive free radicals as well as generation of intense turbulence associated with liquid circulation currents.[8–10] It should be noted here that the mechanical effects rather than the chemical effects of cavitation are usually more responsible for deciding the efficacy of the cell disruption operation.

Cavitation is generally classified into four types based on the mode of generation, namely acoustic, hydrodynamic, optical and particle, but only acoustic and hydrodynamic cavitation have been found to

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be efficient in bringing about the desired chemical/physical changes in processing applications. Optical and particle cavitation are typically used as analytical techniques to understand cavitation phenomena. The spectacular effects of cavitation phenomena generated using ultrasound (acoustic cavitation) have been effectively harnessed in physical and chemical processing applications in food and bioprocessing industries.[11] Similar cavitation phenomenon can also be generated relatively easily in hydraulic systems. Generally, in the case of hydraulic devices, the main aim has been to avoid cavitation owing to the problems of mechanical erosion, and all the initial effort to understand it was with the objective of suppressing it.[12] However, careful design of the system allows generation of cavity collapse conditions in hydraulic devices, similar to acoustic cavitation, thereby enabling different applications that have been carried out successfully using acoustic cavitation phenomena, but with much lower energy inputs compared with sonochemical reactors.[13]

REACTOR DESIGNS

Cavitational reactors in which the generation of cavitation events is by use of ultrasound are usually described as sonochemical reactors, whereas reactors in which the generation of cavities is by virtue of the fluid energy are described as hydrodynamic cavitation reactors.

Sonochemical reactors

Ultrasonic horns are the most commonly used reactor designs among the sonochemical reactors. These are typically immersion-type transducers with very high cavitational intensities (pressures of the order a few thousand bar) observed very near to the horn, i.e. the vibrating surface. The cavitational intensity decreases exponentially on moving away from the horn and vanishes at distances as low as 2 to 5 cm, depending on the maximum power input to the equipment and on the operating frequency.[14] Ultrasonic horn systems can work effectively if operated in geometry where most of the working liquid is constrained within the longitudinal high-intensity region or where the liquid is stirred vigorously in addition to the ultrasonic irradiation.

Horst *et al*.[15] reported a novel modification in terms of using high intensity ultrasound from a concentrator horn. It has been shown that the concept of a conical funnel fits the demands for near-perfect radiation effectiveness and good reaction management. The design used by Dahlem *et al*.[16,17] also deserves special mention here. The Telsonic horn, which has radial vibrations as against conventional longitudinal vibrations for the immersion system, gives the dual advantages of higher irradiating surface (lower intensity of irradiation resulting in better yields) coupled with good distribution of the energy in the radial direction. Moreover, even if the horn is vibrating radially, local measurements just below the horn[17] have also indicated high cavitational activity, which will again enhance the overall cavitational effects. The scale-up prospects of the horn type system are very poor as it cannot effectively transmit the acoustic energy into large process fluid volume at one time. Also, they suffer from erosion and particle shedding at the delivery tip surface owing to the high surface energy intensity (W m^{-2}). They may also be subject to cavitational blocking (acoustic decoupling), and the large transducer displacement (amplitudes) which increases stress on the construction material, resulting in the possibility of stress-induced fatigue failure. Thus, ultrasonic horn type systems are generally recommended for laboratory-scale investigations to obtain scale-up and other design parameters, such as the relationship between the required cavitational intensity and the strength of the cell wall of the microorganisms to be disrupted.

Ultrasonic horns cannot be used efficiently for large-scale applications and reactors based on the use of multiple transducers irradiating at the same or different frequencies seems to be a logical approach. Use of multiple transducers also results in lower operating intensity (at similar levels of power dissipation) and hence results in higher cavitational intensity.[18] The position of the transducers can also easily be modified so that the wave patterns generated by the individual transducers overlap, resulting in an acoustic pattern that is spatially uniform and noncoherent above the cavitational threshold throughout the reactor working volume. Arrangements such as triangular pitch in the case of ultrasonic baths,[19] tubular reactors with two ends either irradiated with transducers or one end with transducers and the other acting as a reflector,[20] parallel plate reactors with each plate irradiated with either the same or different frequencies[21,22] and transducers on each side of a hexagon[23–25] can be constructed (Fig. 1).

The use of low-output transducers gives the additional advantage of avoiding the phenomenon of cavitational blocking (acoustic decoupling) and surface erosion, which arises when the power densities close to the delivery point are very high. In addition, these multi-transducer units very effectively concentrate ultrasonic intensity towards the central axis of the cylinder and away from the vessel walls, thus reducing problems of wall erosion and contamination due to particle shedding. The vessels can be operated in batch mode or, for larger-scale work, in continuous mode whereby units can be combined in a modular fashion for 'scale-out' and increased residence time. In summary, a plurality of low electrical and acoustic power (1–3 W cm^{-2}) transducers produces 25–150 W L^{-1}, but ideally 40–80 W L^{-1}. The power can be applied continuously or in pulsed mode.[26]

The work of Keil and coworkers[27–30] appears pioneering in terms of simulations of the steady and dynamic pressure fields existing in the reactor.

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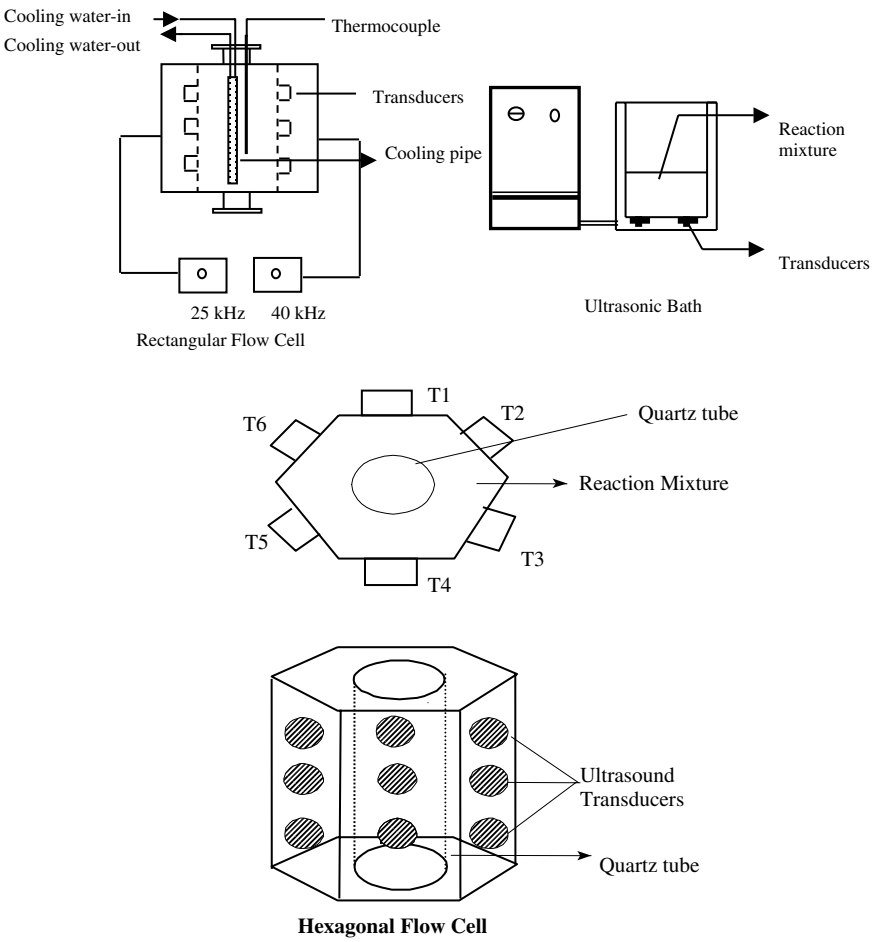

Figure 1. Multiple transducer based sonochemical reactor.

Such a detailed analysis can be used to identify regions with the required pressure fields in a large-scale reactor for optimum cavitational intensities, and then small secondary reactors can be placed strategically at these locations to gain maximum benefits. It might so happen that the threshold required for cell disruption applications is obtained at these locations, but if considered globally, these effects will be marginalized, resulting in much lower overall yields from such cavitational reactors. Thus, the location of the transducers on the irradiating surface and the location of micro-reactors will also depend on the required cavitational intensity for release of the desired intracellular enzymes.

HYDRODYNAMIC CAVITATION REACTORS

Hydrodynamic cavitation can simply be generated using a constriction such as orifice plate, venturi or throttling valve in a liquid flow. At the constriction, the kinetic energy/velocity of the liquid increases, with a corresponding decrease in the local pressure. If the throttling is sufficient to cause the pressure around the point of vena contracta to fall below the threshold pressure for cavitation (usually vapor pressure of the medium at the operating temperature), cavities are generated. Subsequently, as the liquid jet expands, the pressure recovers and this results in collapse of the cavities. During the passage of the liquid through the constriction, boundary layer separation occurs and substantial amounts of energy are lost in the form of permanent pressure drop due to local turbulence. Very high intensity fluid turbulence is also generated downstream of the constriction; its intensity depends on the magnitude of the pressure drop and the rate of pressure recovery, which, in turn, depend on the geometry of the constriction and the flow conditions of the liquid, i.e. the scale of the turbulence. The intensity of turbulence has a profound effect on the cavitation intensity.[31] Thus, by controlling the geometrical and operating conditions of the reactor, the required intensity of cavitation for the desired physical or chemical change can be generated with maximum energy efficiency.

Liquid whistle reactors

The first reactor operating on the principle of hydrodynamic cavitation used a liquid whistle reactor, generally suitable for mixing and homogenization. Here, vibrations are generated in a steel plate as liquid passes over it at high velocity. The liquid couples itself with the vibrations to produce cavitation in the flow, which results in highly efficient mixing. The major feature of the operation of liquid whistle reactors is that the transfer of power is reverse, i.e. power is transferred from medium to the device. A main disadvantage of

these reactors is that they do not offer flexibility in terms of selecting different operating geometries to control cavitational intensity as the optimum results are obtained when the plate is made to vibrate at its resonant frequency.

High pressure homogenizer (HPH)

The high pressure homogenizer is basically a high pressure positive displacement pump with a throttling device that operates according to the principle of high-pressure relief technique. Typically, a HPH reactor consists of a feed tank and two throttling valves designated as first stage and second stage, to control the operating pressure in the hydrodynamic cavitation reactor. There is a critical discharge pressure at which cavitation inception occurs and significant cavitational yields are obtained beyond this discharge pressure. It should be noted that the value of the critical discharge pressure leading to the desired cavitational effect is also dependent on the type of application and the geometry of the throttling valve. Shirgaonkar *et al*.[32] used these reactors for cell disruption purposes and reported that operating pressures up to 4000 psi are not very effective either in disrupting yeast cells or in iodine liberation (which indicates the existence of cavitating conditions), whereas at a discharge pressure of 5000 psi substantial cell disruption and iodine liberation occur, which has been attributed to the onset of cavitation phenomena.

HPH are especially suitable for the emulsification processes in Food, Pharmaceutical and Bioprocess industries. Again, there is not enough control over the cavitationally active volume and the magnitude of the pressure pulses generated at the end of the cavitation events (cavitational intensity), thereby limiting the possibility of selective release of intracellular enzymes based on the relative location of the enzymes in the cell. These reactors are also regularly used for cell disruption operations but suffer from the problem of generation of very fine cell debris, creating subsequent separation problems.

High speed homogenizer (HSH)

Cavitation can also be generated in rotating equipments. When the tip speed of the rotating device (impeller) reaches a critical speed, the local pressure near the periphery of the impeller falls and becomes closer to the vapor pressure of the liquid. This results in the generation of vaporous cavities. The original research on hydrodynamic cavitation can be attributed to this effect, namely cavitation occurring on the propellers of ships and erosion of the propeller blades caused by this. Subsequently, as the liquid moves away from the impeller to the boundary of the tank, the liquid pressure recovers at the expense of the velocity head. This causes the cavities which have travelled with the liquid bulk to collapse.

Kumar and Pandit[33] found that the critical speed for the inception of cavitation to occur in this design of HSH is 8500 rpm (quantified by the rate of oxidation of potassium iodide) for the size and geometry of the stator–rotor used by them and it depends upon the dissolved gas content. Thus the operation of high speed homogenizers should be at speeds higher than the critical speed. Shirgaonkar *et al*.[32] used these reactors for cell disruption purposes and reported a significant release of intracellular proteins only beyond an operating speed of 8500 rpm.

It should be noted that the energy consumption in these types of reactors is much higher, and also flexibility over the design parameters is hardware dependent compared with reactors based on the use of multiple plate orifice plates, to be discussed next.

Orifice plates setup

In these type of reactors (Fig. 2), the flow through the main line passes through a constriction where the local velocities suddenly rise due to the reduction in flow area, resulting in lower pressures, which may even go below the vapor pressure of the liquid medium generating the cavities. The constriction can be a venturi,[33] a single hole orifice[34] or multiple holes on an orifice plate.[35] Multiple hole orifice plates having different combinations of number and diameter of holes, varying the free area offered for flow are represented in Fig. 3. Such an arrangement helps in achieving different intensities of cavitation and also the number of cavitational events generated in the reactor is different. Thus, the orifice plate setup offers tremendous flexibility in terms of the operating (control of the inlet pressure, inlet flow rate, temperature) and geometrical conditions (different arrangements of holes on the orifice plates varying in peripheral fluid shear layer area). Thus, depending on the type of application and cavitational intensity required for release of specific enzymes, geometry and operating conditions can be selected in the hydrodynamic cavitation reactor. The dependency of the extent of release on the cavitational intensity has been explained by Balasundaram and Pandit[36,37] on the basis of the concept of location factor, which has been defined as the ratio of release rate of specific enzyme to the release rate of proteins. It has been reported[36,37] that enzymes that are located in the periplasm or outer layer of the cell wall require much lower cavitational intensity for release than enzymes that are typically located in the cytoplasm area of the cell. Thus, achieving a specific cavitational intensity for selective release of the enzymes is an important requirement, which can best be achieved using the orifice plate setup. Some recommendations for selection of the operating parameters to achieve a desired cavitational intensity are detailed later.

Sampathkumar and Moholkar[38] recently proposed a conceptual design of novel hydrodynamic cavitation reactor that uses a converging–diverging nozzle to create pressure variation in the flow necessary for driving bubble motion, instead of the orifice plates as

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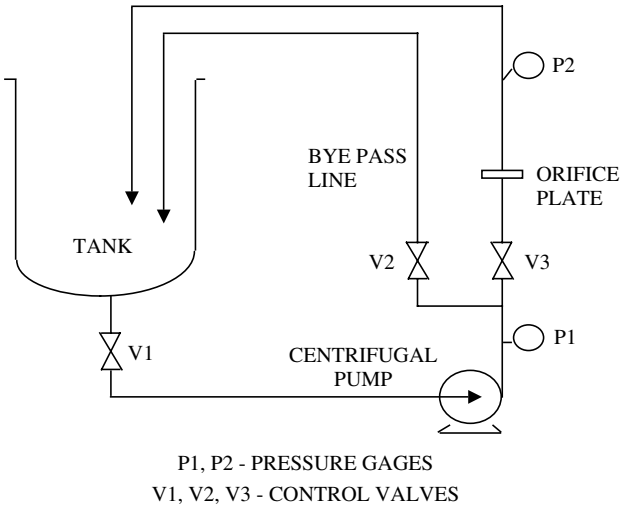

Figure 2. Orifice plate hydrodynamic cavitation setup.

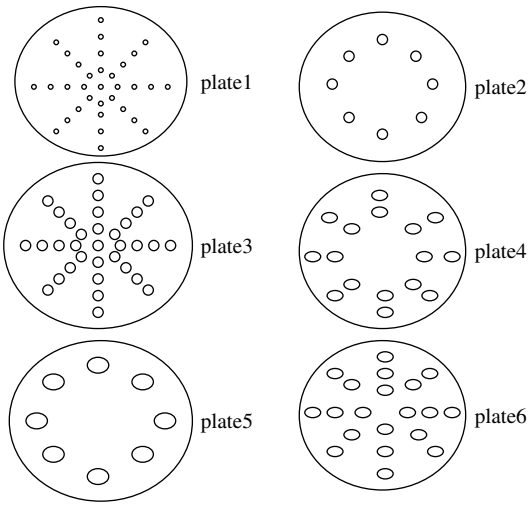

Figure 3. Multiple hole orifice plates having different combinations of number and diameter of holes.

discussed earlier. The cavitation bubbles or nuclei are introduced in the water flow externally, upstream of the nozzle using a sparger. Different gases can be used for the introduction of bubbles. Also, the size of the gas distributor (usually a glass frit), flow rate of gas and pressure of gas in the reservoir (or source) from which gas is withdrawn can be suitably controlled to control the initial size of the cavitational nuclei, which significantly affects the resultant cavitational intensity. The aim should be to generate as small a size of nuclei as possible to maximize the intensity and hence the net cavitational effects. However, compared with the orifice plate setup, the flexibility in terms of controlling the cavitational intensity, is substantially reduced as the length and diameter of the nozzle are the only geometric parameters that can be varied in this case, whereas the number, size and shape of the holes in the orifice plate can be varied. It might be worthwhile using a combination of converging–diverging nozzle and orifice in tandem depending on the intensity requirements for the specific application under question.

From the above discussion about various hydrodynamic cavitation reactors, it can be easily concluded that the orifice plate set-up offers maximum flexibility and can also be operated at relatively large scale. It should also be noted that the scale-up of such reactors is relatively easier as the efficiency of the pump increases with increase in size (flow rate and discharge rate), which will necessarily result in higher energy efficiencies. In earlier work,[22] it was clearly pointed out that the energy efficiency (indicating the transfer of supplied electrical energy into available energy for cavitation phenomena) as well as the cavitational yield (quantification of the net cavitational effects) for the pilot plant scale orifice plate setup is higher than that with high pressure and high speed homogenizers.

OPTIMIZATION OF OPERATING PARAMETERS IN CAVITATIONAL REACTORS

The magnitudes of collapse pressures and temperatures as well as the number of free radicals generated at the end of cavitation events are strongly dependent on the operating parameters of sonochemical reactors, namely intensity and frequency of irradiation along with the geometrical arrangement of the transducers and the liquid phase physicochemical properties, which affect the initial size of the nuclei and the nucleation process. The effect of these parameters on the collapse pressure generated and the maximum size of the cavity during the cavitation phenomena have been studied using the bubble dynamics equation, which considers the compressibility of the medium and a single bubble in isolation,[18,39] In the present work only important considerations regarding the selection of operating parameters have been presented (Table 1) also indicating ways and means to manipulate cavitating conditions for maximum effect. Detailed discussion of the bubble dynamics approaches in cavitational reactors is beyond the scope of this present work; however, the readers may refer to earlier work[18,33,39,40] for better understanding.

In earlier work,[40] a detailed theoretical analysis of the bubble dynamics in hydrodynamic cavitation type reactors was presented. The numerical simulations are based on Rayleigh–Plesset equations similar to acoustic cavitation, the only difference being the fact that surrounding fluctuating pressure field is driven by hydrodynamic conditions existing downstream of the constriction, whereas in the case of acoustic cavitation, it is dependent on the frequency of irradiation (sinusoidal variation in the pressure field). The optimum set of operating parameters as obtained from these theoretical investigations for the case of hydrodynamic cavitating conditions is given in Table 2.

Table 1. Optimum operating conditions for the sonochemical reactors

No.	Property	Affects	Favorable Conditions
1	Intensity of irradiation (Range: 1 to 300 W cm^{-2})	Number of cavities, collapse pressure of single cavity	Use power dissipation till an optimum value and over a wider area of irradiation
2	Frequency of irradiation (Range: 20 to 200 kHz)	Collapse time of the cavity as well as final pressure/temperature pulse	Use enhanced frequencies till an optimum value
3.	Liquid vapor pressure (Range: 40 to 100 mm of Hg at 30 °C)	Cavitation threshold, Intensity of cavitation, rate of chemical reaction.	Liquids with low vapor pressures
4.	Viscosity (Range: 1 to 6 cP)	Transient threshold	Low viscosity
5.	Surface tension (Range: 0.03 to 0.072 N m^{-1})	Size of the nuclei (Cavitation threshold)	Low surface tension
6.	Bulk liquid temperature (Range: 30 to 70 °C)	Intensity of collapse, rate of the reaction, threshold/nucleation, almost all physical properties.	Optimum value exits, generally lower temperatures are preferable
7.	Dissolved gas A. Solubility B. Polytropic constant and thermal conductivity	 Gas content, nucleation, collapse phase Intensity of cavitation events.	 Low solubility Gases with higher polytropic constant and lower thermal conductivity (monoatomic gases)

Table 2. Optimum operating conditions for the hydrodynamic cavitation reactor

No.	Property	Favorable conditions
1	Inlet pressure into the system/Rotor speed depending on the type of equipment	Use increased pressures or rotor speed but avoid super-cavitation by operating below a certain optimum value
2	Physicochemical properties of the liquid and initial radius of the nuclei	The guidelines for selecting the physicochemical properties so as to achieve lower initial sizes of the nuclei are similar to those used for the sonochemical reactors
3	Diameter of the constriction used for generation of cavities, e.g. hole in the orifice plate	Optimization needs to be carried out depending on the application. Higher diameters are recommended for applications which require intense cavitation whereas lower diameters with large number of holes should be selected for applications with reduced intensity
4	Percentage free area offered for the flow (ratio of the free area available for the flow, i.e. cross-sectional area of holes on the orifice plate to the total cross-sectional area of the pipe)	Lower free areas must be used for producing high intensities of cavitation and hence the desired beneficial effects

OVERVIEW OF EARLIER WORK ON CELL DISRUPTION USING CAVITATION

Harrison and Pandit,[6] were the first to report the use of cavitational reactors for cell disruption processes using a configuration whereby cavitation was generated using a throttling valve. Save *et al*.[41] used a similar configuration of hydrodynamic cavitation reactor for disruption of Baker's yeast and brewer's yeast cells in a pressed yeast form and the extent of cell disruption was monitored in the form of the increase in soluble protein content in the media (water) used for the preparation of the cell suspension. A detailed study of the effect of operating parameters, such as initial cell concentration, time of treatment and number of passes in the flow loop system, on the extent of cell disruption has been reported. As expected, an increase in the time of treatment and number of passes resulted in a corresponding increase in the extent of cell disruption, with an observed linear variation. The concentration of cells in the suspension influenced the disruption process significantly. An increase in concentration beyond 5% by weight was reported to reduce the quantity of energy advantage of the pump setup, although no quantification has been done. Optimization for operating pump discharge pressure (related to the strength of the cell wall) and the concentration of the yeast cells is recommended to obtain energy efficient disruption operation. The growth stage of the yeast cells is another parameter that affects the energy efficiencies. Preliminary experiments with fresh fermentation broth indicated that the cells in an exponential growth phase are far more susceptible to disruption than those which are either stored or frozen. Comparison of the energy efficiencies, for different operations including hydrodynamic cavitation, mixer-blender and

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ultrasonication, indicated that the energy requirement of the hydrodynamic cavitation setup is lower than that for the other two methods by more than two orders of magnitude, for an equivalent protein release. Quantitatively speaking, energy utilization per milliliter of yeast suspension to observe the same level of protein release was 20.7 J mL^{-1} for the hydrodynamic cavitation reactor, 1500 J mL^{-1} for ultrasonic irradiation and 900 J mL^{-1} for the mixer-blender.

In a progression of earlier work, Save *et al.*[42] investigated the process of cell disruption using hydrodynamic cavitation operating at a capacity of 50 and 200 L. It was established that to obtain a similar extent of protein release at higher cell concentrations in the reactor, higher operating pressures are required. Comparison of the energy efficiency of the hydrodynamic cavitation reactor with the conventional mixer-blender system and acoustic cavitation induced using ultrasound confirmed the earlier findings of the order of magnitude higher energy efficiencies for hydrodynamic cavitation, even at large-scale operation. Save *et al.*[42] also established that even though cavitation is known to generate conditions of very high temperature and pressure locally, along with the generation of free radicals, the activity of the enzymes released from the cells remains unaltered. The activity of glucosidase and invertase enzymes was not affected under normal circumstances but prolonged exposure to severe conditions of cavitation (at severe operating conditions as reported in the work) resulted in a marginal decrease in the activity of the enzymes. Thus, it is important to control the intensity of the cavitation phenomena by suitably adjusting the operating and geometrical parameters of the system.

It should also be noted that the mechanism of the cell disruption process is also different, depending on the equipment used.[32,36,43] The cell disruption process can proceed via complete breakage of the individual cells releasing the intracellular enzymes in certain devices or can be shear driven where only the cell wall breaks so that enzymes present at the wall or periplasm will only be released (leached slowly). Cavitation phenomena mainly control the extent of cell disruption and release of enzymes and generating cavitating conditions in the system is important to maximize the release of intracellular enzymes for a given energy input.[32,33]

Balasundaram and Pandit[36] investigated the release of invertase enzyme by disruption of *S. cerevisiae* cells using sonication, high pressure homogenization and hydrodynamic cavitation. Invertase is an enzyme located in the periplasmic space of *Saccharomyces cerevisiae* cells. The experimental setup was similar to that used earlier[42] except for the use of an orifice plate instead of a throttling valve for the generation of cavitation. The plate consisted of 33 holes of 1 mm diameter. A total of 50 L of 1% yeast suspension in acetate buffer of pH 5 (100 mmol L^{-1}) was disrupted at 75 psig pump discharge pressure for 50 min. The extent of release of the enzyme invertase was found to be higher than total soluble protein. This could be due to the periplasmic location of the enzyme. Based on the release pattern of the enzyme and protein, a selective release of invertase (periplasmic) is expected in the early stages of disruption by hydrodynamic cavitation before complete mutilation of the cells, releasing all the available proteins (cytoplasmic as well). For the case of ultrasonic induced cavitation, the rate of release of invertase enzyme was comparable with proteins, which can be attributed to the higher cavitational intensity in the case of acoustic cavitation compared with hydrodynamic cavitation. Severe cavitation results in complete breakage of the cells whereas the mild cavitational intensity associated with the hydrodynamic cavitation reactor results in an impingement/grinding action at the cell arising from the shear, and causing breakage of the cell wall rather than the complete cell. Comparison of hydrodynamic and acoustic cavitation modes for release of enzyme indicated that hydrodynamic cavitation resulted in 44 times more specific yield (mg of enzyme released per unit of energy supplied) than sonication when compared on the basis of overall energy consumption.

Balasundaram and Harrison[44] investigated the application of hydrodynamic cavitation for the partial disruption of *E. coli* cells and selective release of specific proteins relative to the total soluble protein. The effects of cavitation number, number of passes, and specific growth rate of *E. coli* on the release of periplasmic and cytoplasmic proteins has been studied. At the optimum cavitation number of 0.17 for this experimental configuration, 48% of the total soluble protein, 88% of acid phosphatase, and 67% of β-galactosidase were released by hydrodynamic cavitation, and the specific activity of the enzymes, acid phosphatase and β-galactosidase, were 70% and 33% higher, respectively, for hydrodynamic cavitation than that obtained using multiple passes through the French Press. The higher release of acid phosphatase over total soluble protein suggested preferred release of periplasmic compounds. This was supported by SDS-PAGE analysis. *E. coli* cells cultivated at a higher specific growth rate (0.36 h^{-1}) were more easily disrupted than slower grown cells (0.11 h^{-1}). The specific activity of the enzyme of interest (β-galactosidase) released by hydrodynamic cavitation, defined as the units of enzyme in solution per milligram of total soluble protein, was greater (43 units mg^{-1} at an operating cavitation number of 0.22) than that obtained on release by the French Press (17 units mg^{-1}), high-pressure homogenization (10 units mg^{-1}), osmotic shock (1 unit mg^{-1}), and EDTA treatment (4 units mg^{-1}). The selectivity offered indicates the potential of selective enzyme release by hydrodynamic cavitation to ease the subsequent purification in downstream processing operations. These studies have clearly established the dependency of the extent of release of enzymes on the location of the enzyme in the cell.

Balasundaram and Pandit[37] quantified the dependency of the position of enzymes in the cell using the concept of location factor. Disruption of yeast cells for selective release of invertase, alcohol dehydrogenase (ADH) enzymes and disruption of *E. coli* cells for release of penicillin acylase was achieved using a sonicator, hydrodynamic cavitation reactor and high pressure homogenizer. For the release of invertase and penicillin acylase, the location factor was observed to be greater than 1 for all cavitation equipments, which confirms the periplasmic location of the two enzymes in the yeast and *E. coli* cells, respectively.[45,46] Further, it was observed that the location factor is higher for the hydrodynamic cavitation reactor than for the other two, confirming that the mechanism of cell disruption in this case is by impingement/grinding action on the cell wall due to the fluid shear, as discussed earlier. For the ADH enzyme, the location factor value was around 0.5, confirming that ADH is present mostly in the cytoplasmic space of the cell.[45,47] Heating the cell suspension as a pre-treatment strategy for translocation of the enzyme was found to be effective for the ADH enzyme.[48] Even the time of culture in the fermentation process was found to affect the location factor of the enzyme. For studies with *E. coli* cells, longer culturing times resulted in periplasmic location of penicillin acylase whereas short culturing times resulted in cytoplasmic location of the enzyme. The concept of location factor is very useful for identifying the suitability of disruption equipment for differential product release.

Balasundaram and Harrison[49] investigated the effect of process variables including cavitation number, initial cell concentration of the suspension and the number of passes across the cavitation zone on the release of enzymes from various locations in Brewers' yeast. The release profile of the enzymes studied include β-glucosidase (periplasmic), invertase (cell wall bound), alcohol dehydrogenase (ADH; cytoplasmic) and glucose-6-phosphate dehydrogenase (G6PDH; cytoplasmic). An optimum cavitation number C_v of 0.13 for maximum disruption was observed across the range of C_v from 0.09 to 0.99. The optimum cell concentration was found to be 0.5% (w/v, wet wt) when varied over the range 0.1–5%. The sustained effect of cavitation on the yeast cell wall when re-circulating the suspension across the cavitation zone was found to release the cell wall bound enzyme invertase (86%) to a greater extent than enzymes from other locations of the cell (e.g. periplasmic β-glucosidase at 17%). Localized damage to the cell wall could be observed using transmission electron microscopy (TEM) of cells subjected to less intense cavitation conditions. Absence of the release of cytoplasmic enzymes to a significant extent, absence of micronization as observed by TEM and presence of a lower number of protein bands in the culture supernatant on SDS-PAGE analysis following hydrodynamic cavitation compared to disruption by high-pressure homogenization confirmed the selective enzyme release offered by hydrodynamic cavitation. Thus, controlling the intensity of cavitation phenomena in the same cavitational element or using different cavitational elements can result in the selective release of enzymes from different locations in the cell. Some pretreatment strategies can be used for modification of the location of the enzyme in the cell before the cell suspension is subjected to cell disruption. Translocation of enzymes by a pretreatment step can be exploited to improve the efficacy of cell disruption because most of the target product is usually produced in the cytoplasm of the cells and cannot be obtained readily without spending a large amount of energy in completely mutilating the cell wall and the cytoplasm during disruption. More energy is required to recover the cytoplasmic enzymes than the periplasmic enzymes.[1] Thus, translocation of enzymes from cytoplasmic space to periplasmic space could result in a large saving in energy requirements. Not only can the energy requirements be reduced, the problem of production of fines observed in conventional cell disruption devices is substantially reduced as the extent of cell breakage required for recovery of all the intracellular enzymes is also reduced due to the selective translocation of the enzymes to outer cell wall locations.

Some possible pretreatment strategies that can be used to intensify the release of enzymes is now discussed. The studies described used ultrasound-induced cavitation but should be equally applicable to hydrodynamic cavitation reactors.

Farkade *et al.*[50] reported that heat stress was found to induce the translocation of the target enzyme (β-galactosidase) and also other proteins, although their translocation rates were found to be different. The location factor varied between 0.4 and 2 depending on the time of heat treatment and temperature of the treatment. Heat treatment also resulted in the formation of insoluble protein aggregates, which could be removed by a centrifugation step, thereby reducing the total protein release in the suspension during cell disruption by sonication, thus increasing the specific enzyme activity. The translocation rate was found to be different at different treatment temperatures. It increased with increase in time and temperature of the treatment. However, treatment at temperatures above 50 °C results in substantial deactivation of the target enzyme. Heat treatment can therefore be a good pre-treatment step to improve the energy efficiency of the mechanical cell disintegration step but requires a heat stable target product or an optimum pre-treatment temperature needs to be established. The results of Farkade *et al.*[50] clearly demonstrated that by using an optimum heat treatment, cell disintegration can be considerably improved in terms of its energy efficiency.

Farkade *et al.*[51] investigated the release of intracellular β-galactosidase by ultrasonic disruption of cells treated with aqueous solutions of different pH as a pre-treatment step. Pretreatment of the cells at different pH levels for various time intervals was investigated

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with the aim of optimizing the pretreatment step for more efficient release of target enzyme, i.e. β-galactosidase. The maximum yield ($95 \pm 3\,U\,mL^{-1}$) was obtained by pretreatment of the cells at pH 4.4 for 6 h and subsequent ultrasonic cell disruption for 40 min. The maximum yield on ultrasonic disruption without pretreatment was $7.2 \pm 0.6\,U\,mL^{-1}$. The energy efficiency of the disruption process for releasing β-galactosidase using different pre-treatments has been calculated and compared. It was observed that the energy efficiency increased more than 19-times for cells pretreated at pH 4.4 for 6 h compared with the untreated cells.

Anand *et al*.[52] reported that the combination of chemical treatment and high pressure homogenization (under the operating conditions it is not clear whether cavitating conditions are present but the efficacy of chemical treatment would be equally applicable to cavitational reactors) can increase the release of intracellular components and decrease the exposure to mechanical disruption required for breakage to attain maximum intracellular release. The energy requirement of microbial cell disruption can be decreased along with a reduction in the micronization of cell debris. Pretreatments to permeabilize or weaken the cell envelope were selected and the optimum conditions determined through a screening process. The permeabilization of *Escherichia coli* with EDTA was successful in achieving maximum intracellular protein release at a lower pressure of 13.8 MPa with high pressure homogenization, compared with 34.5 MPa in the absence of EDTA. Significant reduction in energy input required was observed with the use of this combination method. Pretreatment with guanidium hydrochloride (G-HCl) and Triton X-100 also resulted in increased intracellular release and decreased energy use. Chemical pretreatment can be useful in enhancing mechanical disruption, however, careful selection of pretreatment conditions is required to avoid protein deactivation and chemical interference in the protein assay.

Overall, it can be said that use of cavitational reactors, in particular hydrodynamic cavitation reactors, for cell disruption has been conclusively proven at pilot scale to give much higher energy efficiencies than conventional techniques used currently. A particular reactor configuration in terms of geometry of the cavitation chamber and operating parameters such as inlet pressure, circulation flow rate can be chosen based on the location of the specific enzymes in the cells and cell concentration in the medium. Pre-treatment strategies such as heat, pH and chemical treatments can enhance the selectivity of the release of the target enzyme and at the same time significantly decrease energy requirements.

EFFORT NEEDED IN THE FUTURE

It should be noted that, in spite of the extensive research at laboratory scale and the immense industrial potential, the application of cavitational reactors at an industrial scale is still a daunting task. The main problems for efficient scale-up and operation include lack of design-related information and well-established methodology for scale-up. Also, there is the inherent limitation that high concentration (>5%) cell suspensions do not allow an adequate level of cell disruption. Efforts are required in the following directions so as to realize the dream of applying cavitational reactors in industrial practice.

1. Detailed theoretical analysis of the bubble dynamics phenomena under different operating conditions is essential to predict the cavitational intensity and its effect on the rates of physical or chemical processing applications.
2. Design and fabrication of different types of cavitational reactors differing in flow field, turbulence characteristics and geometry to study the effect of these on cavity/bubble/cluster dynamics and hence on the cavitational activity.
3. In the case of hydrodynamic cavitation reactors, realistic modelling of the turbulence phenomena which can then be used to model the cavity/bubble dynamics either in isolation or in the form of cavity clusters in high velocity flow. Modern sophisticated CFD codes can be employed to obtain flow field information, i.e. mean and fluctuating velocity components, Reynolds stresses, turbulent pressure fluctuations, which can then be used to understand the role of these flow field parameters in altering cavity dynamics.
4. Combination of hydrodynamic cavitation reactors and ultrasonic irradiation, in which the cavity is generated using hydrodynamic means and the collapse of the cavities takes place in the ultrasonic flow cell, needs to be tested for cell disruption operations with different enzyme locations.
5. The effect of process intensifying parameters such as the presence of dissolved salts and gases should be studied in detail at different operating scales with the aim of intensification of the cell disruption operation to minimize operating costs and to increase the applicability of cavitational reactors to high concentration cell masses.

CONCLUDING REMARKS

Cavitational reactors appear to be a promising alternative for cell disruption operations. For various cell disruption applications illustrated in the present work, the energy efficiency is much higher for hydrodynamic cavitation reactors than for their acoustic counterparts. Also, the scale-up of these reactors is comparatively easier as vast amounts of information about the fluid dynamics downstream of the constriction are readily available and the operating efficiency of the circulating pumps, which is the only energy dissipating device in the system, is always higher at large operating scales. Among the different

hydrodynamic cavitation reactors, the orifice plate type configuration appears to be most suitable as it offers tremendous flexibility for controlling the intensity of cavitation for desired applications so that considerable energy savings are possible. The optimization strategies on the basis of theoretical analysis reported earlier should serve as useful guideline to design engineers for selection of the optimum set of operating parameters and design configuration to achieve maximum benefits. Overall, it can be said that cavitation is a well established technology at laboratory/pilot scale and combined efforts of microbiologists, engineers and physicists are required to effectively harness this technology for cell disruption on an industrial scale of operation.

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