Ann Microbiol (2015) 65:1447–1456
DOI 10.1007/s13213-014-0983-z

ORIGINAL ARTICLE

Evaluating the effect of heat transfer on cell disruption in ultrasound processes

Talal Yusaf

Received: 29 May 2014 / Accepted: 11 September 2014 / Published online: 7 October 2014

Abstract Ultrasound is one of the mechanical methods used for disruption of microorganisms. Ultrasonic treatments of microorganisms are sensitive to a wide range of parameters such as net thermal power, residence time distribution (RTD), and the biological structure of the target microorganism. A commercial ultrasonic processor attached to a stainless steel processing cell was used in this research work. To evaluate the net heat dissipated in a small volume of the commercial yeast *Saccharomyces cerevisiae*, the suspension was subjected to 117 W at 20 kHz; the ultrasound cell was operated in a batch configuration with a Perspex base. Mixing of the yeast suspension and the RTD were evaluated using image processing techniques. The results of the present study showed that the heat lost through the stainless steel wall, Perspex base, and the Sonotrode (Titanium) was around 13.5 % of the total power. The yeast disruption results were found to be positive. The yeast disruption test showed that complete yeast reduction can be achieved at 117 W and a specific energy of 1,146 kJ kg^{-1}. Further study is needed to understand the real causes of microorganism disruption using ultrasound.

Keywords Heat dissipated · Yeast suspension · Ultrasound · Log reduction · Residence time distribution · Power

Introduction

The cytoplasm of the *Saccharomyces cerevisiae* yeast cell is a rich source of bio-products (proteins, cytoplasmic enzymes, polysaccharides) that are valuable for the food industry. For good recovery of these intracellular bio-products, efficient breakage of the cell walls is a necessary step (Liu et al. 2013). During the 1980s, researchers studied the effects of ultrasound in combination with other treatments for use in the food industry (Ordoñez et al. 1984; Garcia et al. 1989; Wrigley and Liorca 1992). Structural changes in the cell walls of corn straw and oil-palm fibers under intense mechanical treatment were studied by Bychkov et al. (2012). The destruction mechanism was shown to be dependent on the structure of the cell walls and the lignin content (Bychkov et al. 2012). Furthermore, the impact of low frequency ultrasound combined with an increase in temperature on the disruption of microorganisms in milk suspended in water has been investigated (Ciccolini et al. 1997). Different species of microorganisms exhibit different resistance to ultrasound. Large-sized microorganisms are generally more sensitive to ultrasound as the area directly in contact with ultrasound is larger. Coccal forms are more resistant than rod-shaped bacteria (Jacobs and Thornley 1954; Alliger 1978; Ahmed and Russell 1975). A number of factors influence cavitation intensity (Earnshaw et al. 1995): (1) liquid temperature, (2) frequency of the ultrasound, (3) amplitude of the ultrasound, and (4) viscosity of the liquid environment. Moreover, ultrasound can be used for other purposes such as emulsification. The preparation of emulsion fuel with and without fresh water microalgae *Chlorella vulgaris* (FWM-CV) cells was conducted using ultrasound to overcome the problems of large-sized microalgae colonies and to form homogenized emulsions. The emulsified water fuels, prepared using ultrasound, were found to be stable and the size of FWM-CV colonies were effectively reduced to pass through the engine nozzle safely (Allwayzy et al. 2014).

When ultrasonic sound waves pass through a medium, thermal effects can occur. However, it has been found that the thermal impact is insignificant in terms of producing a

T. Yusaf (✉)
National Centre for Engineering in Agriculture, Faculty of Health Engineering and Sciences, University of Southern Queensland, 4350 Toowoomba, Australia
e-mail: yusaft@usq.edu.au

temperature rise in biological systems when using an ultrasonic frequency of 26 kHz at various levels of intensity (Scherba et al. 1991). According to research conducted by Scherba et al. (1991), the maximum rate of temperature rise due to ultrasound is too small to inflict significant harm on the biological systems of the microorganism. Therefore, ultrasonically induced thermal effects are not responsible for altering biological systems of microorganisms under the exposure conditions used. Sanz et al. (1985) successfully used an ultrasound device that can generate a power of 120 W at 20 kHz. In general, the level of cell damage is higher when the power is higher. It is known that the velocity and pressure of any particle is increased when power is increased (Chambers and Gaines 1932). In addition, Chambers and Gaines (1932) stated that if the power provided by the oscillator is high enough, cavitation will occur even in the absence of gas. They also pointed out that cavitation does not occur throughout the entire cell sample (i.e. it is confined to restricted regions), and occurs only in the region adjacent to the free ends of the ultrasound probe. Gao et al. (2014a) studied high-frequency (850 kHz) ultrasound to investigate the inactivation of bacteria and yeast at different growth phases under controlled temperature conditions. On one hand, the study showed that high-frequency ultrasound is highly efficient in inactivating bacteria, with more than 99 % inactivation achieved. The mechanism of bacterial inactivation is due mainly to acoustic cavitation generating free radicals and H_2O_2. On the other hand, the yeast *Aureobasidium pullulans* was found to be more resistant to high-frequency ultrasound treatment (Gao et al. 2014a).

A study by Liu et al. (2013) investigated the disruption and protein releasing kinetics of *Saccharomyces cerevisiae* cells. An ultrasonic technique (probe system) was used and the following parameters were considered: acoustic power, and duty cycle of the sonicator. The cell disruption efficiency was evaluated by measurement of electrical conductivity, UV-spectroscopy and cell size. It was found that by increasing acoustic power and duty cycle, a higher degree of cell disruption was observed. Also, different sonication systems have been compared, with a bath-type sonicator being less effective for yeast cell disruption and protein release compared to a horn-type sonicator (Liu et al. 2013).

Koda et al. (2009) used an ultrasound power of 12.8 W at a frequency of 20 kHz for 15 mins to treat 50 cm^3 water contaminated with a long reduction of *Streptococcus mutans* and reported a 97 % reduction in the microorganisms. The results of Koda et al. (2009) indicate that ultrasonic waves do not completely destroy the cells but damage some of them by increasing the cells' sensitivity to heat. The optimum ultrasonic power for maximum deactivating effect was found to be around 100 W. Microorganisms become more sensitive to heat treatment if they have already undergone an ultrasonic treatment (Garcia et al. 1989; Ordoñez et al. 1987). The combination of heat and ultrasonic treatments is called "thermo-sonication" (Earnshaw et al. 1995). Ordoñez et al. (1987) studied the effect of thermo-sonication on the survival of a strain of *Staphylococcus aureus* in a phosphate buffer. Garcia et al. (1989) found that thermo-sonication (5 W/mL) was not significantly effective in killing spores of *Bacillus subtilis* in water at temperatures close to boiling point (100 °C), with the low effectiveness being attributed to a decrease in the violence of bubble collapse due to the higher vapour pressure acting like a cushion (Garcia et al. 1989; Earnshaw et al. 1995). Zhang et al. (2014) used a 20 kHz high-intensity ultrasound for the selective release of polysaccharide and protein from yeast cells. The release of polysaccharide and protein was found to be affected by sonication time, temperature and ionic strength, of which temperature had the greatest influence. The release selectivity, which is the ratio of polysaccharide released to that of protein designated as T/P value, was investigated. It has been found that the T/P value at 85 °C was a factor of 9.3 of that at 25 °C. The underlying mechanism of this selectivity is speculated to be the thermal denaturation and aggregation of protein within yeast cells at elevated temperatures leading to the decrease of protein release by ultrasound (Zhang et al. 2014).

A theoretical model based on shear forces generated by the collapse of the ultrasound cavities near the surface of a microorganism was proposed by Gao et al. (2014b). This model considers two parameters: the number of acoustic cavitation bubbles, and the resistance of the cell wall of the microorganism to the shear forces generated by bubble collapse. A high-power, low frequency (20 kHz) ultrasound was used to inactivate many microorganisms including yeast. The results showed that the Log of the inactivation ratio decreases linearly with sonication time, and the rate of inactivation increases with the increase in sonication power (Gao et al. 2014b).

From the literature, it can be concluded that bacterial cells generally become more sensitive to heat treatment after being subjected to ultrasound treatment. Sequential or simultaneously applied ultrasonic and heat treatments result in the destruction of bacteria at much lower temperatures than would be required for heat treatment alone. Earnshaw et al. (1995) demonstrated that the elimination of bacteria can be improved by subjecting them to a combination of ultrasonic and heat treatments compared with bacteria that are subjected only to ultrasonic treatment. The conclusions of Raso et al. (1994) were consistent with those of Earnshaw et al. (1995). Garcia et al. (1989) reported a 43 % reduction in the heat resistance of *Bacillus subtilis* when it was subjected to ultrasonication in hot water at temperatures from 70 °C to 95 °C. When the temperature of a liquid exceeds the boiling point, a loss in the cavitation effect takes place due to the high vapour pressure (Garcia et al. 1989). In order to overcome this problem, pressure is often applied to thermo-sonication. This kind of combination treatment is known as Mano-thermo-

sonication (MTS). For the MTS technique, cavitation can be generated using ultrasound despite the high temperature of the liquid (Ahmed and Russell 1975). Apart from thermosonication and MTS treatment, there are other combinations that have proven effective against microorganisms, such as ultrasound combined with pH or chemical control. There is a lack of understanding regarding the actual reasons for microorganism disruption using ultrasound and whether it is caused by shock or shear. A study by Yusaf (2013) showed that the shear apparatus can efficiently and effectively disrupt *Saccharomyces cerevisiae* at different treatment times, suspension temperatures and rotor speeds. Experimental work suggests that maximum yeast log reduction was achieved when the maximum power dissipation of 2.095 kW was recorded at 10,000 rpm, while suspension temperature was controlled below 35 °C. The corresponding shear stress at 10,000 rpm was 2,586.2 Pa (Yusaf 2013).

High power ultrasound can generate high temperatures and localised pressure. The high pressures generated are thought to be responsible for cell disruption (Sanz et al. 1985; Yusaf 2011) and the high temperatures are formed during cavitation bubble collapse, which have some effect on the suspension treatment process. Other researchers contend that the combination of pressure and temperature generated by high power ultrasound contribute to the death of the microorganism (Balachandran et al. 2006). Evaluating the net ultrasonic energy dissipated into the suspension and the heat loss to the surrounding area through the chamber, probe, and the chamber base is very important in this work. The aim of this work was to assess the overall heat loss through the walls of the container when ultrasonic power was applied. This was achieved by measuring the convection heat transfer at the surface of the processing cell. It was anticipated that quantification of the heat transfer associated with ultrasonic processing would enable future experimental results on microorganism disruption to be reported with greater clarity. These results would also contribute to an accurate assessment of the economic viability of any future proposed ultrasonic treatment processes. The research work presented in this paper aims to cover the following:

1. Theoretical and experimental work to conveniently evaluate heat transfer through the ultrasound chamber wall, Perspex and the ultrasound probe surface. Simplicity in determining energy-related matters in ultrasound applications is an important aspect and has been one of the demands in both research and industrial fields. For example, Kimura et al. (1996) pinpointed the necessity of finding easy and convenient methods for periodic measurements of ultrasonic energy in all reaction systems.
2. The ultrasound experimental apparatus based on the results obtained from the heat transfer and mixing section. This section will cover the log yeast reduction due to ultrasound treatment and discussion.

Materials and methods

Experimental apparatus

The ultrasonic treatment apparatus consisted of a commercial ultrasonic processor (Hielscher, Teltow, Germany, type: UIP500) attached to a 316 stainless steel processing cell (Fig. 1). The cell was operated in a batch configuration with a Perspex base that did not have a flow port. Perspex is a synthetic polymer of methyl methacrylate, is a transparent thermoplastic that can be used as a lightweight or shatter-resistant alternative to glass. The ultrasound generator can provide electrical oscillations of 400 W to power an ultrasonic device with a frequency of 20 kHz. The ultrasonic processor provided approximately 117 W of power (at 20 kHz) to a sample of approximately 4 mL water in the processing cell. Three thermocouples (type K) were located at various points around the processing cell as illustrated in Fig. 1. The most important thermocouples are the water temperature thermocouple (giving the value T_w) and the thermocouple located at the surface of the Perspex in contact with the water (giving the value T_{sp}). The thermocouple located on the lower surface of the Perspex (giving the value T_l) was used to indicate the time at which the heat transfer within the Perspex departed from the assumed semi-infinite process. Signals from the thermocouples were amplified using an integrated circuit with cold junction compensation (Analogue Devices, AD595, http://www.analog.com) and the temperature signals (voltages) were recorded at 20 samples/s using an A/D card and Lab-View software (http://www.ni.com/labview/). In this work there was a need for adequate cooling arrangements to reduce the temperature of the liquid to ensure that the microorganism would be inactivated by ultrasound without thermal influences.

Ultrasonic device

The ultrasound machine consists of a generator, transducer and a sonotrode with the generator providing an electrical oscillation of 400 W to power an ultrasonic device with a frequency of 20 kHz, which is transferred to the transducer. The sonotrode was immersed in a suspension of the commercial yeast *Saccharomyces cerevisiae*. The ultrasound device generates heat, which is dissipated into the suspension. Results obtained from the heat transfer experiment will be used to evaluate the correct net power dissipated into the suspension. A temperature control was also included in the present configuration so that there would be no thermal effect

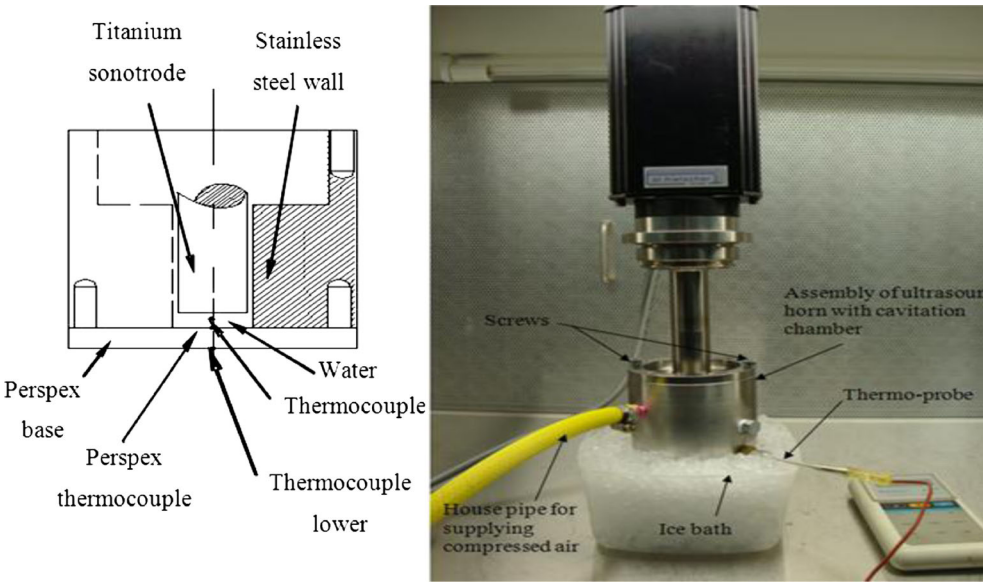

Fig. 1 Illustration of the ultrasonic processing cell for heat transfer experiments

on the microorganism's disruption. As a result an ice bath configuration was added to the original design to maintain a sufficiently low suspension temperature in order to avoid thermal disruption of the microorganism.

Yeast preparation and test procedure

Saccharomyces cerevisiae was chosen as the test yeast species as it is easy to grow, has well established mechanical properties, and the cells are large enough for counting, readily available and inexpensive. The lysogeny broth (LB) was prepared using 5 g yeast extract with 10 g NaCl and 1 g glucose per 1 L solution. *Saccharomyces cerevisiae* was grown in a shaker incubator at 25 °C for 24 h and then yeast suspensions at different concentrations were subjected to around 117 W ultrasound power. With each test, an untreated sample was also reserved to provide an accurate means of comparing different tests with different test conditions. The number of yeast cells as colony forming units (CFU)/mL was determined before and after each treatment using a viable count standard procedure.

Experimental work

The work can be divided into three major sections: (1) heat transfer through the ultrasound chamber, (2) suspension mixing time and residence time distribution (RTD), and (3) yeast disruption using ultrasound. The second part of this work was covered thoroughly by our previous paper (Yusaf and Buttsworth 2007).

Heat loss through the ultrasound chamber (steel, perspex and titanium)

Measurements provided by the three thermocouples over a period of 5 min (300 s) are presented in Fig. 2. Time 0 in Fig. 2 corresponds to the point at which the ultrasonic processor was switched on. The temperature differences relative to the initial (pre-run) level are presented here also. Measurement of differences in temperature are necessary in transient heat flux analysis. The initial temperatures indicated by each thermocouple were: T_w=15 °C, T_{ps}=17 °C, and T_l=18 °C. Two relatively large disturbances appeared on the signal from the water temperature thermocouple—the first at about 15 s and the second at around 140 s on the time scale in Fig. 2. The second of these disturbances has been removed from the signal and hence the data appears smooth in this region. These disturbances were attributed to thermocouple sensitivity from the ultrasonic treatment (causing gaseous bubbles at the suspension surface); however, this did not affect the results of this test because only average water temperatures were required.

Heat loss through the Perspex surface Provided the substrate into which heat is transferred can be regarded as semi-infinite, the surface heat flux can be identified from the surface temperature (Carslaw and Jaeger 1959). In the case of a flat surface without any lateral conduction effects, Schultz (1973) demonstrates that the appropriate expression is:

$$q = \frac{\sqrt{\rho c k}}{\sqrt{\pi}} \int_0^t \frac{dT_s}{d\tau} \frac{1}{\sqrt{(t-\tau)}} d\tau, \quad (1)$$

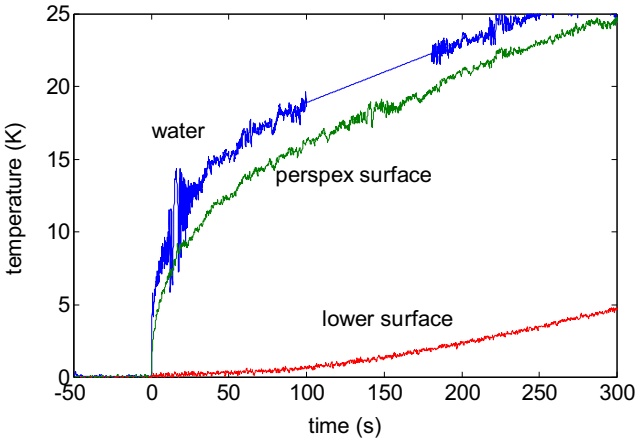

Fig. 2 Temperature measurements from thermocouples in the heat transfer experiment of water, and the upper and lower Perspex surface

A numerical implementation of Eq. 1 was used to identify the heat flux (q) to the surface of the Perspex from the T_s results (in Fig. 2) for a certain period of time (τ). The Perspex thermal properties of density, specific heat and conductivity ρ, c, and k, respectively, are presented in Table 1. Assuming the calculated value of heat flux applies across the entire Perspex surface that was exposed to the water (531 mm^2), the heat transfer to the Perspex surface was around 0.4 W.

From Fig. 2, it is apparent that a measurable increase in temperature at the lower surface of the Perspex occurs approximately 1 min after heating begins. This is to be expected since the thickness of the Perspex was x=12.7 mm and the thermal diffusivity of Perspex (Table 1) was $\alpha=0.11\times10^{-6}$ m^{-2} s^{-1}, giving the heat penetration time [20] being:

$$t=\frac{x^2}{16\alpha}=92\text{ s}. \quad (2)$$

Thus, approximately semi-infinite conditions persist for about 100 s after the heating started (the time at which the ultrasonic processor was switched on). Provided the induced flow and thermal transport conditions within the processing cell remain constant during the experiment, the surface heat flux should be proportional to the difference in temperature between the water and the surface,

$$q=h(T_w-T_s), \quad (3)$$

where h is the convective heat transfer coefficient.

The Perspex heat flux results from Eq. 3 were used in conjunction with the water and Perspex surface temperature measurements to estimate the heat transfer coefficient. Results from this section are presented in Fig. 3. The convective heat transfer coefficient data, prior to the start of the ultrasonic processor, is insignificant and has not been included here.

Table 1 Properties of materials used in the processing cell construction

Material	ρ (kg m^{-3})	c (J kg^{-1} K^{-1})	k (W mK^{-1})	α (10^{-6} m^{-2} s^{-1})
Perspex	1,200	1,450	0.2	0.11
Stainless steel	8,300	470	13	3.3
Titanium	4,500	520	22	9.4

From Fig. 3, it can be observed that the apparent heat transfer coefficient is not exactly constant, but decreases steadily from a value of approximately 500 W m^{-2} K^{-1} at the start of heating to approximately 300 W m^{-2} K^{-1} at a time of 100 s after the start of heating. In this experiment, the water temperature changed by around 18 °C in the first 100 s. The associated changes in viscosity and thermal conductivity would be around 30 % and 5 %, respectively, and thus some variation in the heat transfer coefficient would be expected. Another effect that may contribute to the apparent variation in the heat transfer coefficient is the fact that the ultrasonic processor may actually require a few minutes to reach a steady operating condition. Another factor that may contribute to the apparent variation in heat transfer coefficient with time is lateral conduction. Such effects have been assumed to be negligible.

Stainless steel surface

The relationship between the heat flux and the measured surface temperature was evaluated using Eq. 4 (Buttsworth and Jones 1997),

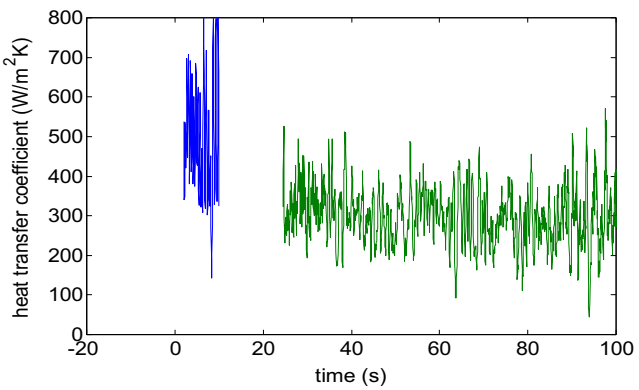

Fig. 3 Heat transfer coefficient at the Perspex surface from the heat transfer experiment. *Green line* Heat flux at the start of the experiment, *blue line* heat flux at the middle and end of the experiment

$$q = \frac{\sqrt{\rho ck}}{\sqrt{\pi}} \int_0^t \frac{dT_s}{d\tau} \frac{1}{\sqrt{(t-\tau)}} d\tau \quad + \frac{k}{2R}(T_s - T_i), \tag{4}$$

Taking the Laplace transformation of Eq. 4 and assuming that the convective heat transfer coefficient is constant, the Laplace transformation can be used to evaluate the surface temperature:

$$\overline{T_s} = G(s)\overline{T_w}, \tag{5}$$

with the transfer function between the water temperature and the surface temperature given by

$$G(s) = \frac{h}{\sqrt{\rho ck}} \frac{1}{\sqrt{s} + a}, \tag{6}$$

where,

$$a = \frac{k + 2Rh}{2R\sqrt{\rho ck}} \tag{7}$$

The inverse Laplace transformation of Eq. 6 is

$$g(t) = \frac{h}{\sqrt{\rho\, ck}} \left(\frac{1}{\sqrt{\pi\, t}} - a e^{a^2 t} \operatorname{erfc}\left(a\sqrt{t}\right) \right). \tag{8}$$

The surface temperature history can therefore be obtained from Eq. 5 using the convolution integral,

$$T_s = \int_0^t g(\tau) T_w(t-\tau) d\tau. \tag{9}$$

No thermocouple was placed on the stainless steel surface; however, the surface temperature history can be estimated using Eq. 9 if the heat transfer coefficient on the stainless steel is assumed to be constant and equal to the heat transfer coefficient measured at the surface of the Perspex. Since the system reached its steady state in a very short time, it is reasonable to assume the heat transfer coefficient was constant and equal to the maximum. The maximum heat transfer coefficient (500 W m^{-2} K^{-1}) was selected in the calculation. The constant value adopted for the convective heat transfer coefficient was h=500 W m^{-2} K^{-1}. This assumption is reasonable and acceptable as shown in Fig. 4. Having estimated the stainless steel surface temperature history (Fig. 2), the surface heat flux can be calculated using Eq. 1. Heat transfer to the stainless steel as determined with this method was 11 W, as shown in Fig. 5. As was the case with the Perspex results in Fig. 3, the heat flux results (expressed in W m^{-2}) have been scaled by the relevant surface area (1,633 mm^2 in this case) for presentation in Fig. 4. Limitations of the above analysis include the approximate nature of Eq. 3, which produces results within 1 % of the actual solution for heating times such that $(\alpha\, t)/R^2 \approx 0.1$, according to Buttsworth and Jones (1997).

Heat lost through Titanium surface (sonotrode tip)

Heat transfer to the titanium surface can be estimated using the analysis outlined in the previous section. Slight adjustments to this analysis would need to be made to accommodate the flat surface of the titanium ($R \to \infty$), which has significantly different thermal properties to the stainless steel (see Table 1). When this is complete, the resulting heat transfer across an area of 380 mm^2 (the area of the sonotrode) is obtained as presented in Fig. 6.

Due to the transient nature of the present experiments, the heat transfer to the surfaces of the processing cell tends to vary with time. The proposed heat transfer model in this work is intended to capture convective heat losses from the reaction vessel during the first few seconds of the ultrasound run, as this period of time is valid for the semi-infinite assumption and is of importance to the calorimetric measurements which are usually conducted in such a period (Kimura et al. 1996; Hodnett and Zeqiri 1997). Hence, to obtain some indication of relative magnitudes, the time 100 s after the start of the ultrasonic processor is considered. As shown in Fig. 6, the average heat lost to the titanium surface was around 2.1 W.

Although the lateral conduction heat loss across the processing cell walls after 100 s is not important for ultrasonic power measurements, it would be useful to calculate this portion of heat loss using convenient methods. Due to the low thermal diffusivity of Perspex, the lateral heat conduction through Perspex is marginal and can be ignored. The lateral heat conduction through the steel wall after 100 s can be estimated as follows:

The energy balance of the steel wall is expressed by the following equation (Kreith et al. 2010)

$$E_{in} = E_{out} + E_{accum} \tag{10}$$

Where E_{in} is the energy entering the wall, E_{out} is the energy leaving the wall and E_{accum} is the accumulated heat energy in the wall. The difference between the energy entering the cylindrical wall and the energy leaving the wall corresponds to the energy associated with the lateral conduction, and the equation above can be rewritten in the form below;

$$-KA\frac{dT}{dx} = mC_p\frac{dT}{dt} \tag{11}$$

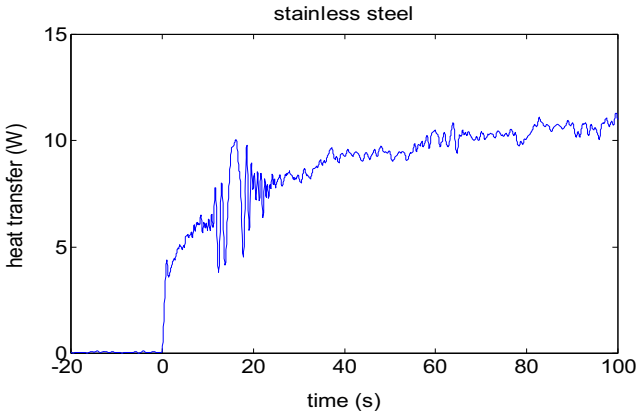

Fig. 4 Heat transfer to the stainless steel deduced from the heat transfer experiment

where, K is thermal conductivity of the steel (W/mK), A is the surface area of the steel cylinder (m^2) and dT/dx is the temperature rise across (x) the thickness of the cylinder (K/m), m is the mass of the cylinder wall (3 kg), Cp is the specific heat of the steel (given in Table 1) and dT/dt is the slope of the temperature rise of the steel wall after 100 s (K/s). The temperature rise inside the steel wall was determined by averaging the inner steel wall temperature (estimated from Eq. 9) and the outer steel wall temperature (average of inner wall temperature and ambient temperature). This means that when the heat loss from the outer wall of the cylinder is negligible, the lateral heat conduction across the cylinder wall is the main cause of the change in the internal energy of the construction materials of the wall. From the equations given above, the lateral conduction heat loss was calculated to be 20.68 W.

Mixing rate and RTD

To adequately assess the potential of high power ultrasound in the present application, it was necessary to have some

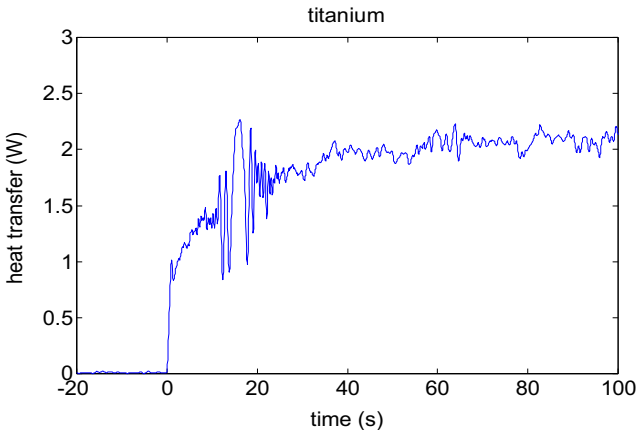

Fig. 5 Heat transfer to the titanium deduced from the heat transfer experiment

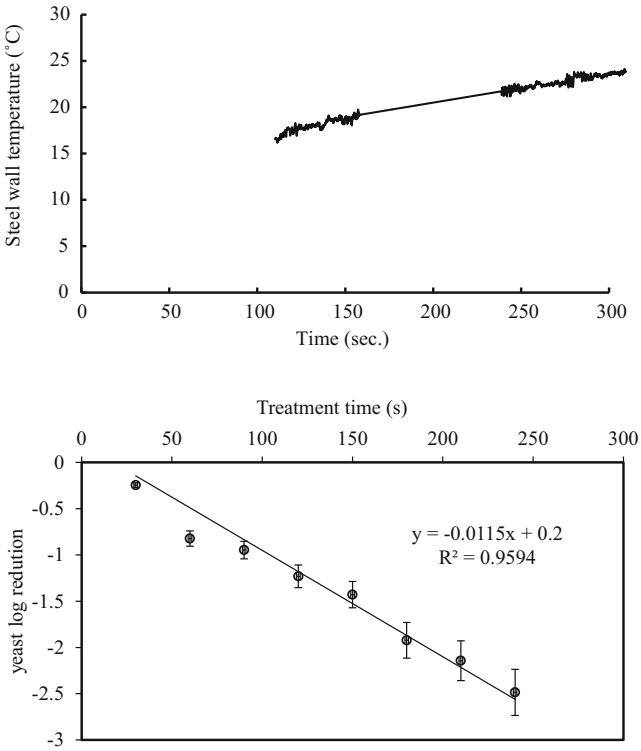

Fig. 6 The effect of treatment time on yeast log reduction at an amplitude of 20 (74 W), at a suspension temperature of between 30 °C and 40 °C

knowledge of the uniformity of the treatment. To achieve uniform ultrasound treatment for yeast in the suspension, a theoretical and experimental mixing investigation was conducted. Evaluating the mixing time is a very important factor to ensure that the yeast cells in the suspension are subjected to uniform ultrasound energy. A new technique for the identification of mixing characteristics associated with high power ultrasonic treatments at 20 kHz has been detailed in Yusaf and Buttsworth (2007). The mixing rate within the batch arrangement (30 mL) is a function of the applied ultrasonic power; however, the macroscopic mixing is substantially completed within 1 s for absorbed ultrasonic power levels greater than 40 W (thermal dissipation). A detailed description of this work was published elsewhere (Yusaf and Buttsworth 2007).

Yeast disruption results

An initial test was conducted at an amplitude of 20 (ultrasound power of 74 W) for different treatment times ranging between 0 (no treatment) to 300 s. Due to several factors relating to the experimental conditions such as suspension temperature and the way in which yeasts were counted, the test was repeated three times to ensure the reliability of the results. The initial concentration of the suspension prior to the treatment was 6.3×10^4 CFU/mL, 1.53×10^6 CFU/mL, and 2.17×10^7 CFU/mL for a period of 30 s at an ultrasound power of 74 W. The

suspension temperature was closely monitored and recorded over time. The results of this test indicated that a yeast log reduction of 0.25 was achieved at a treatment time of 30 s as shown in Fig. 6, while the maximum suspension temperature was around 40 °C. The treatment time was then increased to 60 s at the same ultrasound power with the yeast log reduction found to be 0.8, confirming that some improvement was achieved. The treatment time was then increased gradually to 240 s with results showing that the maximum yeast log reduction of 2.5 was achieved when the treatment time was 240 s and the suspension temperature was 45 °C. The results confirmed that the ultrasound apparatus was able to achieve a yeast log reduction of 2.5 at 240 s at 74 W, 20 kHz, and suspension temperatures between 30 °C to 40 °C.

Further tests were conducted using different amplitudes and treatment times while the suspension temperature was monitored carefully and controlled below 35 °C. In this test, the ultrasound chamber was submerged in an ice bath to ensure that yeast disruption occurred in the absence of thermal effects. Three amplitudes of 20, 70 and 100 were used in this test, and these amplitudes correspond to the ultrasound powers of 74 W, 104 W and 117 W, respectively. The treatment time ranged from 240 s and 900 s. Table 2 illustrates the yeast log reduction results for different treatment times and different amplitudes. As shown in Table 2, higher yeast log reduction occurred at higher treatment times. A yeast log reduction of 4 was achieved when the treatment time was around 365 s at the ultrasound power of 117 W (amplitude=100). A yeast log reduction of 2.5 was achieved at a treatment time of 292 s and an ultrasound power of 117 W. The results of this work also show that a complete yeast reduction can be achieved at a maximum ultrasound power of 117 W at a specific energy of 1,150 kJ kg^{-1}, or neat dissipated energy of 103 W at neat specific energy of 1,146 kJ kg^{-1}.

Table 2 Summary of the yeast log reduction results using different amplitude, ultrasound power and treatment time, suspension temperature was controlled to around 30–40 °C

Amplitude	Power (W)	Neat power (W)	Log reduction 2.5 Treatment time (s)	Log reduction 4 Treatment time (s)
20	74	60.5	540	900
70	104	90.5	360	600
100	117	103.5	292	365

Discussion and analysis

Heat transfer

The heat transfer to the Perspex, stainless steel and titanium surfaces was found to be approximately 0.4, 11, and 2.1 W, respectively, giving the combined heat transfer from the water at 13.5 W of the applied ultrasonic power. Estimates of the current configuration suggested that around 13.5 % of the applied ultrasonic power was removed from the processing volume in the form of heat. Such heat transfer can have a significant impact on efficiency calculations for the ultrasonic processor based on calorimetric experiments in this and related configurations. Evaluation of this lost power had to be considered in the subsequent experimental phase of this research where the net ultrasound power dissipated in the suspension. One of the limitations of the present data and its analysis is that the heat transfer coefficient appears to vary with time; however, the variation was not significant and the impact on the final heat transfer was marginal. The modelling deficiencies, such as the semi-infinite one dimensional heat conduction assumption, may also contribute to the apparent variation in time.

Mixing quality in ultrasound processing

As indicated in the previous section, an image process technique was used to identify the mixing characteristics associated with high power ultrasonic treatment at different powers. It was found that for a batch arrangement of 30 mL, homogeneity was achieved in approximately 1 s (Yusaf and Buttsworth 2007).

Yeast disruption

Ultrasound can create cavitation in the yeast suspension. If the ultrasound power is sufficiently high, the cavitation bubbles will expand until they reach a critical radius at which they collapse. Collapse of the cavitation bubbles releases energy into the suspension, which is presumably the reason for microorganism disruption. The relationship between yeast disruption and the specific energy dissipated into the yeast suspension is an important factor in this type of research. The yeast disruption results showed that heat transfer will have a significant impact on efficiency calculations for the ultrasonic processor. Although the subsequent disruption experiments were performed in an ice bath, the heat transfer must be determined to make sure that the cell wall temperature and the yeast suspension temperature is maintained below 40 °C to avoid yeast disruption due to thermal stress. The material properties of the ultrasound chamber had no effect on cell disruption in this work.

The specific energy is the energy dissipated into the suspension per unit mass, where the mass of suspension was 0.03 kg, and the power measured in Watts ranged between 74 and 117 W. In the case of ultrasound treatment; depending on the treatment time, the neat specific energy dissipated into the suspension was calculated and found to be in the range of 298 kJ kg^{-1} to 1,146 kJ kg^{-1}. Results presented in Fig. 7 show

that the approximate maximum yeast log reduction was 4 when the specific energy was 1,128 kJ kg^{-1}

The power intensity of ultrasound is measured in Watts per unit area (cross sectional area of the ultrasound probe), which is 238 kW/m^2 in this work. In a similar study, an ultrasound yeast treatment was carried out at 100 W (203 kW/m^2) for at a suspension temperature of between 50 °C and 60 °C. The effect of using 203 kW/m^2 ultrasound power intensity (on a 60 mL suspension) led to a yeast disruption of log 2, but disruption was less marked at 50 °C (Ciccolini et al. 1997). Comparing this result with the present results, a yeast log reduction of 2 was achieved at a specific energy of around 700 kJ kg^{-1} or a power intensity of 238 kW/m^2 (when the suspension volume was 30 mL) as shown in Fig. 7. The results of the present work were achieved when the yeast suspension temperature was around 40 °C. Taking into consideration the temperature difference between the present and previous works, the specific energy required to achieve yeast log reduction of 2 was close.

Earnshaw et al. (1995) demonstrated that using ultrasound specific power of 1.65 kW/kg resulted in no yeast disruption as no cavitation was expected to be developed, and therefore the log reduction was zero. In Earnshaw's experiment, the cross sectional area of the probe diameter was 25 mm, resulting in an ultrasound power intensity of about 101.8 kW/m^2 when 50 W was used. It was reported that the 101.8 kW/m^2 power intensity was just enough to generate a very small number of bubbles, but these were not able to produce significant energy upon collapse (as reported by Earnshaw et al. 1995). These cavitation bubbles were reported to be insufficient to even weaken the yeast (Earnshaw et al. 1995). Similarly, in the present work, the yeast log reduction at approximately 50 W was insignificant.

Hodnett and Zeqiri (1997) and Cameron et al. (2008), conducted an experiment to examine the effect of ultrasound power and treatment time on *Saccharomyces cerevisiae*. A 40-mL yeast suspension was subjected to 100 W ultrasonic power with a probe of diameter of 13 mm, giving a power intensity of 744 kW/m^2. Both works used the same ultrasound power of approximately 100 W, but in the work produced by Cameron et al. (2008), the possibility of generating cavitation and ultimately microorganism disruption was higher due to the increased power intensity. Ultrasound power intensity influences the effectiveness of the treatment. The ultrasound probe diameter is another factor that affects power intensity, leading to an influence on the cell rupturing efficiency under constant power. For example, a probe with a diameter of 15 mm can be more effective in cell damage in comparison to a probe of 25 mm diameter because the probability of generating cavitation is higher due to the increased power intensity. This outcome demonstrates that cavitational effects are more significant closer to the vibrating surface (Cameron et al. 2008).

The results generated from the experimental ultrasonic work indicated that ultrasonic treatment is capable of destroying yeast over different treatment times and amplitudes. The results of this work demonstrate that a complete yeast reduction can be achieved at maximum power when the probe is very close to the suspension surface.

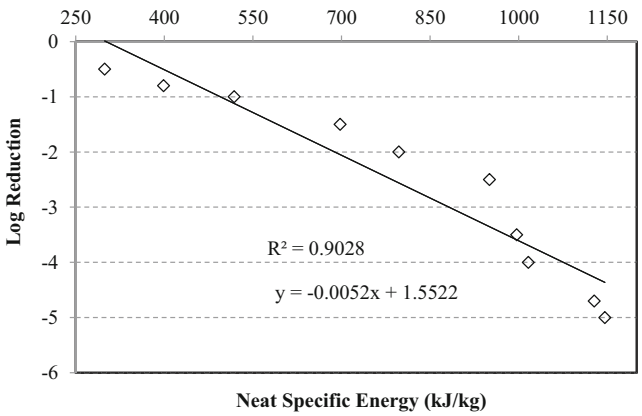

Fig. 7 Changes in yeast log reduction against the specific energy using ultrasound treatment

Conclusion

The heat losses associated with the ultrasound components—steel chamber, Perspex base and sonotrode (titanium)—were evaluated experimentally using a transient one-dimensional heat conduction model. The results obtained using this model indicated that heat losses through the Perspex, stainless steel, and titanium surfaces are approximately 0.4, 11, and 2.1 W, respectively. The combined heat lost through solid surfaces was approximately 13.5 W of the total input of applied ultrasonic power of 117 W. The maximum electrical power input was found to be 117 W and the minimum power, 74 W. Three different power inputs with different exposure times were investigated to determine disruption of *S. cerevisiae* when subjected to ultrasound. The results showed that the concentration of yeast cells in the suspension decayed with time of exposure and that, by increasing the power input, the rate at which the cells are destroyed is similarly increased. The yeast disruption test showed that a complete yeast reduction can be achieved at 117 W and a specific energy of 1,146 kJ kg^{-1}. Further study is needed to understand the real cause of microorganism disruption using ultrasound.

Acknowledgement T.Y. would like to thank Professor David Buttsworth for his scientific advice at the early stage of this project.

References

Ahmed FIK, Russell C (1975) Synergism between ultrasonic waves and hydrogen peroxide in the killing of micro-organisms. J Appl Microbiol 39:31–40

Alliger H (1978) New methods in ultrasonic processing. Am Lab 10:81–87

Al-lwayzy SH, Yusaf T, Al-Juboori RA (2014) Biofuels from the fresh water microalgae *Chlorella vulgaris* (FWM-CV) for diesel engines. Energies 7:1829–1851

Balachandran S, Kentish SE, Mawson R, Ashokkumar M (2006) Ultrasonic enhancement of the supercritical extraction from ginger. Ultrason Sonochem 13:471–479

Buttsworth DR, Jones TV (1997) Radial conduction effects in transient heat transfer experiments. Aeronaut J 101:209–212

Bychkov AL, Ryabchikova EI, Koroleva KG, Lomovsky OI (2012) Ultrastructural changes of cell walls under intense mechanical treatment of selective plant raw material. Biomass Bioenergy 47:260–267

Cameron M, McMaster LD, Britz TJ (2008) Electron microscopic analysis of dairy microbes inactivated by ultrasound. Ultrason Sonochem 15:960–964

Carslaw HS, Jaeger JC (1959) Conduction of heat in solids, 2nd edn. Oxford University Press, New York

Chambers LA, Gaines N (1932) Some effects of intense audible sound on living organisms and cells. J Cell Comp Physiol 1:451–473

Ciccolini L, Taillandier P, Wilhem AM, Delmas H, Strehaiano P (1997) Low frequency thermoultrasonication of *Saccharomyces cerevisiae* suspensions: effect of temperature and of ultrasonic power. Chem Eng J 65:145–149

Earnshaw RG, Appleyard J, Hurst RM (1995) Understanding physical inactivation processes: combined preservation opportunities using heat, ultrasound and pressure. Int J Food Microbiol 28:197–219

Gao S, Lewis GD, Ashokkumar M, Hemar Y (2014a) Inactivation of microorganisms by low-frequency high-power ultrasound: 2. A simple model for the inactivation mechanism. Ultrason Sonochem 21(1):454–460

Gao S, Hemar Y, Ashokkumar M, Paturel S, Lewis GD (2014b) Inactivation of bacteria and yeast using high-frequency ultrasound treatment. Water Res 60:93–104

Garcia ML, Burgos J, Sanz B, Ordonez JA (1989) Effect of heat and ultrasonic waves on the survival of two strains of *Bacillus subtilis*. J Appl Bacteriol 67:619–628

Hodnett M, Zeqiri B (1997) A strategy for the development and standardisation of measurement methods for high power/cavitating ultrasonic fields: review of high power field measurement techniques. Ultrason Sonochem 4:273–288

Jacobs SE, Thornley MJ (1954) The lethal action of ultrasound waves on bacteria suspended in milk and other liquids. J Appl Bacteriol 17: 38–55

Kimura T, Sakamoto T, Leveque JM, Sohmiya H, Fujita M, Ikeda S, Ando T (1996) Standardization of ultrasonic power for sonochemical reaction. Ultrason Sonochem 3:S157–S161

Koda S, Miyamoto M, Toma M, Matsuoka T, Maebayashi M (2009) Inactivation of *Escherichia coli* and *Streptococcus mutans* by ultrasound at 500 kHz. Ultrason Sonochem 16:655–659

Kreith F, Manglik RM, Bohn MS (2010) Principles of heat transfer: SI edn, http://www.cengagebrain.co.uk

Liu D, Zeng XA, Suna DW, Hana Z (2013) Disruption and protein release by ultrasonication of yeast cells. Innov Food Sci Emerg Technol 18: 132–137

Ordoñez JA, Sanz B, Hernandez PE, Lopez-Lorenzo P (1984) A note on the effect of combined ultrasonic and heat treatments on the survival of thermoduric streptococci. J Appl Microbiol 56:175–177

Ordoñez JA, Aguilera MA, Garcia ML, Sanz B (1987) Effect of combined ultrasonic and heat treatment (thermoultrasonication) on the survival of a strain of *Staphylococcus aureus*. J Dairy Res 54:61–67

Raso J, Condon S, Sala-Trepat FJ (1994) Mano-thermosonication: a new method of food preservation. In: Leistner L, Gorris LGM (eds) Food preservation by combined processes. FLAIR Final Report, EUR 15776 EN, European Commission, Brussels

Sanz B, Paklacios P, Leopes P (1985) Effect of ultrasound wave on the heat resistants of *Bacillus Stearothermophilus* spore. Fed Eur Microbios Soc 18:251–259

Scherba G, Weigel RM, O'Brien WD (1991) Quantitative assessment of the germicidal efficacy of ultrasonic energy. Appl Environ Microbiol 57:2079–2084

Schultz DL (1973) Heat transfer measurements in short-duration hypersonic facilities, AGARD-AG-165. Advisory Group for Aerospace Research and Development, Paris

Wrigley DM, Liorca NG (1992) Decrease in *Salmonella typhimurium* in skim milk and egg by heat and ultrasonic wave treatment. J Food Prot 55:678–680

Yusaf T (2011) Mechanical treatment of microorganisms using ultrasound, shock and shear technology, thesis. University of Southern, Queensland

Yusaf T (2013) Experimental study of microorganism disruption using shear stress. Biochem Eng J 79:7–14

Yusaf T, Buttsworth DR (2007) Characterisation of mixing rate due to high power ultrasound. Ultrason Sonochem 14:266–274

Zhang L, Jin Y, Xie Y, Wu X, Wu T (2014) Releasing polysaccharide and protein from yeast cells by ultrasound: selectivity and effects of processing parameters. Ultrason Sonochem 21(2):576–581