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Low frequency thermo-ultrasonication of *Saccharomyces cerevisiae* suspensions: effect of temperature and of ultrasonic power

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Abstract

The combined effect of low frequency ultrasound (20 kHz) with temperature on the survival of a strain of *Saccharomyces cerevisiae* suspended in water was studied. The treatment temperatures tested were 45 °C, 50 °C and 55 °C; the actual ultrasonic powers tested were 50 W, 100 W and 180 W. Application of ultrasonic waves at a non-lethal temperature did not display a deactivating action; a higher effect of ultrasound at higher temperatures was observed, and the synergy between ultrasound and temperature was confirmed. These results proved that the ultrasonic waves do not destroy the yeast's cells; they damage them, thus increasing their sensitivity to heat. The existence of an optimal ultrasonic power for a maximal deactivating effect was shown, and was found to have a value of around 100 W (actual power). © 1997 Elsevier Science S.A.

Keywords: Low frequency thermo-ultrasonication; *Saccharomyces cerevisiae*; Ultrasonic power

1. Introduction

Heat treatment is the most common method used for the processes of pasteurization and sterilization of food products, beverages, fermentation media, etc. Reduction of the temperature applied and/or of the time of treatment would mean a lower energy consumption and, above all, reduced detrimental effect on food. Therefore, combining heat with other physical or chemical agents in order to increase the lethal action remains a subject of interest.

The application of ultrasonic waves generating cavitation in microbial suspensions often has lethal results. Cavitation is an effect of high power ultrasound propagating in a liquid; above a certain minimal input intensity, the low pressure generated during the rarefaction phase of the pressure wave set up in the liquid can momentarily exceed the cohesive strength of the liquid. This results in cavities containing gas and vapor in the liquid. The threshold and intensity of cavitation depend on a number of factors, the most important being the presence of cavitation nuclei. These are microbubbles or suspended particles creating points of weakness in the liquid at which cavities may form [1]. Once formed, these bubbles will oscillate according to the pressure wave. For high acoustic pressures, the growth and collapse are violent (transient cavitation), as for lower pressure levels, the evolution of the bubble diameter is weak and occurs over a long period of time, resulting in slow growth of the bubble (stable cavitation).

The effects of cavitation observed [2] on microbial suspensions are: dispersion of clumps of micro-organisms; modification of the cellular activity; puncturing of the cell wall; increased sensitivity to heat; existence of synergy between temperature and ultrasound. The biological parameters which influence the intensity of the effects are: the microbial strain tested, the suspending medium, the size of the cell [3,4]. The mechanism for biological cell destruction may be explained by the collapse of cavitation bubbles.

The study presented here was carried out in order to confirm and identify the mechanisms by which ultrasound and cavitation display deactivating and destructive action, as well as setting the basis to enable their generalization to any microbial suspension.

The study was focused on the yeast strain *Saccharomyces cerevisiae* VL1 suspended in water. The effect of two parameters is described here: temperature and power.

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2. Materials and methods

2.1. Ultrasonic equipment

A submerged ultrasonic horn, with a tip diameter of 13 mm and working at a fixed frequency (20 kHz), was used. A generator (Sonics & Materials Vibracell) converting 50 Hz electric energy into 20 kHz and was equipped with a wattmeter allowing the power to be changed in the range 0–500 W. A transducer containing the piezo-electric element enabled the conversion of the 20 kHz electric energy to vibrating mechanical energy of the same frequency.

2.2. Reactors

Each experiment consisted of a thermo-ultrasonication carried out in parallel with a control thermal treatment; therefore, two reactors were used. These were sterilizable jacketed glass reactors, and contained 300 ml of suspension. They were provided with magnetic stirrers. Agitation in the control reactor was maintained at 250 rpm, while for the thermo-ultrasonication reactor it was kept at 100 rpm since the immersed probe increased vortex formation, leading to a low propagation of the ultrasonic waves into the microbial suspension.

2.3. Micro-organisms and inoculum preparation

The yeast strain studied was *Saccharomyces cerevisiae* VL1, provided in freeze-dried form (Lallemand Inc.) of concentration 20×10^9 cells g^{-1} (dry weight). 3 g of yeast were added to 30 ml of sterile water at 40 °C. The suspension was left at ambient temperature to rest for 5 min before being stirred for 20 min. The inoculum was then divided into two equal volumes, one being submitted to the thermo-ultrasonic treatment, and the other to the thermal (control) treatment.

2.4. Reaction media

The yeast inoculum was suspended in 300 ml of water; the reactor and the water contained therein were sterile.

2.5. Treatment procedure

Ultrasound was applied. Inoculation was done when the suspending media had reached the temperature selected for the treatment. Samples were taken at regular intervals and the treatments lasted 10 min (for some control treatments, sampling was carried out for 20 min).

The ultrasonic frequency was 20 kHz. Experiments were carried out at the three temperatures 45 °C, 50 °C, 55 °C; at each temperature, three ultrasonic powers were tested (actual value): 50 W, 100 W, 180 W.

2.6. Assessment of microbial viability

Samples were diluted, to between 30 and 300 colonies per plate, in sterile water and were plated (0.1 ml) on glucose agar of the following composition:

agar	20 g l^{-1}
glucose	20 g l^{-1}
yeast extract	10 g l^{-1}

For each sample, three dilutions were plated in duplicate. Spreading was done with sterile glass beads which were then removed and the petri dishes were incubated for 48 h at 30 °C. The number of colonies then counted allowed calculation of the concentrations.

2.7. Analysis of the results

2.7.1. Concentration profile

The resultant plate counts were averaged and the concentration of the cells was calculated using the formula

initial concentration

= (number of colonies/volume plated out)

× dilution factor

The concentration is plotted as a function of time.

2.7.2. Evaluation of the decimal reduction time D

D is the time required, at a given temperature, to kill 90% of the microbial population, or the time to reach 1/10 of the initial number of viable cells. The equation is

$$\log (N_t/N_0) = -(t/D)$$

Plotting $\log (N_t/N_0)$ vs. time gives a line of slope $(1/D)$.

A percentage reduction was calculated as the percentage of the D-value reduction of the heat treatment when a thermo-ultrasonic treatment was applied.

2.7.3. Calculation of Z

Z is the number of degrees change in temperature necessary to achieve a 10-fold change in the decimal reduction time, D:

$$\frac{\log D1 - \log D2}{T2 - T1} = 1/Z$$

The above equation was used to calculate the Z values for the control and for the thermo-ultrasonic treatments.

3. Results and discussion

3.1. Effect of a direct ultrasonic treatment

Ultrasound was applied at the non-lethal temperature of 45 °C. For the control and ultrasonic treatments at the three ultrasonic powers, the concentrations of the yeast suspensions did not decrease; they remained constant and, depending on

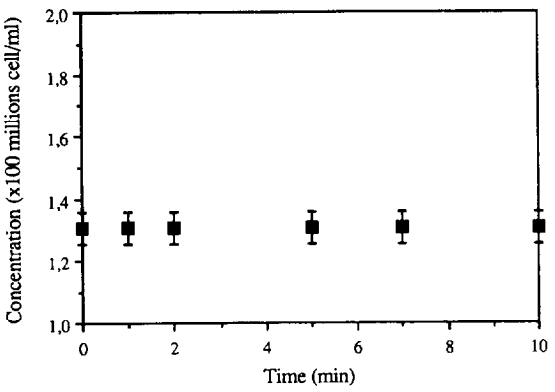

Fig. 1. Sonication at 45 °C.

the initial concentration of the suspension, were in the range 1.0–1.5 10^8 cell ml^{-1}. Fig. 1 shows the concentration profile for the sonication at 45 °C at an effective ultrasonic power of 100 W.

A temperature of 45 °C approaches the lethal temperature range for yeasts in water (pH 7), but it is still non-destructive for short exposure times. As expected, no decrease in the microbial population was obtained for the controls.

No destruction was observed when sonication was carried out, independently of the ultrasonic power selected. Treated and untreated samples were observed under microscope, and it was seen that treated cells were entire but reacted differently to vital staining with methylene blue if compared to untreated cells. These results prove that ultrasonic waves generating a cavitation field in the microbial suspension do not deactivate, break up or kill the yeasts; they damage the cell wall and maybe also the cytoplasmic membrane, thus affecting the cellular permeability. Furthermore, they lead to modifications in the cellular structure of the yeasts [5,6].

3.2. *Effect of temperature and ultrasonic power during thermo-ultrasonication treatments*

Figs. 2 and 3 show the concentration profiles for the thermo-ultrasonication treatments at 50 °C and 55 °C, respectively. These are plotted using as a starting point the *D* values obtained experimentally.

Table 1 presents the values of calculated decimal reduction times.

At both temperatures (50 °C and 55 °C), the action of ultrasound in increasing the sensitivity of yeast to heat [6,7] is strong for an actual power of 100 W, but is less marked at 50 W and 180 W. These results prove that a range of optimal ultrasonic powers exists for ultrasound applied in microbiology. The strongest microbial destruction, compared with the control treatment, was reached for an actual ultrasonic power of 100 W.

At a power of 50 W, the intensity of ultrasonic waves in the suspension is too low to allow a good development of cavitation. This leads to the formation of a smaller number of bubbles, and to vibrations of smaller amplitudes. The level of damage of the yeast is not sufficient to increase the sensitivity of the *Saccharomyces cerevisiae* cells to heat. For an actual power of 180 W, a sensitizing action due to ultrasound is visible, but is much smaller than that reached at an actual power of 100 W. Cavitation unloading is being reached: at such a power the energy of the transmitted waves is not well propagated; bubbles coalesce, absorbing the acoustic energy which is not, therefore, released in the suspension.

The lower values of *D* obtained for the thermo-ultrasonication treatments can be attributed to the following phenomena associated with ultrasonic waves and cavitation:

1. cavitation bubbles imploding close to micro-organisms and causing very violent jets of liquid; these act on the cell wall of *Saccharomyces cerevisiae*;
2. the eddies, releasing the energy carried by larger eddies, which might be similar in size to the yeast cells; the cells would therefore be submitted to shear stresses and normal forces.

These damaging agents do not break the cells into fragments, but cause puncturing of the cell wall and cell membrane, which is why the permeability of the micro-

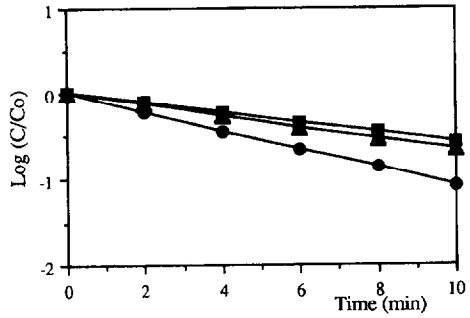

Fig. 2. Variation of the concentrations for treatments at 50 °C: (■) control and P = 50 W; (▲) = 180 W; (●) P = 100 W.

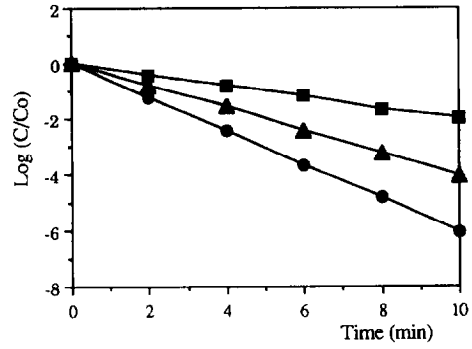

Fig. 3. Variation of the concentrations for treatments at 55 °C: (■) control and P = 50 W; (▲) = 180 W; (●) P = 100 W.

Table 1
Values of decimal reduction time for the various treatments of *Saccharomyces cerevisiae* VL1 suspensions

Treatment	*D* (50 °C)	*D* (55 °C)
Control	16 min	4.3 min
Pact = 50 W	16 min	4.3 min
Pact = 100 W	9 min	1.6 min
Pact = 180 W	15 min	2.4 min

organism changes and becomes more fragile sensitive to high temperatures.

Furthermore, the yeast cell is eucaryotic, and is therefore a rather complex cell which could contain cavitation nuclei [2]; sonication could cause an internal cavitation as well as internal microstreaming; the cellular structure might be modified.

Also, solid particles placed in a turbulent field are surrounded by a layer where streaming is stationary. This layer is a barrier to heat transfer during heat treatment. Microstreaming generated by acoustic cavitation affects this layer which becomes thinner, and thus heat transfer from the suspension to the centre of the cell is accelerated, resulting in faster cellular deactivation and destruction. Such a phenomenon also explains the more pronounced destructive effect of ultrasound at higher temperatures.

Table 2 shows the values of D (control) / D (thermo-ultrasonication) (D_C/D_{TU}) at 50 °C and 55 °C, and of Z calculated for the yeast suspensions, at the three ultrasonic powers applied.

The levels of destruction reached during thermo-ultrasonic treatment at a power of 50 W are the same as those reached with thermal treatment alone. Thus, under these working conditions, an actual power of 50 W is not enough to obtain an effect of sensitization on the yeast cells. The treatment is not a thermo-ultrasonication but a thermal treatment: the destruction obtained is only due to the effect of the lethal temperature.

For higher temperatures, the values (D_C/D_{TU}) are higher, proving that the destructive effect of ultrasonic waves becomes more marked as the temperature is increased. A phenomenon of synergy is thus observed.

When the actual powers applied were 100 W and 180 W, a reduction in D with respect to those values obtained for the control treatments was observed. The Z values calculated are lower than that determined for the control treatment. The decrease in the value of the decimal reduction times D proves that ultrasound at this power increases the level of destruction of micro-organisms in the suspension. Moreover, the values of Z obtained for the thermo-ultrasonications are lower than that of the control treatments. The ratios (D_C/D_{TU}) increase as the temperature increases. These results show that a synergy between temperature and ultrasound exists.

Fig. 4 is an illustration of the variation of the ratios (D_C/D_{TU}) as a function of the actual ultrasonic power. Since at present only three powers have been tested, no lines are plotted to interpolate the points obtained since the actual pattern is not known.

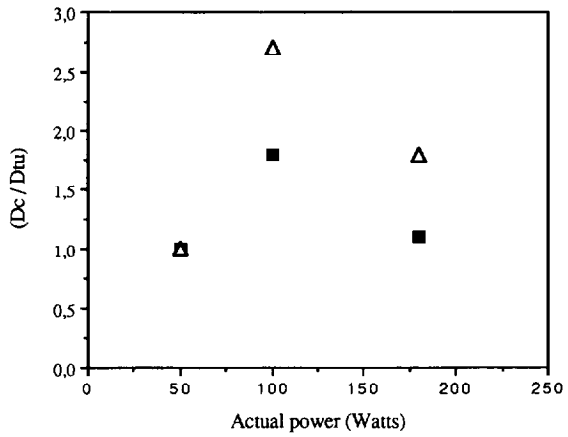

Fig. 4. Variation of the ratio (D_C/D_{TU}) as a function of the actual ultrasonic power in the suspension: (■) 50 °C, (△) 55 °C.

It can be seen that an optimal ultrasonic power to reach the maximal microbial destruction exists. For the conditions chosen here, the optimal actual ultrasonic power was around 100 W; applying this ultrasonic power leads to good propagation of the ultrasonic wave, generating the effects responsible for the sensitization of cells at an efficient level.

4. Conclusion

The action of ultrasound on *Saccharomyces cerevisiae* VL1 is to damage the cell wall and the cytoplasmic membrane, affecting the internal structure of the cell. These modifications lead to cells which are more sensitive to heat. Therefore, during thermo-ultrasonication, the yeast cells are destroyed more easily than are those submitted to control thermal treatment.

The increased sensitivity to heat of the micro-organisms could be linked to the following ultrasonic phenomena.

1. The immense local variations of temperature and pressure in the sites where bubbles implode.
2. The jets of liquid caused by the implosion of a bubble near an interface.
3. Microstreaming generating turbulent eddies which break up until they reach a size η, dependent on the medium's viscosity, at which they release the carried energy and are sources of stresses. If the size η is similar to that of the yeast cells, both shear stresses and normal forces damage the cell [8,9].

Table 2
Values of (D_C/D_{TU}) for *Saccharomyces cerevisiare* VL1 submitted to thermal or thermo-ultrasonic treatments. The percentage reduction of D is also presented

Treatment	(D_C/D_{TU}) at 50°C	(D_C/D_{TU} at 55°C	Z (°C)	% reduction of D at 50°C	% reduction of D at 55°C
Control			8.8		
Pact = 50 W	1.0	1.0	8.8	0	0
Pact = 100 W	1.8	2.7	6.7	43.8	62.8
Pact = 180 W	1.1	1.8	6.3	6.25	44.2

4. The thinning of the viscous layer surrounding the microorganism and the reduction of the possible resistance to heat transfer.
5. The possible internal cavitation in the eucaryotic yeast cell if this contains cavitation nuclei. Consequently, the internal cellular structure might be modified. The synergy existing between temperature and ultrasound was confirmed by our work. The existence of an optimum for the applied ultrasonic power was proved. The aims of the studies on thermo-ultrasonications are to enable a decrease in the temperatures used or the times of the treatments.

5. List of symbols

C concentration (cells ml^{-1})
C_0 concentration at time zero (cells ml^{-1})
D decimal reduction time (min)
D_C decimal reduction time for a control treatment (min)
D_{TU} decimal reduction time for a thermo-ultrasonication (min)
N_0 number of viable cells at time zero
N_t number of viable cells at time t
P_{act} actual ultrasonic power in the microbial suspension (watts)
t time (min)
T temperature (°C)
Z increase in temperature to reach (1/10) D (°C)

5.1. Greek letters

η Kolmogoroff's microscale of turbulence (m)

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