American-Eurasian J. Agric. & Environ. Sci., 8 (6): 656-661, 2010
ISSN 1818-6769

Effect of Thermo-Ultrasonication on the Survival of Baker's Yeast

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Abstract: The combined effect of low frequency ultrasound (20 kHz, with waves of amplitude 20 and 40) with temperature (45°C for periods of 5 and 10 min) on the survival of a baker's yeast suspended in water was studied. Irradiation was conducted for periods of 2-10 min. Colony counting analyses in Sabouraud agar media were performed for comparison. Application of ultrasonic waves at a non-lethal temperature (45°C) did not display a deactivating action to initial 4min irradiation time and higher CFU/gr values at higher heating time was observed. These results proved that the ultrasonic waves do not destroy the yeast's cells especially in initial 4min ultrasonication, but the consequence also showed the resistance of cells decreased as ultrasonic amplitude and irradiation time increasing.

Key words: Baker's yeast · Thermo-ultrasonication · Colony counting · Amplitude · Temperature

INTRODUCTION

An ultrasonic, 20 kHz to above 25 MHz is widely used to apply in many applications. The ultrasonic power used for material testing, measuring and communication are considered the behavior or character of the received ultrasonic waves [1]. Ultrasonic power is inclusive utilized to investigate the change in liquid by the sound wave analysis such as sound velocity, attenuation, monitoring the kinetics of invertase hydrolysis and improvement biological process [2-5]. In the recent year, the ultrasonic for liquid characterization is a becoming technique to apply in a biotechnology. Especially in food and biotechnology fields [6], the advantages of ultrasonic characterization are rapid measuring and noninvasive technique to the products. In biotechnology, both of high and low ultrasonic powers are applied to the products of fermentation in several applications such cell growth and permeability [7-9]. It is a point of view to categorize the research of the ultrasonic effects into three groups. First, the high power of ultrasonic are used to destroy the microorganisms to get the product inside them such as enzyme or protein. Several researches play into the different technique to use of the ultrasonic powers, disruption techniques and filtering the products off the dead cell. The aromatic compounds of wine were extracted with a new rapid using ultrasound by showing good recovery, linearity and reproducibility for most of the compounds, together with rapidity and simplicity better than those resin extraction method [10]. Second, various ultrasonic power are utilized to inactivate the microorganisms for food, beverage or liquid purification by propagation and standing wave technique. The inactivation with using of ultrasonic waves in comparison with chlorination technique has been investigated [11-12]. Third, the effects from ultrasonic to the microorganism are studied by some researches and give details of the reasons and results from ultrasonic effects. Viability of yeast cells subjected to ultrasonic standing wave field in acoustic cell retention system was studied by varying the pressure amplitude of ultrasonic [2]. This paper deals with the investigation of yeast viability effected from low frequency (20 kHz) ultrasonic technique. A concept idea of a research was stated that the ultrasonic energy transmitted in to liquid possibly affects to viability. However, the effects of ultrasonic energy to the microorganism growths are still indistinct. The valuable of fast growth rate of yeast will be increased the product of the fermentation such as ethanol or alcohol for energy problems solving in the present and the future [9, 13]. By

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using 20 kHz ultrasonic transducer to stimulate the suspension included baker's yeast cell strain *S. cerevisiae* and heating 45°C were setup the experiment. The change of viability was investigated then; the results were analyzed and discussed thermo-ultrasonication effect on yeast viability.

MATERIALS AND METHODS

Materials: In the experiment, The Iran mellas baker's yeast strain *S. cerevisiae* was used to culture in the Sabouraud agar media. Sabouraud agar media purchased from Merck Company. Salt powder for saline 0.8% ringer solution (diluting media) prepared from Fluka Company product. All solutions for the experiments were prepared with Millipore water purified by a Millipore Milli-Q system.

Preparation of Microbial Suspension: In the experiment baker's yeast powder suspended in sterilized water. 1gr yeast mixed with 9 ml water in screwed top tubes and these tubes was used for treatments.

Heating Treatment: A Memmert thermostatically-controlled water bath with shaker whose temperature was fixed to attain 45°C in the screwed top yeast suspension tubes. Temperature of medium during heat treatment period time (5 and 10 min) was continuously monitored by a thermocouple to ensure from a constant pre-determined temperature value. After heat treatment samples immediately cooled in cold water and transferred to ultrasonic treatment.

Ultrasonic Irradiation: A horn-type sonicator (UP200H, Dr. Hielscher) operating at a fixed frequency of 20 kHz and an energy density of 650W/cm^2 was used. Samples of 10 mL screwed top yeast suspension tubes were placed in glass container and subject to continuous ultrasound irradiation emitted through a 1 mm in diameter tip. An ultrasound 20% and 40% of 260 micrometer of wave amplitude was applied to the medium with an immersed 10-mm diameter probe. During period time (2, 4, 6, 8 and 10min) of the experiment, the temperature was controlled with cold water [14]. Due to a lot of bubbles generated by the cavitations process, the systems were always highly mixed since the beginning of the experiments. After the ultrasonic treatment, samples transferred to the dilution tubes.

Colony Counting: Yeast survivors were diluted 1:10 with sterile saline 0.8% ringer solution to -8, then plated on to Sabouraud Agar by spread plating with 0.1 ml of sample suspension. Plates of two serial dilutions (-7 and -8) were incubated at 30°C for 2 days. Three plates were used for each dilution. Survival curves were obtained by plotting Log number of survivor number [15]. Conventionally, the number of microorganisms per gram of sample is calculated using the following formula: $C/[(n_1+0.1 * n_2)* d]$,

In which C is the sum of the colonies counted over all the plates and selected in two successive dilutions; d is the degree of the first dilution, the degree of the second dilution being equal to 0.1* d, n_1: number of plates counted and selected at the first dilution and n_2: number of plates counted and selected at the second dilution.

Statistics: The experiment utilized a randomized complete block design with irradiation time, amplitude and heating time as factors. Experiments were individually repeated three times. The number of cells was transformed into log CFU/gr before analysis by Minitab statistical software. Microsoft_ Excel and Minitab soft wares were used for data analysis

RESULTS AND DISCUSSION

Analysis of colony formation of *Saccharomyces cerevisiae* cells irradiated with ultrasound and heat treated. Cell survivals determined by colony counting were compared. Figure 1 shows that the survival of yeast cells during initial 2-6 min ultrasonic irradiation increased and then decreased approximately linearly with increasing ultrasonic irradiation time. Hence, it was suggested that the yeast cells were inactivated by shock wave after 6min. As shown in the Figure 2, the survival of yeast cells after 4 min decreased with increasing radiation time. This value was closely related to the threshold time obtained from Figures 1 and 2. Figures 3 and 4 respectively show the survival of yeast cells as a function of ultrasonic irradiation time in the 20 and 40% amplitudes(10min heating in 45°C) and 20 and 40% amplitudes (5 min heating in 45°C temperature). Figure 5 shows the higher heating time had increasing effect on number of survival yeast cells, in 10 min heating (45°C) higher values observed ($P<0.05$). Figure 6 shows the higher amplitude had reducing effect on number of survival yeast cells, in 20% amplitude higher values observed ($P<0.05$). Figure 7 shows the irradiation time had significant effect on number of survival yeast cells, this figure shows from 2 to 4 min had increasing effect, after 4 min had decreasing effect ($P<0.05$) and combined effect of irradiation time with

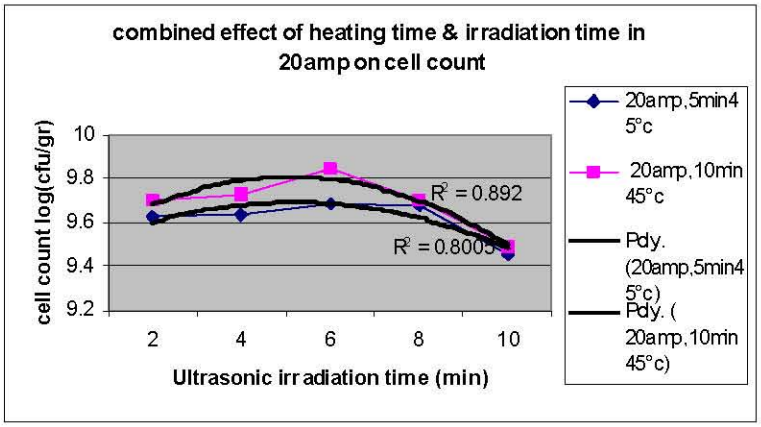

Fig. 1: Survival of yeast cells as a function of ultrasonic irradiation time in the 20% amplitude and (5 and 10 min) 45°C temperature.

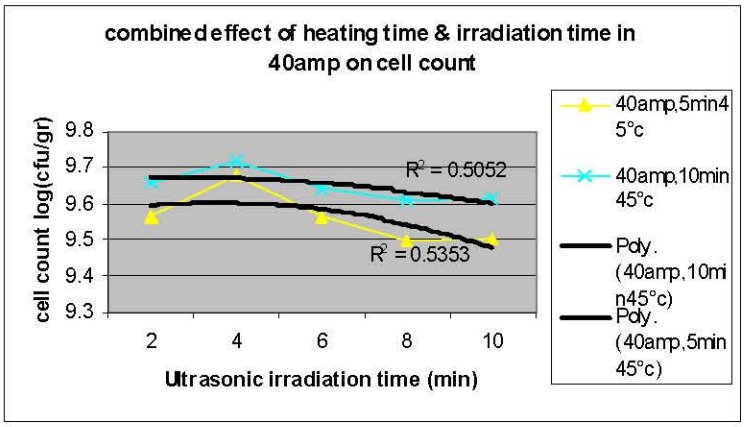

Fig. 2: Survival of yeast cells as a function of ultrasonic irradiation time in the 40% amplitude and (5 and 10 min) 45° C temperature.

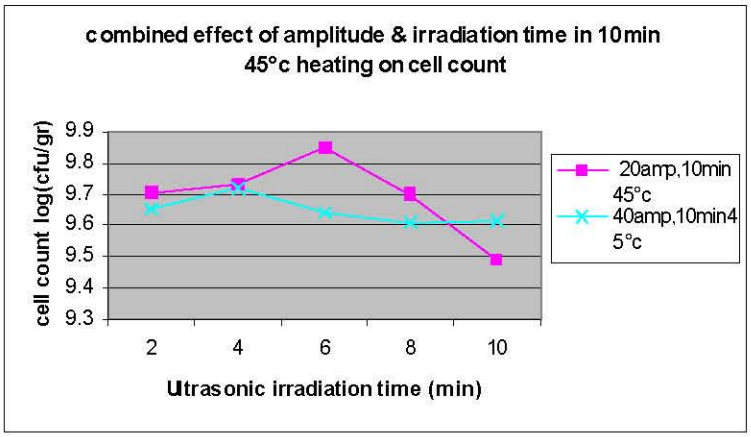

Fig. 3: Survival of yeast cells as a function of ultrasonic irradiation time in the 20 and 40% amplitudes (10min heating in 45° C)

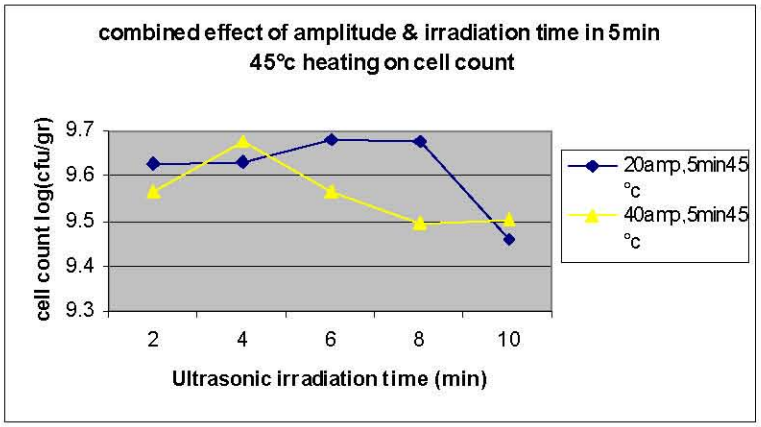

Fig. 4: Survival of yeast cells as a function of ultrasonic irradiation time in the 20 and 40% amplitudes (5min heating in 45°C)

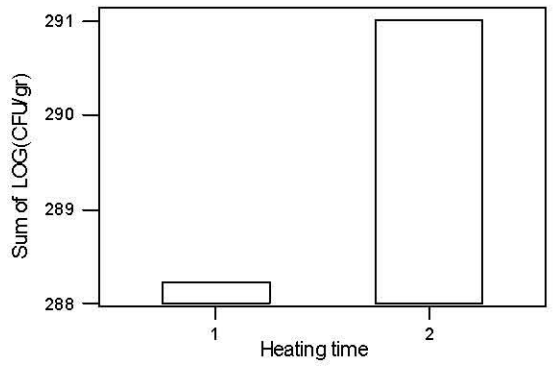

Fig. 5: Effect of heating time (1:5min, 2:10min) on number of survival yeast cells

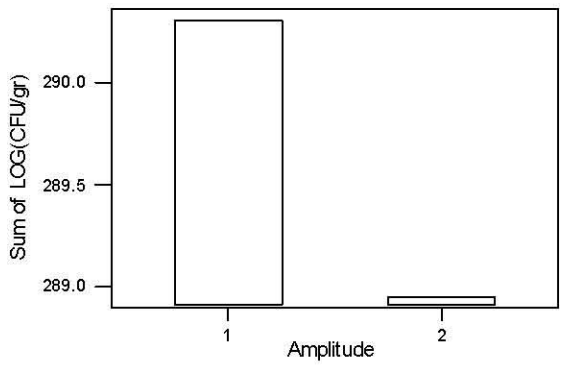

Fig. 6: Effect of amplitude (1:20%, 2:40%) on number of survival yeast cells

amplitude was significant (P<0.05). Figures 8, 9 and 10 show combined effects of irradiation time (1, 2, 3, 4 and 5), amplitude (A1 and 2) and heating time (H 1 and 2) on CFU/gr value.

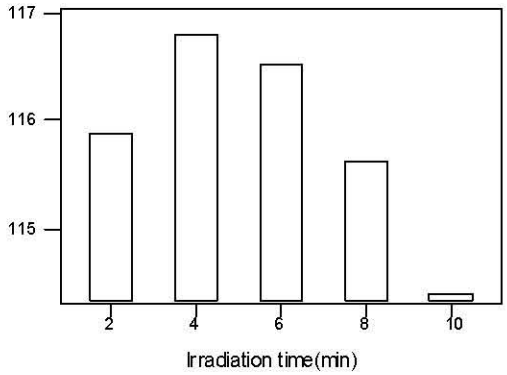

Fig. 7: Effect of irradiation time (1:2min, 2:4min, 3:6min, 4:8min, 5:10min) on number of survival yeast cells

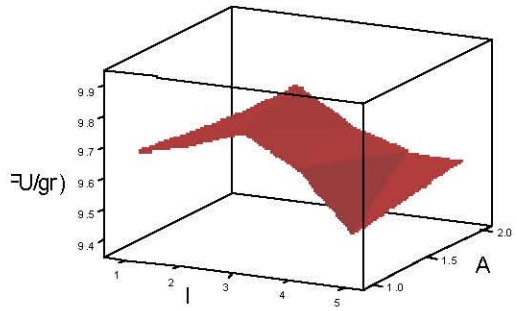

Fig. 8: Combined effect of irradiation time (I) with amplitude (A) on number of survival yeast cells

CONCLUSION

The ultrasonic stimulation that affects to *S. cerevisiae* yeast growth was studied at the surrounding temperature of 45°C. The colony numbers curves were generated by the surface plate count. The results were

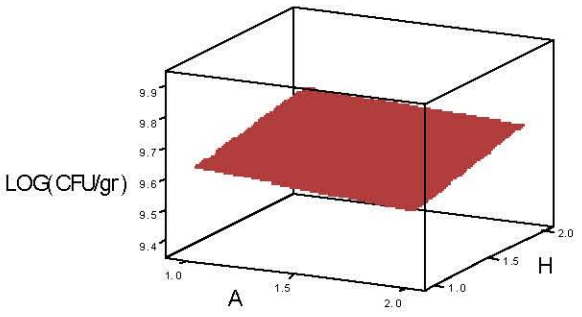

Fig. 9: Combined effect of heating time (H) with amplitude (A) on number of survival yeast cells

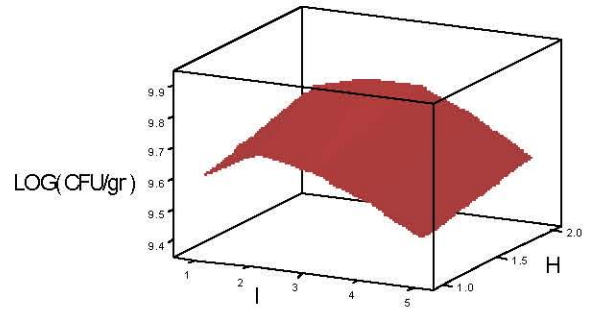

Fig. 10: Combined effect of heating time (H) with irradiation time (I) on number of survival yeast cells

proved the statement and shown the different values from ultrasonic stimulations at times, amplitudes and heating time. In particular, in initial 4min irradiation time and 10min heating (45°C), CFU values raises advanced. Afterward, the low-frequency ultrasonic effects in liquid including compression/rarefaction, irradiation force and acoustic streaming were discussed to explore the consequences of affecting to the yeast viability. The resistance of *S. cerevisiae* to 20 kHz ultrasonic stimulation with different irradiation time and amplitude was analyzed. The consequence showed the resistance of cell decreased as ultrasonic amplitude and irradiation time increasing especially after initial 4 min like to results of some researches [15-16].

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