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Ultrasonic Field on The Sublethal Injury of *Saccharomyces cerevisiae* Cell

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Abstract. This study aims to investigate the sublethal injury of *saccharomyces cerevisiae* cell caused by ultrasonic field with osmotic pressure selective plate method and preservation experiment. The absorbance of cell suspensions and preservation activity are studied for sublethal injury. According to the results, increase of intensity of ultrasonic field lead to higher extension of cell exudation and structure destruction, which indicated the positive correlation between intensity of ultrasonic field and degree of cell injury. Cellular damage and destruction of its structure may cause cell death. However, the rate of cellular injury was found to be less than 5% under high ultrasonic field, indicating an insignificant sublethal effect. In conclusion, treatment of ultrasonic field at high intensity may potentially aid in the non-thermal sterilization of food.

Keywords: ultrasonic field, *Saccharomyces cerevisiae* cell, sublethal injury

1. Introduction

Food safety is an issue of great concern worldwide and foodborne illness is one of the most important symptoms, which are responsible by foodborne microorganisms. *S.cerevisiae* cell is the most commonly used bacteria in fermentation with widely relations with human. Currently, heating and non-thermal sterilization are commonly used in food sterilization technology. In recent years, ultrasonic field is used as non-thermal sterilization with simple, mature equipment and non-toxic byproducts [1]. Ultrasonic field is a kind of sound waves with penetrating ability and directivity, whose frequency is 2×10^4~2×10^9Hz [1]. Tremendous energy is produced in the interaction of ultrasonic field and transmission media, able to destroy the cell structure and physiological function [2]-[4]. The main effect of ultrasonic field in liquid is hot action, mechanical effect and cavitation [5]. The cavitation is the major mechanism of cell death of ultrasonic field treatment [6]. The kind of sublethal cell is physiologically defective, which is able to be repaired and recurred in appropriate conditions [7], [8]. The adaptability of microorganism to stress conditions may potentially cause harm to the processing and preservation. Thus ultrasonic field on the sublethal injury effects of *S.cerevisiae* cell is of much importance in food fields and may provide significant guidance for ultrasonic field sterilization.

Up to date, researches of ultrasonic field have been limited within the extraction of active ingredient, structures of food component and rheological properties [9]-[11], with sublethal injury still unclear. Damar reported that sublethal injury of *S. aureus* cell with osmotic selective plate method by PEF [12]. Lu studied the increase of *Escherichia coli* cell membrane by ultrasonic field significantly [13]. Somoinos studied self repair of *S.cerevisiae* cells by PEF [14].

In this paper, *S.cerevisiae*, one of common strains in liquid food contamination, is selected as experimental object. Research model is established to verify the existence of sublethal cells with colony

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count and determine the selective medium lethal critical osmotic pressure. The preservation test is further studied to confirm *S.cerevisiae* cells viability.

2. Materials and Methods

2.1. Materials and Preparation of Bacterial Cells

S.cerevisiae cell is obtained from Chinese industrial culture collection center. Yeast peptone dextrose medium (1% yeast extract, 2% bacterial peptone, 2% dextrose, 2% agar powder. YPD) is used for bacterial growth. Medium components are dissolved in distilled water, with packaging and sterilization at 115℃ for 20 min. Sodium chloride, A.R., is purchased from Guangzhou chemical reagent. Agar powder, biochemical reagent, is purchased from Shanghai Boao Biotech Corp. Speeding refrigerated centrifuge 3K30 (Germany Sigma Co.), UV spectrophotometer UV2102PC (Shanghai UNICO Co.), Digital display electric incubator HPX-9162MBE (Shanghai Industrial Co., Ltd. Boxun), Transmission electric microscope TECNAI G2 12(Netherlands FEI Electron Optics Ltd.), High pressure steam sterilizer MLS-3020 (Japanese SANYO company), Clean Benches SW-CJ (Suzhou Purification Equipment Factory), Ultrasonic generator UP 400S(Germany Dr. Hielscher GmbH). The *S.cerevisiae* cells were inoculated into liquid YPD medium, shaking culture at 28℃ to a certain period. The cells were collected by centrifugation in 4000 rpm for 5min, washed twice with sterile distilled water and then the cells were resuspended in sterile distilled water. A concentration of cell suspension were prepared by adjusting the absorbance of bacterial suspension in A600. Normally the absorbance in A600 is 0.1 OD, that is, the concentration of yeast cell is 3.0×106 cells/ml [15].

2.2. The UV Detection of Cell Suspension and Cell Sublethal Injury

50ml *S.cerevisiae* cells suspension were treated by ultrasound for 10 min with the frequency from 40 to 200 W. The samples treated and untreated by ultrasound were centrifuged for 10 min in 8000 rpm with the temperature of 4℃. The supernatant was collected and detected by UV. The supernatant of sample untreated by ultrasound is blank, the absorbance of all samples were measured in 280 nm. The sublethal effect of ultrasound was tested by osmotic pressure selective plate method. Sodium chloride with the concentration of 3%, 4%, 5%, 6%, 7% was added to YPD medium and the solid medium was configured, while the medium with no sodium chloride was negative control. The cell suspension with the concentration of 10^6-10^7 cells/ml was diluted to an appropriate concentration. According to viable count operation, the same amount of cell suspension was applied to six medium plates and placed into incubator for 4d at 28-30℃. Then the growth of cells was observed for colony counting. The death rate=lgN0/N (N0: the initial number of viable cells. N: the number of viable cells with ultrasound treatment).

2.3. The Preservation Test with Ultrasound Treatment

The bacterial suspension with ultrasound and no ultrasound treatment were centrifuged in 3000 rpm for 5 min at 4℃. The cell pellet was collected and suspended with the same volume of sterile 1×PBS buffer containing 0.1% peptone. The mixed cell suspension was divided to two equal volume samples. The sample, respectively, was preserved for 7d at 4 or 30℃, then the sample was selected for colony counting.

3. Results and discussion

3.1. Effects of Ultrasound on the Cell Structure and Cell Sublethal Effects

S.cerevisiae cells are rich in protein substances with characteristic absorption at 280 nm. When cell structure is damaged, the absorbance of cell suspensions will reach 280 nm. As shown by Figure 1, increase of intensity of ultrasonic field lead to higher extensive of cell exudation and structure destruction. Positive correlation between intensity of ultrasonic field and degree of cell injury was found. Slight correlation between electric conductivity of cell suspensions and intensity of ultrasonic field was seen. Electric conductivity is directly related with ion concentration. This may be explained by that the cell exudation is not ionic materials completely. Sublethal cells can grow in YPD medium instead of selective medium with high osmotic pressure. Colony counts method is used to determine whether sublethal injury exists. Difference of colony counts indicates the occurrence of sublethal injury. Ultrasound field leads to dead or

sublethal injury. Sublethal cells are not detected firstly. However, repairrment, exudation and growth were observed later. The outbreak of viable cell may cause potential toxins to food process.

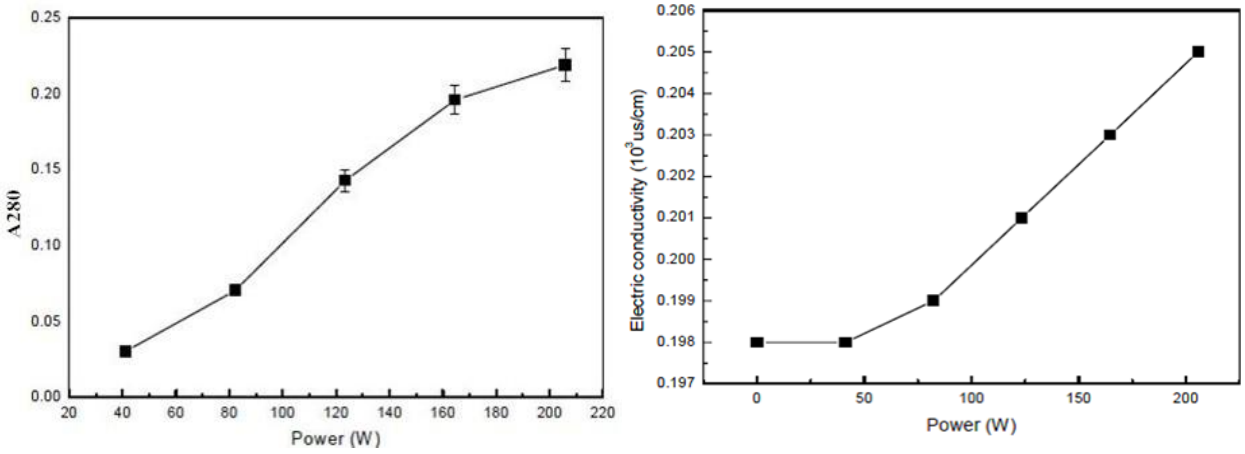

Fig. 1: Absorbance of cell exudation by ultrasound treatment and effects of ultrasound on electric conductivity of cell exudation.

3.2. Determination of Additional Concentration of NaCl

The growth of *S.cerevisiae* cells is shown in Figure 2. The colonies in YPDN medium are less than those in YPD medium. No significant difference among colony counts of a, b, c and d medium and no colony in e and f medium were observed. The shape of colony is affected by low concentration of NaCl and high doses inhibit cell viability. Negative correlation between the concentration of NaCl and counts of colony are found.

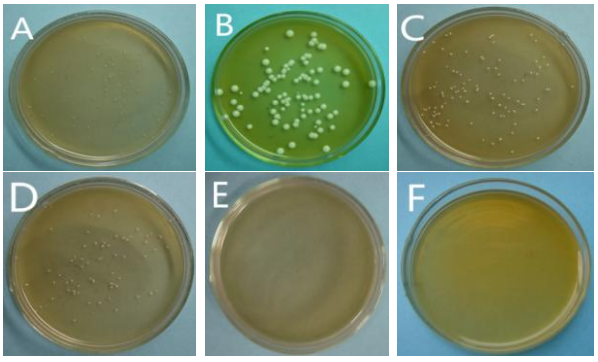

Fig. 2: The viability of *S.cerevisiae* cells in YPDN medium. A:CK, 0%NaCl; B:3% NaCl; C:4% NaCl; D:5% NaCl; E:6% NaCl; F:7% NaCl.

Table I: Sublethal injury of cells after ultrasound treatment with irradiation time and intensity.

Irradiation time（min）	Injured cells （%）	Irradiation intensity（W）	Injured cells （%）
5	0.91±0.05	40	3.55±0.18
10	1.16±0.06	80	2.15±0.11
15	1.75±0.09	160	0.00
20	0.00	320	0.00

3.3. Ultrasound on Cell Sublethal Injury Effects

Sublethal injury of *S.cerevisiae* cells with different irradiation time and intensity is shown in Table I. With ultrasound intensity of 160 W, slight correlation between irradiation time and the rate of damaged cells is obtained. However, no damaged cells exist in irradiation time of 20 min. Such finding indicates that cells may be completely dead. As irradiation time reaches 20 min, with the ultrasound power increasing, the injury rate decreases to 0%. This may be due to bactericidal effects of ultrasound intensity. With ultrasound power increases, the number of dead cells rises instead of injured cells. The rate is lower than 5%, indicating the sublethal effect is not significant. Treated cells are either completely dead or intact to cell activity. However,

even small amount of damaged cells are likely to lead to food risks from perspective of microbiological safety. Such finding indicates that some damaged cells exists, and the sublethal injury of preservation of cells is thus required to be further evaluated.

3.4. Preservation of Cells by Ultrasound Treatment

Two preservation temperatures are selected for the experiment, with 4℃ commonly used for cryopreservation. 30℃, in the scope of normal temperature, is the optimum temperature for *S.cerevisiae* cell growth. Both typical temperatures are representative in food process and preservation. Figure 3 shows that with the bacterial suspension by ultrasonic treatment preserved for 7 d at 4℃, live cells count decrease by different ultrasound power. At 200 W, cells are completely dead and no live cells occur. This reason may be that 4℃ is not the optimum temperature to grow and sire for cells or that damaged cells by ultrasound are hard to repair by self. Preservation for 7 d at 30℃, the number of live cells by 160 or 200 W ultrasound treatment doesn't increase. This may be no damaged cells by treatment. The viable cell counts of samples increase and the up degree is decreasing with the ultrasound power increasing. Cells are easy to grow and sire at 30℃ and even damaged cells are not hard to repair. Therefore counts of samples increase in different degree. However the degree is contrary with ultrasound power. This effect is corresponding with the result of UV detection.

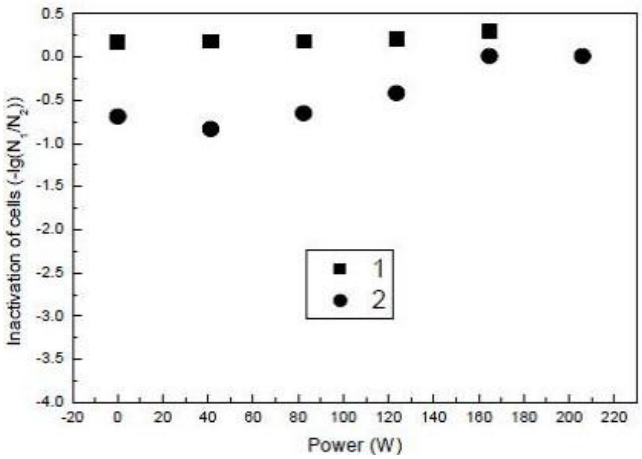

Fig. 3: The inactivation of ultrasound treated cells at different storage temperatures. 1:4° C; 2:30° C. N_1: viable cell number after preservation; N_2: viable cell number before preservation; cfu.mL^{-1}.

4. Conclusions

The absorbance of *S.cerevisiae* cells by ultrasound treatment is found to increase with UV detection, which indicates that cell structure has been damaged and cell components flow out. In food sterilization, sublethal effect of microorganisms may be a potential danger to food quality. In this paper, detection of sublethal effect with osmotic selective plates indicates damaged cells appear in some ultrasound treatment though insignificantly. Considering biological safety, preservation test is selected to sublethal damage of target microorganisms. The result shows that viable cell counts of samples with preservation at 30℃ increase but the up degree decreases with the ultrasound power. This may be that high ultrasound power leads to serious damage to cells or makes cells directly to death. Sublethal death occurs less.

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