Innovative Food Science and Emerging Technologies 18 (2013) 132–137

Contents lists available at SciVerse ScienceDirect

Innovative Food Science and Emerging Technologies

journal homepage: www.elsevier.com/locate/ifset

Disruption and protein release by ultrasonication of yeast cells

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ARTICLE INFO

Article history:
Received 8 January 2013
Accepted 9 February 2013

Editor Proof Receive Date 8 March 2013

Keywords:
Ultrasonication
S. cerevisiae yeast
Cell disruption
Protein release
Kinetics

ABSTRACT

Protein is an important intracellular component of yeast cells with many beneficial functions to human health. In this study, disruption and protein releasing kinetics of *Saccharomyces cerevisiae* cells were investigated using an ultrasonic probe system. The effects of acoustic power and duty cycle of a sonicator on cell disruption and protein release were examined. The efficiency of cell disruption was evaluated by measurements of electrical conductivity, UV-spectroscopy and cell size. Higher degree of cell disruption was observed with increasing acoustic power and duty cycle. The relationship between protein release and cycle time at various process conditions was studied; and the data were fit to a first order kinetic expression. The analysis of these kinetic data led to the proposing of a simple model for protein releasing kinetics that involves acoustic power and duty cycle as parameters. Finally, different sonication systems have been compared, with the bath-type sonicator being less effective for yeast cell disruption and protein release compared to horn-type sonicator. *Industrial relevance:* The cytoplasm of (*S. cerevisiae*) yeast cell is a rich source of bio-products (proteins, cytoplasmic enzymes, polysaccharides, etc.) valuable for the food industry. For good recovery of these intracellular bio-products, efficient breakage of the cell walls is a necessary step. In this study, disruption and protein releasing kinetics of *S. cerevisiae* cells were investigated using an ultrasonic probe system.

1. Introduction

The cytoplasm of (*Saccharomyces cerevisiae*) yeast cell is a rich source of bio-products (proteins, cytoplasmic enzymes, polysaccharides, etc.) valuable in biotechnology, pharmacology, and food industry. *S. cerevisiae* is a simple eukaryotic cell with a relatively rigid cell wall in addition to cell membrane. Because of the existence of cell wall, efficient breakage of the cell walls is a necessary step to recover intracellular compounds (Liu, Lebovka, & Vorobiev, 2013). The basic structural components of yeast cell wall have been identified as glucans, mannoproteins and chitins, which provide mechanical strength to the cell (Smith, Moxham, & Middelberg, 2000). Complete disruption of the cell wall and release of intracellular components require destruction of the strength-providing components of the wall, namely glucan in yeast. Various cell disruption methods have been developed to accommodate efficient and cost effective release of these products from their hosts.

Disruption methods available can be mainly classified into mechanical (employing shear force) and non-mechanical (chemical or enzymatic lysis) methods (Geciova, Bury, & Jelen, 2002; Harisson, 1991; Middelberg, 1995). Due to operational and economic limitations at process scale, the non-mechanical methods are commonly used in the laboratory scale for intracellular protein recovery. Mechanical methods are easy to scale up and relatively cheaper to operate. Thus, the mechanical methods are often favored for large-scale cell disruption. Ultrasonication (US) is a technique that can be used to enhance food processes (Li & Sun, 2002; Sun & Li, 2003; Zheng & Sun, 2006; Delgado, Zheng, & Sun, 2009; Kiani, Zhang, Delgado, & Sun, 2011; Kiani, Sun, Delgado, & Zhang, 2012; Kiani, Sun, & Zhang, 2012, 2013; Tao, García, & Sun, 2013), thus US is one of the most commonly used mechanical cell-disruption methods for extraction of intracellular products. This method creates high shear force by high-frequency ultrasound (above 16 kHz) (Chemat, Huma, & Khan, 2011). In ultrasonication, an intense shear action induced by high frequency sonic waves is used for cell wall disintegration. Mechanism of cell disruption by ultrasound is normally associated with the cavitation phenomena (Doullah, 1977; Vargas, Piao, Domingos, & Carmona, 2004). Cavitation is the combination of formation, growth and collapse of gas and vapor bubbles, which are induced by the action of intense sound waves (Gogate, 2011). In the collapse phase of cavitation bubbles, a large quantity of sonic energy is converted to mechanical energy in the form of intense elastic shock waves, in which the pressure reaches as high as thousands of atmosphere near the point of collapse (Borthwick et al., 2005; Feliu, Cubarsi, & Villaverde, 1998; Garcia, 1993). As a consequence, the use of ultrasound leads to a better cell disruption and faster mass transfer between the solvent and host material. US has been extensively used to obtain intracellular proteins from microbial cells. Apar and Obek (2008) studied the kinetics of protein release from brewer's yeast. They found that protein release was

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1466-8564/$ – see front matter
http://dx.doi.org/10.1016/j.ifset.2013.02.006

independent of cell concentration, and to be nearly proportional to the acoustic power. It was found that the kinetic rate constant of the protein release increases linearly with the acoustic power, as higher US power has stronger cavitational effect and better cell disruption, thus enhancing the protein release rate. Additionally, the effect of acoustic power and cell suspension volume on cell disruption was investigated by Ho et al. (2006), who found that the cell disruption and *HBcAg* release rates increased with the increase of acoustic power, and 15 mL suspension volume and 90 min of sonication time were the optimal conditions for the disruption of *E. coli* cells and the release of intracellular *HBcAg*. Kapucu, Gulsoy, and Mehmetoglu (2000) examined the release kinetics of *Acetobacter peroxydans* by ultrasonication, and found an increase in the cell disruption with increasing acoustic power. The rate constant of the protein release was found to be 0.022 min^{-1} using the first order protein release kinetic equation (Kapucu et al., 2000). In the study of Iida, Tuziuti, Yasui, Kozuka, and Towata (2008), a saturation of protein release from yeast cells at higher power conditions was observed. Although most of the ultrasound induced cell disruption studies are carried out successfully, its application is limited mainly due to inadequate knowledge of cell disruption and product release mechanism, and unavailability of kinetic equation for predicting the rate of cell disruption and product release. The kinetic studies appearing in the literature so far have mainly dealt with the effect of acoustic power on protein releasing (Apar & Obek, 2008; Doullah, 1977; Kapucu et al., 2000). Despite the above studies, to the best of our knowledge, no consideration has been made to study the synergic effect of duty cycle and acoustic power on protein releasing to determine the kinetic parameters. Therefore, our interest is to analyze and propose a kinetic model for protein releasing experiments.

In this study, effects of ultrasonic parameters including acoustic power and duty cycle on the cell disruption and protein releasing kinetics were investigated systematically. The effectiveness of cell disruption was determined by conductivity disintegration index, cell size and protein release. An attempt was made to explain protein releasing kinetics by establishing a kinetic model involving both acoustic power and duty cycle. Finally, the effect of irradiation mode was also investigated using an ultrasonic bath to compare with the results obtained by direct irradiation using an ultrasonic horn.

2. Materials and methods

2.1. Yeast cells

Dry wine yeast *S. cerevisiae* (Maurivin, Mauri Yeast Australia Pty Ltd, NSW, Australia) was used throughout this study. Active dry yeast was derived from washed biomass and the intact yeast cell structures were kept. The dry commercial powder was stored in a refrigerator at 5 °C. No further cultivation or incubation of yeast cells was made.

2.2. Preparation of yeast suspensions

Suspensions at 1% were prepared by mixing the dry yeast cells and distilled water. The gentle agitation conditions were set at rotation speed of 100 rpm in order to prevent any damage of the yeast cells. The yeasts were swelled in an aqueous suspension at room temperature and agitated for 0.5 h before ultrasound treatment.

2.3. Ultrasonication process

A horn-type sonicator (JY98-IIIN, Ningbo Scientz Biotechnology Co. Ltd., Ningbo, China) was used for ultrasonic irradiation, which operates at 1200 W and 20 kHz frequency (Fig. 1). The amplitude is adjustable from 1% to 99%. The instrument can be used in cycle mode, the pulse duration and pulse interval refer to "ON" time and "OFF" time of the sonochemical reactor. The total time of a pulse duration period plus a pulse interval period is the cycle time. A duty cycle (*DC*) expressed as % is the proportion of the pulse duration period to the cycle time. The

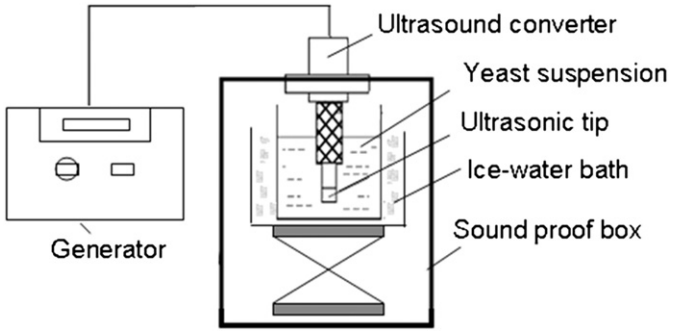

Fig. 1. Ultrasonic cell disrupter.

disruption experiments were performed at various acoustic powers (from 120 to 600 W) and duty cycle (from 10 to 80%) at 25 °C in 200 mL of yeast solution. The tip of the sonicator horn (20 mm diameter) was located in the center of the solution. The temperature of the yeast solution was checked and kept constant by the use of a cooling bath containing ice–water mixture to prevent overheating. In case of experiments involving ultrasonic bath, a bath-type sonochemical reactor (SB-600 DTY, Ningbo Scientz Biotechnology Co. Ltd., Ningbo, China) was used for ultrasonic irradiation. The generator can generate the ultrasonic waves of 25 kHz frequency and the power was fixed at 600 W.

2.4. Protein release kinetics

Protein release by ultrasonication obeys the first order kinetics law (Doullah, 1977) and can be described by:

$$\ln\left(\frac{R_m}{R_m - R}\right) = kt \tag{1}$$

where R_m is the maximum amount of protein released (mg/mL), and it was reached after 30 min of treatment by US at a fixed power of 600 W and 80% duty cycle in the current study. R is the amount of protein released (mg/mL) during sonication, k is the release rate constant (s^{-1}), and t is the sonication time (s).

Generally speaking, US produces intense local shock waves in the proximity of the sonication probe. The absorption of shock wave energy by the suspension induces intense local isotropic turbulence. The higher the applied ultrasonic power is, the more intense the local shear stress is (Lorincz, 2004). Thus, constant k is dependent on ultrasonic parameters. The dependence of the protein release constant k on acoustic power input and duty cycle was established in the following forms:

$$k = k_0(p)^{\alpha} \exp(\beta \cdot DC) \tag{2}$$

where k_0 is the pre-exponential constant (J^{-1}), p is the ultrasonic power (J/s), DC is the duty cycle, and α and β are empirical constants.

2.5. Analysis

The electrical conductivity of suspensions was measured at 25 °C using a conductivity instrument (DDS-11A, Shanghai Leici-Chuangyi Instrument And Meter Co. Ltd., Shanghai, China). The electrical conductivity of suspensions was related to the extraction of ionic intracellular components from damaged cells. The degree of yeast damage can be evaluated by the conductivity disintegration index Z (Shynkaryk et al., 2008):

$$Z = \frac{\sigma - \sigma_i}{\sigma_{max} - \sigma_i} \tag{3}$$

where σ is the electrical conductivity of yeast suspension after ultrasound treatment, σ_i is the initial electrical conductivity of yeast