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Effects of Ultrasonic Waves on Filtration Performance and Fermentation in an Internal Membrane-Filtration Bioreactor

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Abstract Ultrasonic wave technology was employed to improve filtration performance and ethanol production in a bioreactor equipped with an internal ceramic-membrane filter module. The filtration performance was found to depend on the power and the pattern of ultrasonic wave irradiation. Under the optimized conditions (irradiation time: 25 sec, period: 5 min, and ultrasonic power: 60 W), the flux was improved with the periodic-pause method by 200–700% compared with the control (with no irradiation), while the improvement was only 30 to 90% without the periodic-pause method. The final ethanol concentration also increased slightly. However, in a more severe condition (irradiation time: 2.5 min, period: 5 min, and ultrasonic power: 110 W), the irradiation of ultrasonic waves was observed to disturb cell integrity and viability, and thus to decrease ethanol production.

Key words: Internal membrane-filtration bioreactor, ultrasonic wave, filtration performance, ethanol fermentation

Novel applications of ultrasonic waves or ultrasound have recently been drawing much attention in biotechnology [12]. One such application is in heterogeneous enzyme reaction systems. The reaction rate in these systems was enhanced because of improved mass transfer between the bulk liquid phase and immobilized enzymes [1, 4]. Another application is for homogeneous or quasi-homogeneous bioreaction systems in which the enzymes or cells are dissolved or suspended. An example of such systems is the harvest of intracellular substances without cell disruption, e.g., repeated harvest of vacuole-located secondary product (pigment) from *in vitro* grown plant cells [6]. These interesting examples show the extensive and successful prospects for ultrasound applications in biotechnology.

Cell retention culture using membranes is a very efficient technique for achieving high productivities of biomass and metabolites [3, 5, 7]. However, a membrane process is usually limited by the rapid decline in filtration flux due to membrane fouling or clogging [9, 10]. To-date, only a few studies have addressed methods for improving filtration flux during fermentation. In the case of the external filtration system, cross-flow was introduced to improve filtration performance and to demonstrate the effectiveness to some extent [11]. One of the most recent examples is the backflushing method to reduce membrane fouling, by our group [8].

In this study, we propose a method of cell retention culture using membrane-filtration coupled with an ultrasonic wave system which has many potential advantages, and investigate the possibility of improving the filtration performance and the ethanol production by *Saccharomyces cerevisiae*.

MATERIALS AND METHODS

Microorganism and Cell Preparation

The yeast strain used in this study was an industrial *S. cerevisiae* generously provided by Seoyoung Ethanol Industry, Korea. The seed culture medium contained 50 g/l glucose, 3 g/l yeast extract, 3 g/l malt extract, and 5 g/l bactopeptone. The fermentation medium consisted of 100 g/l glucose, 8.5 g/l yeast extract, 1.3 g/l NH_4Cl, 0.13 g/l $MgSO_4 \cdot 7H_2O$, and 0.06 g/l $CaCl_2$.

Seed culture in a 1-l flask containing 500 ml of medium was carried out in a rotary shaker incubator at 30°C for 24 h. An inoculum of 10% (v/v) of the working volume (40-l) was transfered to a 50-l fermentor (Korean Fermentor

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Co., Korea). The culture broth and cells were recovered separately after the fermentation and used for filtration experiments in a 1.8-l internal filtration fermentor equipped with an ultrasonic wave system.

Membrane and Filter Module

The filter module consists of 13 dead-end ceramic membrane tubes with an inner diameter, outer diameter, and height of 8, 11, and 80 mm, respectively. The total filtration area was approximately 360 cm^2. The tubular ceramic membranes, prepared at the Dalian Institute of Chemical Physics, China, were made of α-Al_2O_3 with a symmetric structure and a mean pore size of 0.3 μm. More details of the filter module are given elsewhere [8].

Bioreactor System and Filtration Performance Test

The experimental setup of the bioreactor system with an internal ceramic filter module is shown in Fig. 1. The ceramic filter module was installed in a jar fermentor with a 1.8-l working volume (Korea Fermentor Co., Korea). An ultrasonic wave generator (Je Il Co., Korea) was placed inside the fermentor, with a frequency fixed at 20 kHz and a power output ranging from 60 to 110 W. The generator consisted of four parts: a power supply, an energy converter, an ultrasonic wave-generating rod, and a timer control system. The generating rod, made of stainless steel to endure high temperature sterilization, was mounted on a side port of the fermentor. The yeast cell suspension was prepared by centrifuging cultivated cells, washing twice with distilled water, and resuspending the harvested cells in a saline buffer solution containing 8.5 g/l NaCl, 6 g/l NaH_2PO_4, and 3 g/l KH_2PO_4. All filtration experiments were performed with 16 g/l yeast cells at pH 7 and at 25°C to maintain the cell viability by lowering the metabolic activity. The liquid in the reactor was removed through the filter module by a suction pump during filtration experiments. The filtrate that accumulated in a graded reservoir was recycled back to the reactor to maintain constant experimental conditions. The effect of ultrasonic waves on flux recovery after fouling was investigated using a periodic regeneration scheme at various levels of power input.

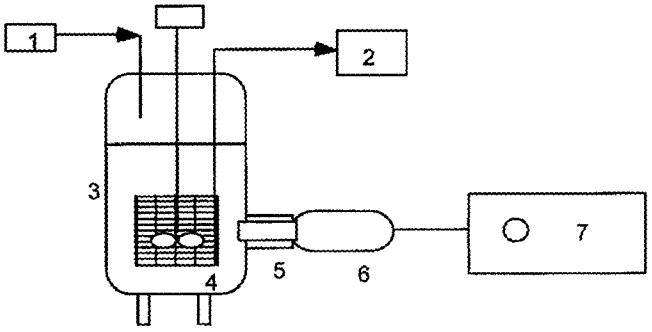

Fig. 1. Ultrasonic wave system coupled with internal filter.
1, Feed tank; 2, Filtrate reservoir; 3, Fermentor; 4, Ceramic filter module; 5, Ultrasonic wave sensor; 6, Ultrasonic wave generator; 7, Power controller.

Fermentation with Ultrasonic Wave Irradiation

The effects of ultrasonic waves on the yeast culture were examined. Cell growth, ethanol production, cell viability, and soluble protein in the broth were monitored during the fermentation for various power levels and ultrasound generation modes. For the purpose of comparison, a control culture without ultrasonic wave irradiation was also performed. The culture medium used in this study contained 50 g/l glucose, 3 g/l yeast extract, 3 g/l malt extract, and 5 g/l bactopeptone. All experiments were performed at 30°C in the same bioreactor systems as in the filtration performance test.

Analytical Methods

Cell concentration was determined by measuring the optical density of samples at 570 nm using a spectrophotometer (Beckman DU-65, U.S.A.). Ethanol concentration was measured by gas chromatography, and glucose was analyzed with a YSI 2700 Select Analyzer (Yellow Spring Instrument, U.S.A.). A methylene blue staining method was used to assess cell viability, while cell integrity was estimated by measuring protein concentration in the supernatant of the fermentation broth after cell removal. Protein concentration was measured by the Bradford method [2] at 595 nm.

Results and Discussion

Effect of Ultrasonic Waves on Filtration Performance Recovery

Ultrasonic waves can induce cavitational phenomena and blast shock in fermentation broth, sweeping away and resuspending cells or particles in the cake layer on the membrane surface. We expected that higher fluxes could be maintained using ultrasonic waves. The improvement in flux may depend on the irradiation frequency, duration, and power of ultrasonic waves. In order to characterize the improvement in flux by using ultrasonic wave technology, a flux improvement index (Fi) was defined as follows for a given filtration time t:

$$Fi(t) = [(\text{Flux with UW})_t - (\text{Flux without UW})_t]/(\text{Flux without UW})_t$$

where UW denotes ultrasonic waves.

Effects of Irradiation Time and Period on Filtration Performance. Figure 2 shows the effect of different ultrasonic irradiation times on Fi at a power of 60 W when the period was fixed at 5 min. In the legend for Fig. 2, 25 sec/5 min, for example, means that the period was 5 min and UW was irradiated during the first 25 sec o

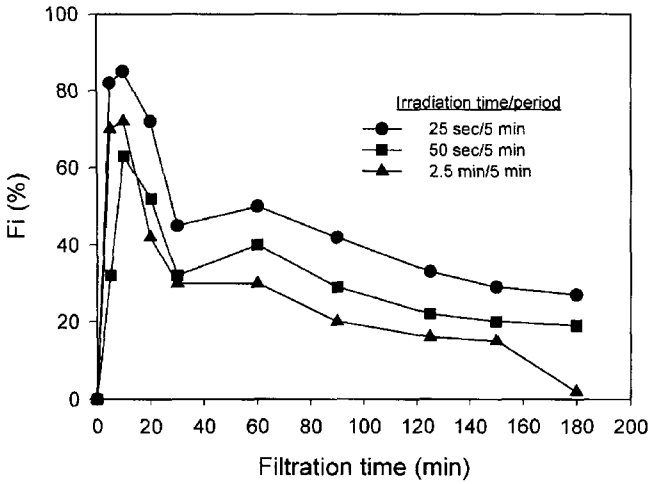

Fig. 2. Effect of ultrasonic irradiation time on Fi at a power of 60 W.

each period. The largest improvement was observed with 25 sec of irradiation time. We considered that an excessively long irradiation time could destroy yeast cells and induce secondary fouling. Some cell debris was observed in the case of 2.5 min/5 min (data not shown).

Figure 3 shows the Fi data for two different ultrasonic irradiation periods, 1 min and 5 min, at a power level of 60 W. In both cases, ultrasound was irradiated only during the first one twelfth of each period. Flux was enhanced with ultrasonic irradiation and the maximum improvement (70–90%) was observed at the very beginning. It seems that at the beginning of filtration, the yeast cell cake layer was quite loose and easily removed by the ultrasonic wave. Later, the cake layer became dense and more difficult to dislodge with a simultaneous decrease in Fi over time. Fi slowly decreased to reach about 30% in 180 min of filtration in both 1 min and 5 min periods. A better improvement was obtained with the period of 5 min.

Effects of Ultrasonic Power on Filtration Performance. The removal of the cake layer on the membrane surface may depend mainly on the power and frequency of the ultrasonic waves. In this study, we examined only the effect of different ultrasonic irradiation powers on Fi with a fixed frequency of 20 kHz (Fig. 4). The irradiation time and period were set at 2.5 and 5 min, respectively. The Fi at a power of 60 W was higher than that at a power of 110 W. An excessively high power and long duration of ultrasonic wave irradiation resulted in a decreased Fi, even to a negative value implying a disimprovement rather than an improvement in filtration performance. Some possible explanation of this could be obtained in the later experiments testing the effects of ultrasonic waves on cell viability and integrity (Figs. 9 and 10). At 110 W, ultrasonic wave irradiation induced more cell death, causing the release of a large amount of soluble proteins into the broth, probably making secondary fouling more significant.

Effect of Periodic-Pause of Filtration. Ultrasonic waves initially improved the filtration performance but the effect decreased with operation times making Fi (Figs. 2 and 3) a negative value, especially for the case of excessive ultrasonic wave irradiation power (Fig. 4). The major reason was thought to be secondary fouling of the membrane due to cell lysis caused by UW. We periodically stopped filtration to solve this problem. In this periodic-pause method, the suction pump for filtration was stopped and UW was irradiated for a certain period of time. During this time period, the cake layer already formed on the membrane was removed by the forces caused by agitation and UW irradiation. While filtration was going on, no irradiation was done. Figs. 5(a) and 5(b) show the Fi and filtration flux data when the periodic-pause method was applied as

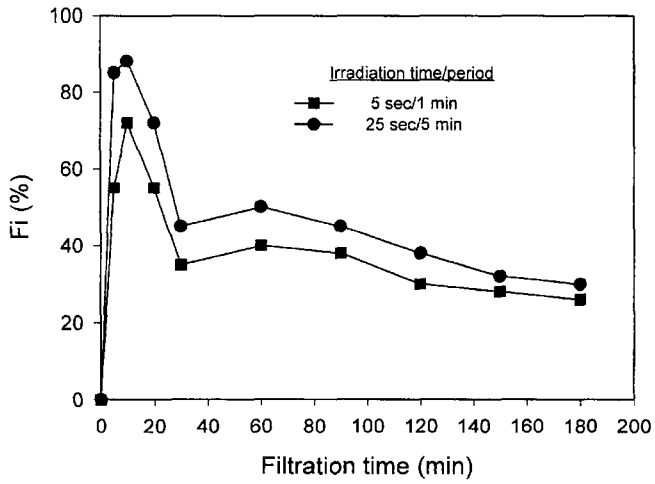

Fig. 3. Effect of ultrasonic irradiation period on Fi at a power of 60 W.

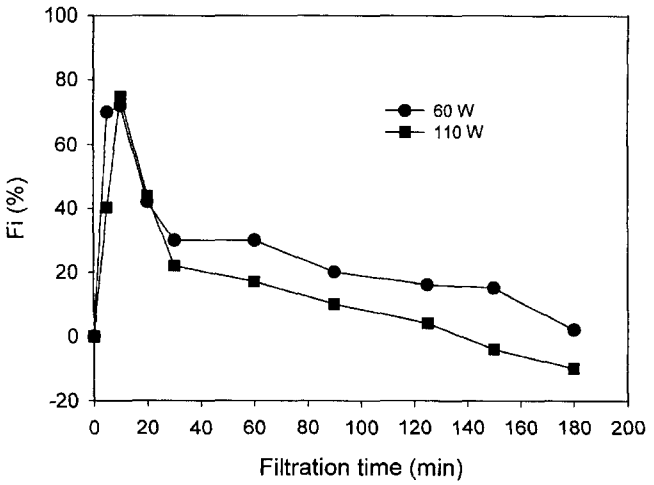

Fig. 4. Effect of ultrasonic power on Fi at an irradiation time/period of 2.5 min/5 min.

compared to the case without it under the irradiation condition of 25 sec/5 min and 60 W. The Fi value with the periodic-pause method was in the range of 200-700%, while it ranged from only 30 to 90% without the periodic-pause method. From these results, the periodic-pause method proved to be very effective for the improvement and maintenance of filtration flux, especially for a long-term operation which is imperative in continuous cultures.

Batch Fermentation of *S. cerevisiae* with Ultrasonic Waves

In order to evaluate the feasibility of an internal filter bioreactor system using ultrasound, the effects of ultrasonic wave irradiation on yeast cell growth, substrate consumption, ethanol production, cell viability, and cell integrity (as measured by soluble protein in the broth) in batch fermentation of *S. cerevisiae* were examined.

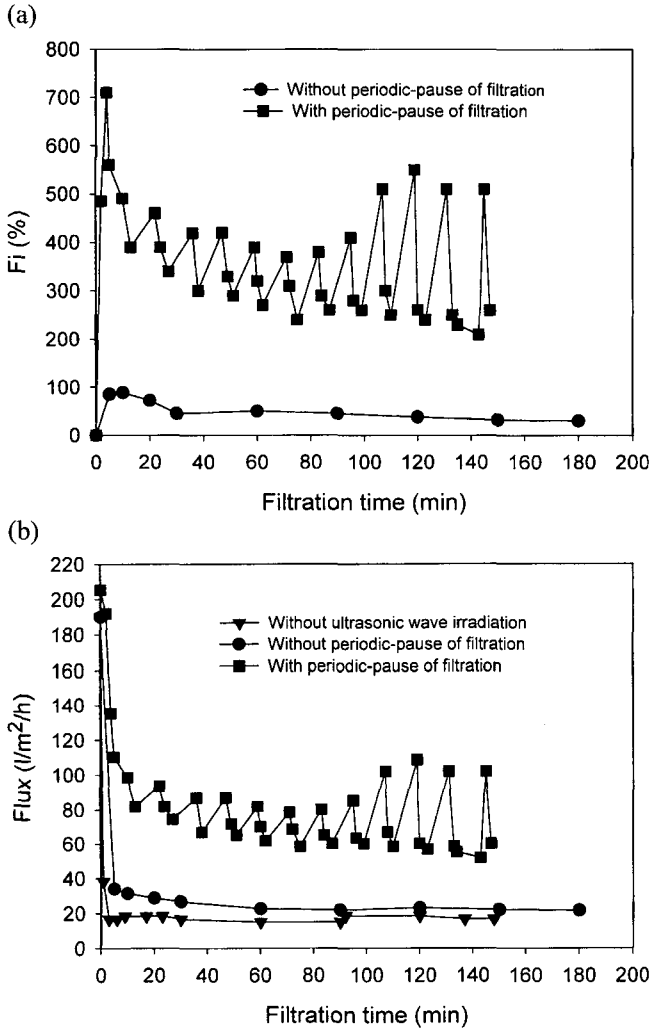

Fig. 5. Effect of periodic-pause of filtration on (a) Fi and (b) actual flux at an irradiation time/period of 25 sec/5 min and a power of 60 W.

Effect of Ultrasonic Waves on Fermentation of *S. cerevisiae*. Figures 6, 7, and 8 show the effects of ultrasonic wave irradiation on yeast cell growth, substrate consumption, and ethanol production, respectively. During the first 7 h, no apparent influence of ultrasonic wave irradiation on yeast growth was observed. However, it decreased the final cell concentration caused by cell disruption, especially at higher powers and longer irradiation times. The final cell concentration was 6.25 g/l without ultrasound, while it was 4.9 g/l with ultrasonic wave irradiation (2.5 min/5 min, 110 W). Glucose consumption was also retarded by the introduction of ultrasound, especially in the last culture stage. The effect on ethanol production seemed to be different. No apparent influence was observed with an ultrasonic wave irradiation of 25 sec/5 min, 60 W. However, a higher ethanol production was obtained with an ultrasonic wave irradiation of 25 sec/5 min, 110 W, which

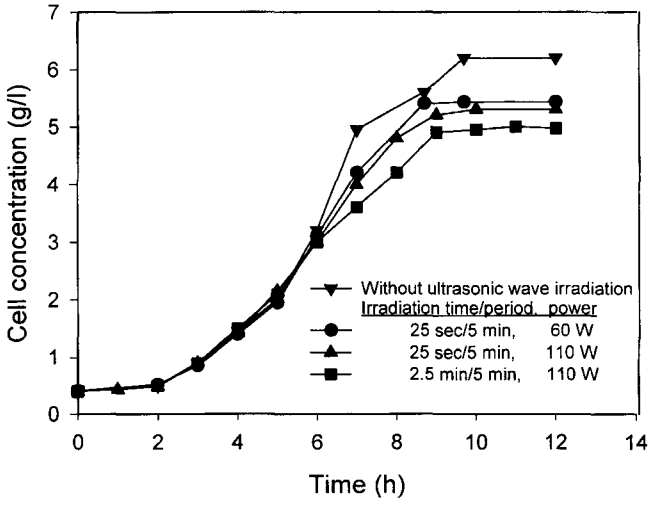

Fig. 6. Effect of ultrasonic wave irradiation on cell growth.

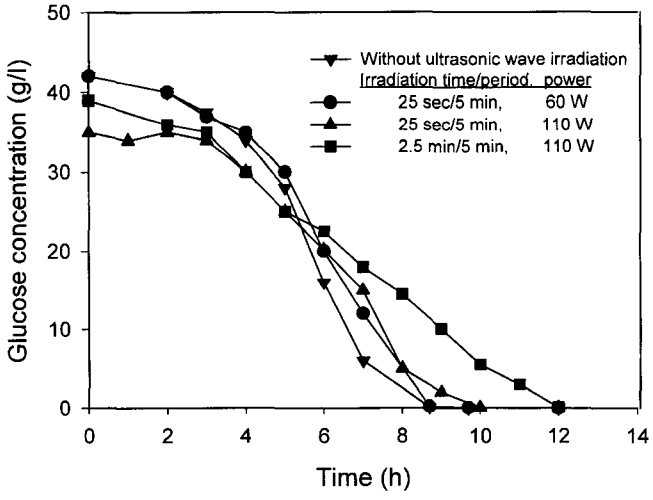

Fig. 7. Effect of ultrasonic wave irradiation on glucose consumption.

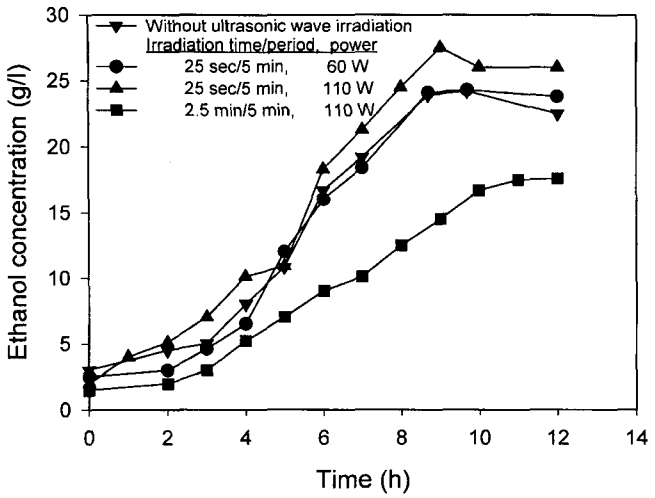

Fig. 8. Effect of ultrasonic wave irradiation on ethanol production.

might be caused by an accelerated transfer of intracellular ethanol into the broth. However, ethanol production decreased significantly with a longer ultrasonic wave irradiation of 2.5 min/5 min, 110 W and the final ethanol concentration was only 80% of that without ultrasound.

Effect of Ultrasonic Waves on Cell Viability and Integrity. The prerequisite for applying ultrasound to internal membrane filtration bioreactor systems is to minimize adverse side effects due to its radiation. Cell viability and integrity can be used as parameters to characterize these effects. Figures 9 and 10 respectively show the effect of ultrasonic wave irradiation on cell viability and integrity in batch fermentation of *S. cerevisiae*. A high level of protein concentration as seen in Fig. 10 indicates a serious cell disruption. Increased power and duration of ultrasonic wave irradiation caused a decreased

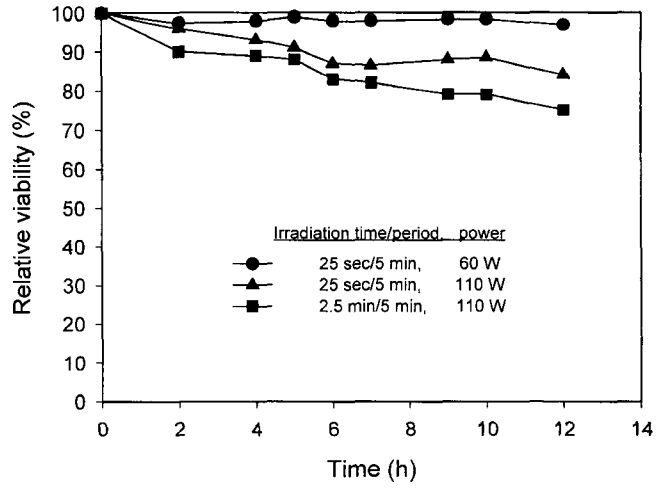

Fig. 9. Effect of ultrasonic wave irradiation on relative cell viability.

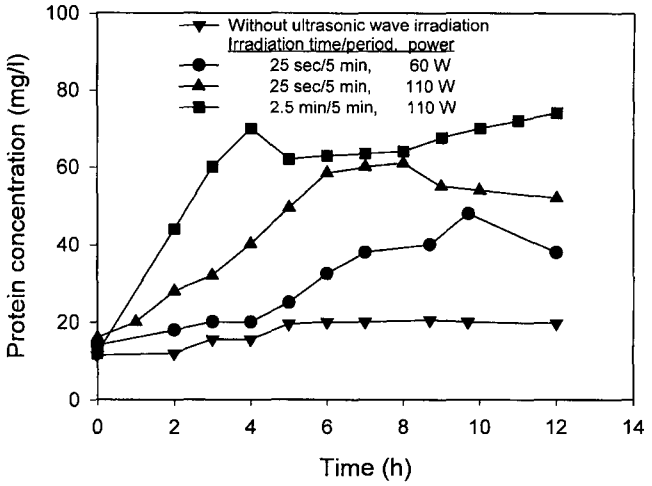

Fig. 10. Effect of ultrasonic wave irradiation on cell integrity.

cell viability and an increased protein concentration in the broth. With an ultrasonic wave irradiation of 25 sec/5 min and 60 W, cell viability higher than 97% was maintained and the protein concentration was twice as high as that of the control, than that without ultrasound. However, for an ultrasonic wave irradiation of 2.5 min/5 min and 110 W, the cell viability decreased significantly, reaching a final value of 75% in 12 h. The protein concentration was 3.5 times higher than that without ultrasound, indicating a more extensive cell disruption. These data on protein concentration can, at least partially, explain the lower Fi values in the case of excessive ultrasound wave irradiation and power, as shown in Fig. 4.

CONCLUSION

Periodic irradiation of ultrasonic waves and intermittent pauses in filtration was proved to be effective in maintaining the filtration flux and enhancing ethanol production by *Saccharomyces cerevisiae* when the appropriate irradiation time and power level were used. However, an excessively long exposure to ultrasonic wave irradiation or an exposure to excessively high power was found to have adverse effects on the filtration performance, cell integrity, and ethanol production. Therefore, the condition for ultrasonic wave irradiation needed to be optimized. In our particular case of yeast culture, the optimum condition was found to be 25 sec of irradiation per 5 min at a power level of 60 W.

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