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Using low-power ultrasonic for enhancing Saccharomyces cerevisiae M30 productivity for ethanol producing from molasses

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Abstract. Low-power ultrasonic in the range of 20-30 kHz was used to enhance ethanol fermentation from molasses. The fermentation was performed under ambient temperature and real-time monitored of environment via pH and liquid temperature as well as ethanol production. The results indicated that ultrasonic power enhanced ethanol production rate by reducing fermentation time by 6-9 h compared to the use of the control bioreactor. Ethanol production increased in proportion to the increase of ultrasonic power. Maximum ethanol concentrations at 13.8%, 15.6% and 13.1% were achieved under ultrasonic power supplied at 20, 25 and 30 kHz, respectively while the control system was 12.0%. The highest specific maximum ethanol production rate (1.55 h^{-1}) was achieved at 25 kHz under normal environment with pH 4.6-5.0 and liquid temperature 30-38°C. It can be noted that continuously ultrasonic power stimulation at 25 kHz was the optimum level to enhance the fermentation performance of *Saccharomyces* cerevisiae ***M30***. At 30 kHz increasing temperature environment was observed to lead decrease in ethanol production rate during prolonged fermentation.

Key words: Ethanol production, Low-power ultrasonic, and Saccharomyces cerevisiae M30

1. Introduction

Fermentation of alcohol from molasses is a biological process. Yeast is the key producer. Ethanol production from molasses has been investigated for several years and many methods have been applied to enhancing bioreactor productivity [1]. The use of ultrasound in specifically designed sonobioreactors can substantially increase the productivity of a biological process. However, there are few studies on the effects of ultrasonic to performance of live microbial [2], especially under fermentation conditions. Particularly, high-power ultrasonic can inhibit microbial activity by breaking macromolecules such as enzymes and possibly from unfolding and scrambling the native protein and breaking the chain into small peptides [3-5]. In contrast, optimum power ultrasonic promotes membrane permeation efficiency on cells and it has been used to induce transfer of genetic material into live animal [6,7] and plant cells [8]. In addition, at sufficiently high acoustic power inputs, ultrasonic is known to rupture cells, and ultrasonication is a well-established laboratory technique of cell disruption [4]. *Saccharomyces cerevisiae* M30 is a high performance yeast on ethanol production, high protein content in living cell, high resistance on stress environment as low pH and high temperature (38°C) [9]. This work studies the effect of low-power ultrasonic on the efficiency of ethanol production as well as activity and efficiency of *S.cerevisiae* M30. Therefore, these results will

provide information on optional technology to improve ethanol production from molasses by *Scerevisiae* M30.

2. Materials and methods

2.1. Microorganism

S. cerevisiae M30 was kindly provided by the laboratory of Professor Dr. Savithree Limthong (Department of Microbiology, Kasetsart University). Starter cultures were prepared by sub-culturing on yeast extract agar 2 times. Then, a loop of the start culture was transferred to 100 ml of medium and incubated at 37◦C with shaking at 100 rpm for 24 h. The medium contained 0.5% yeast extract at pH 5.0. The prepared medium was sterilized at 121 ◦C for 15 min.

2.2. Molasses

The molasses was collected from the molasses tank of a sugar production plant in Thailand. The physical and chemical characteristics were analyzed according to standard method [10] as summarized in Table 1. Before proceeding with the fermentation, molasses was prepared by diluting with distilled water to adjust total sugar concentration at 15 g/l.

Table 1. Molasses characteristics

Characteristics	Value
pH	7.5
Total Solid (g/l)	76.3
Moisture content (g/l)	133.0
Dry matter (g/l)	867.0
Reducing sugar (g/l)	114.7
Total sugar (g/l)	740.6
Total nitrogen (g/l)	8.8

2.3. Batch fermentation

Batch fermentation experiments were carried out in two bioreactors. Molasses contained 15% (w/v) of total sugar solution as carbon source for *S. cerevisiae* M30. Experiments were performed a 10 L stainless tank, with 8 L total liquid volume. One bioreactor was operated without ultrasonic supply (control sample). The other one was operated with ultrasonic generated from ultrasonic transducer with a frequency in the range of 20-40 kHz (NEC TOKIN., Co. Ltd). This reactor was supplied by ultrasonic power at 20, 25 and 30 kHz which was continuous supplied throughout bioreactor operation. Experiments were initiated by transferring 2% of starter culture to both control and supplied ultrasonic tanks with mixing speed at 1 rpm. They were carried out for 48 h under ambient temperature (30-40°C). Fermentation performance was evaluated through the ethanol production rate. Environment of fermentation system was routinely monitored in terms of pH and liquid temperature.

2.4. Analytical methods

pH and liquid temperature were measured in real time by pH/mV/Temp portable meter with accuracy ± 0.01 and ± 0.3 °C, respectively. The determination of ethanol concentration was done using a gas chromatograph equipped with a flame ionization detector (FID).

2.5. Specific maximum ethanol production rate

Ethanol production rate was compared with mathematic model of ethanol production developed by Muenduen et al [11] as follows:

Ethanol: $\frac{dCp}{dt} = vCx$

Cp and *Cx* were ethanol concentration (g/L) and cell concentration (g/L), respectively. In this study, Cx was fixed at 0.48 g/l and *Cp* varied according to the fermentation performance of each batch [11]. The v was specific ethanol production rate (h^{-1}). It can be calculated according to the equation above by substitution of maximum ethanol concentration, fermentation time and cell yeast concentration obtained from the experiment.

3. Results and discussion

3.1. Fermentation performance

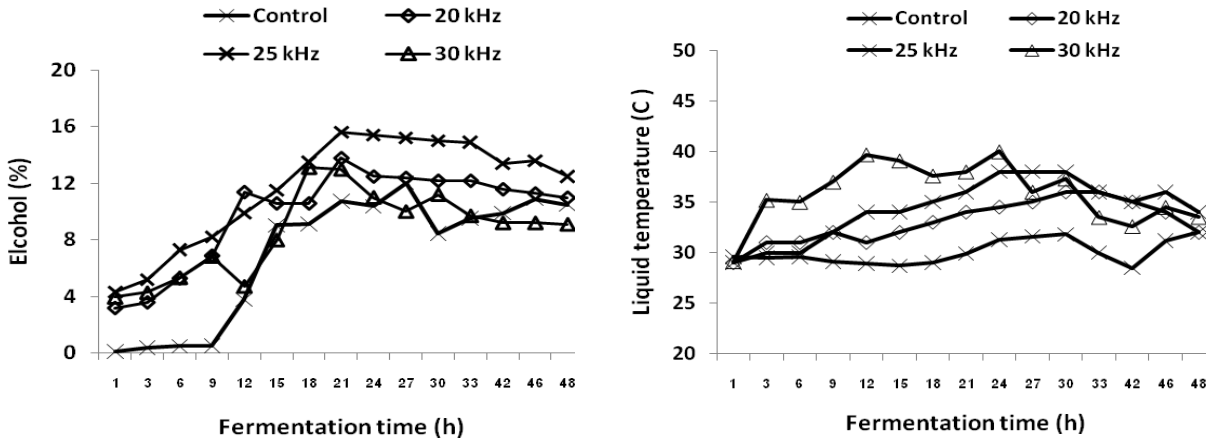
According to the results, low power ultrasonic (20-30 kHz) could increase ethanol production. Ethanol production rates were achieved at 0.66, 0.74 and 1.37 % ethanol/h at ultrasonic power 20, 25 and 30 kHz, while the control sample was 0.44 %ethanol/h. These values are shown in Table 2. Control sample took time 27 h to achieve maximum ethanol production, whereas applied ultrasonic reactors at 20, 25 and 30 kHz took 21, 21, and 18 h, respectively. Maximum ethanol concentration of the control sample was 12.0%, whereas applied ultrasonic samples at 20, 25 and 30 kHz were 13.8%, 15.6% and 13.1%, respectively. It indicated that applied low-power ultrasonic could reduce fermentation time about 6-9 h comparing with control sample. The ultrasonic power mechanism's ability to enhance bacteria living cells has been explained by Chisti and Moo-Young [9] that it disrupted the bacteria living cell by ultrasound probably linking with cavitation and can activated the cell. Cavitation generates microstreaming and other actions in the fluid as interrupts hydrodynamic turbulence in living cell culture. Therefore, low-power ultrasound of short duration can enhance the productivity of living cells. Effects of cavitation were observed on microbial suspensions: 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 [12]

Ultrasonic power at 20 and 25 kHz took time of molasses fermentation to produce maximum ethanol concentration (21 h). Ethanol concentration at 15.6% was achieved at 25 kHz which the maximum value in this study. It can be noted that ethanol fermentation system with stimulation of continuously supplied of ultrasonic power at 25 kHz was the optimum condition, whereas 20 kHz was lower power to enhancing of fermentation performance. This result was supported by Dai Chuanyun et al [13] whose study of low ultrasonic stimulates fermentation found that the optimum power of ultrasonic was about 24 kHz by reducing fermentation time from 72 h to 36 h and increasing of productivity rate of riboflavin about 5 times of control groups. Although continuously supplying of ultrasonic power at 30 kHz could reduce the fermentation time to 18 h, maximum ethanol concentration decreased to 13.1%. It indicated that power ultrasonic could activate yeast activity and enhanced of ethanol production rate in shortly time. However, after 18 h of fermentation time ethanol production decreased and stable approximately at 10% ethanol concentration (Fig 1. (a)). These results investigated the effect of high ultrasonic power on microbial activity in a fermentation system. A previous study reported that the cavitations effect was often accompanied by emission of light, can break apart relatively robust small molecules and bioactive macromolecules, and thus a living cell does not survive cavitations for long [14,15].

Table 2. Ethanol productivity and environmental of fermentation system

Sample	pH	Liquid temperature (C)	Maximum concentration of Ethanol (%)	Fermentation time (h)	Maximum production rate (%ethanol/h)	Specific maximum ethanol production rate (h^{-1})
Control	4.6-4.7	28-32	12	27	0.44	0.93
20 kHz	4.6-4.8	30-36	13.8	21	0.66	1.37
25 kHz	4.6-5.0	30-38	15.6	21	0.74	1.55
30 kHz	4.6-4.7	30-42	13.1	18	1.37	1.52

The environment of liquid fermentations was also monitored. It was found that all systems showed no difference in pH, which was in the range of 4.6 - 5.0. Temperature of liquid fermentations ranged from 28-42°C and increased according to ultrasonic power application. Ultrasonic power at 20 and 25 kHz showed maximum liquid temperature in the range of 36-38°C as shown in Fig. 1(b), with optimum to *S. cerevisiae* M30 activity. Therefore, under these conditions the systems were in normal condition. In contrast with 30 kHz of ultrasonic power supply, it generated maximum liquid temperature at 42°C which improper to *S. cerevisiae* M30 activity. At 30 kHz power ultrasonic might was over power to stimulate yeast activity in fermentation system under continuously supplied and inhibited *S. cerevisiae* M30 activity, finally reducing ethanol productivity.



(a) (b)

Fig 1. Ethanol production (a) liquid temperature (b) at various conditions

3.2. Specific maximum ethanol production rate (v)

The fermentation performance can be determined by both substrate consumption and ethanol production. In this study we used specific ethanol production rate to indicate fermentation performance and it was calculated based on maximum ethanol concentration rate and initial cell yeast concentration as previously reported [2]. The results are reported in Table. 2. Specific maximum ethanol production rate confirmed the results of fermentation performance of control and applied ultrasonic power systems that ultrasonic power could improve ethanol production rate. All applied ultrasonic power systems showed higher specific maximum ethanol production rates than the control system. Maximum ethanol production rate at 1.55 h^{-1} which highest valued was achieved at 25 kHz of ultrasonic power. Control systems, 20 and 30 kHz of ultrasonic power supplied were 0.93, 1.37 and

1.52 h^{-1}, respectively. At 30 kHz of ultrasonic power supplied, the maximum ethanol production rate was closed with 25 kHz but liquid temperature rose to 42°C which affected to yeast activity. Increasing of temperature during ultrasonic application was unclear parameter to enhance fermentation performance. A study at Chulalongkorn University, Thailand reported that the effects of the temperature on the model parameters were clear, because the specific production rate increased exponentially with temperature [12]. Cicolini et al [16] studied the effect of temperature and ultrasonic power and confirmed the synergy between ultrasonic and temperature to increasing their sensitivity to heat and enhanced microbial productivity. Therefore, enhancing fermentation performance might be promoted from a combination of ultrasonic and temperature. Future studies study should separately study and fix temperature throughout fermentation processing both used and not used in ultrasonic stimulation.

4. Conclusion

The results of this experiment demonstrated that continuously supplied low-power ultrasonic in the range of 20-30 kHz could enhance ethanol production via stimulation of *S. cerevisiae* M30 performance. The optimum ultrasonic power was 25 kHz which promoted maximum ethanol concentration at 15.6% within 21 h of fermentation time which was shorter than control system 6 h. Moreover, the highest specific maximum ethanol production rate at 1.55 h^{-1} was achieved at 25 kHz. However, 20 and 30 kHz were lower and over power to enhancing of fermentation performance, respectively. Especially 30 kHz affected to increase of liquid temperature and decreased ethanol production due to *S. cerevisiae* M30 was inhibited by over optimum temperature.

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