1. Introduction

Over the decades, *Saccharomyces cerevisiae* has been used to ferment sugar for ethanol generation. In industries, yeast is used for food, chemical, and pharmaceutical production. The main method of producing ethanol is yeast fermentation due to its short fermentation cycle and high ethanol yield. However, with the rapid advancements of fuel ethanol, demand for ethanol is increasing rapidly [1]. As the traditional craft of producing ethanol in modern society is less efficient, researchers have begun to look at other approaches to increase ethanol production. Yan et al. [2] optimized the fermentation media of *Saccharomyces cerevisiae*, considering pH, temperature, and glucose addition amount to increase ethanol production. However, this has been a traditional method and has faced some problems, such as high osmotic pressure destroying the cells and the likely increase of fermentation costs [3]. Many researchers have employed physical techniques, including ultrasound in microbial fermentation processes, to improve fermentation efficiency [4,5]. This study reports an enhancement of ethanol yield in *Saccharomyces cerevisiae* with low-intensity ultrasonic irradiation using fixed mode frequency generated by a self-developed six-frequencies (20, 23, 25, 28, 33, 40 kHz) ultrasonic device in our group. After sonication treatment, the ethanol production potential was determined. Under the optimal conditions of ultrasonic treatment (ultrasonic frequency 28 kHz, power density 180 W/L, and treatment time 24 h), the maximum ethanol yield increased by 34.87% compared to the control. Transcriptome sequencing showed that the ultrasonic treatment had expression regulations on genes involved in pyruvate metabolism, glycolysis, pentose phosphate pathway, glucose transport, and reducing power production. The quantitative real-time polymerase chain reaction (qRT-PCR) further confirmed the changes in gene expression (up- or down-regulation). Metabolomics revealed that ultrasonic treatments increased intracellular glucose and nicotinamide adenine dinucleotide (NADH) contents, which are key metabolites for ethanol synthesis. Besides, ultrasonic treatments decreased the acetate and its derivatives resulting in lowered reverse consumption of pyruvate and thus promoted ethanol synthesis. These changes in gene expression and metabolites content might be the main reason why the ethanol yield in *Saccharomyces cerevisiae* increased after ultrasonic irradiation.
promoting *Candida tropicalis* proliferation from the genetic level. The results showed that the essential genes RSC7 and RSC8 that regulated *Candida tropicalis* proliferation, genes MFST and CTRG_01806 that encoded the membrane transport proteins of *Candida tropicalis* (MFS transporter) were significantly up-regulated after ultrasonic treatment. This is the main reason for cell proliferation after ultrasonic treatment. However, up till now, there are very few reports using transcriptomic and metabolomic analysis to reveal the effects of ultrasound treatment on cell genes and metabolites. It is no doubt that cooperation between “Next-generation” sequencing technology and metabolomics could reveal more comprehensively the mechanism of sonication effects on microorganism fermentation.

In recent years, our group has developed a piece of ultrasound equipment with six different frequencies. It has two different work modes, viz. sweeping frequency and fixed frequency. The objective of this study was to use ultrasound to increase ethanol yield and explore the mechanism of increased ethanol production by a combined transcriptomic and metabolomic analysis. Thus, this study could be divided into two steps; (1) To increase the ethanol production by ultrasonic treatment; (2) To reveal the mechanism for increased ethanol yield after treatment using transcriptomics and metabolomics together. This study may provide some beneficial results for fermentation processes regulated by ultrasound treatments.

2. Materials and methods

2.1. Ultrasonic equipment

The self-developed ultrasonic device with frequencies, which are 20, 23, 25, 28, 33, 40 kHz, showed in Fig. 1 was employed to stimulate the *Saccharomyces Cerevisiae* for ethanol production. It is handled as follows. A 4 L fermentation medium was added into the fermentation tank and sterilized at 115 °C for 20 min. An ultrasonic generator was chosen, and the flow rate was set to 100 mL/min to obtain stable fermentation conditions (power density) [6,11]. Then the specific parameters (stirring speed, temperature, ventilation) were set in the fermentation tank main console, and the device was started by the control panel.

2.2. *Saccharomyces Cerevisiae* strain and culture media

The *Saccharomyces Cerevisiae* CICC 1048 used in this study was purchased from China Center of Industrial Culture Collection (CICC). *Saccharomyces Cerevisiae* was cultured at 30 °C for 16 h in a 250 mL Erlenmeyer flask containing 100 mL liquid Yeast Extract Peptone Dextrose (YPD) medium in an incubator shaker (HYL-3C, Taicang, China) at 160 r/min. The liquid YPD medium contains 1% Yeast Extract, 2% Peptone, and 2% Dextrose. For ultrasound treatment, 10% of the culture medium was transferred into a 7.5 L fermenter up to the final volume of 4 L medium containing 0.5% Yeast Extract, 1% Peptone, 25% Dextrose, 0.5% Ammonium Sulphate, 1% Potassium Dihydrogen Phosphate, 0.05% Magnesium Sulphate, and 0.015% Calcium Chloride.

2.3. Screening of ultrasonic frequency, power density, and treatment time through one-factor-at-a-time experiments

2.3.1. Ultrasonic frequency optimization

Under the condition that ultrasonic frequency was 28 kHz and sonication-treatment-time was 24 h, the sonication treatments with different ultrasonic power densities (60, 100, 140, 180, 220, 260, and 300 W/L) were used to stimulate *Saccharomyces Cerevisiae* fermentation.

2.3.2. Ultrasonic power density optimization

Under the condition that ultrasonic frequency was 28 kHz and sonication-treatment-time was 24 h, the sonication treatments with different ultrasonic power densities (60, 100, 140, 180, 220, 260, and 300 W/L) were used to stimulate *Saccharomyces Cerevisiae* fermentation.

Fig. 1. External ultrasound irradiation of broth in a recycle bioreactor (1. ultrasonic generator; 2. ultrasonic transducer; 3. slot; 4. injection port; 5. steam inlet; 6. pressure gauge; 7. sampling port; 8. sample outlet; 9. refrigeration machine; 10. peristaltic pump; 11. sample outlet of fermentation tank; 12. injection port of fermentation tank; 13. cooling water outlet; 14. cooling water inlet; 15. air inlet; 16. control system of fermentation tank.) Black, red and blue arrow represent flow direction of fermentation broth, gas and cooling water, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
2.3.3. **Ultrasonic treatment time optimisation**

The condition of ultrasonic frequency was set to 28 kHz, and ultrasonic power density was set to 180 W/L to explore the effects of the sonication treatments with different ultrasonic treatment times (6, 12, 18, 24, 30, 36, and 40 h) on ethanol yield by *Saccharomyces Cerevisiae* fermentation.

The maximum biomass, maximum specific growth rate, ethanol synthesis coefficient $\alpha$, and ethanol yield were used as reference parameters to investigate the effect of different ultrasonic frequencies, power densities, and treatment times on ethanol yield.

2.4. **Determination of ethanol production by *Saccharomyces Cerevisiae* and maximum biomass**

2.4.1. **Determination of ethanol yield**

In this study, the gas chromatography method was used to determine ethanol contents in the fermentation liquor[12]. The ethanol standard solutions of 0.05%, 0.1%, 0.5%, 0.7% and 1% were prepared respectively before the testing. The gas chromatograph (GC-2010 plus, SHI-MADZU, Japan) is equipped with a hydrogen flame detector (FID) and capillary cylinder ($30 \text{ m} \times 0.25 \text{ mm}$, WAX, Agilent). The temperature of the inlet and detector were 250 $^\circ\text{C}$ and 280 $^\circ\text{C}$, respectively. The oven temperature heating program is as below: the initial temperature was 90 $^\circ\text{C}$ and kept 3 min, then increased to 165 $^\circ\text{C}$ for 0.5 min at the rate of 25 $^\circ\text{C}$/min, finally increased to 210 $^\circ\text{C}$ for 2 min at the rate of 15 $^\circ\text{C}$/min. The total analysis time was 11.5 min. The flow rates of nitrogen, hydrogen, and air were 30 mL/min, 40 mL/min, and 300 mL/min, respectively.

2.4.2. **Determination of maximum biomass**

To collect the yeast, 5 mL of the fermentation broth was added into an Eppendorf (EP) tube and centrifuged at 4 $^\circ\text{C}$ and 7000 rpm for 10 min. The deposit of the yeast was washed twice with distilled water and then put into a drying oven at 60 $^\circ\text{C}$ for 4 h. Yeast dry weight was then calculated according to the weight of the same tube with and without the yeast deposit[13].

2.5. **Calculation of the maximum specific growth rate and ethanol synthesis coefficient $\alpha$**

2.5.1. **Maximum specific growth rate**

The growth trend of *Saccharomyces Cerevisiae* meets the characteristics of Logistic regression [14]. The equation is as follows:

$$X = \frac{X_0 \exp(\mu t)}{X_0 - \frac{\mu}{\alpha} X_0 \exp(\mu t)} = \frac{X_0}{1 + \exp(-\frac{\mu t}{\alpha})}$$

The kinetic expression was modified to get the mathematical model:

$$\ln \left(\frac{X}{X_0} - \frac{\mu}{\alpha} \right) = \ln \left(\frac{X_0}{X_0 - \frac{\mu}{\alpha}} \right)$$

where, $t$ is culture time (h) $X_0$ is the initial concentration (dry weight) (g/L) $X$ is current concentration (dry weight) (g/L) $\mu$ is the maximum specific growth rate (mg/h).

The maximum specific growth rate was obtained by calculating the linear regression constants between $\ln \left(\frac{X}{X_0} - \frac{\mu}{\alpha} \right)$ and $t$.

2.5.2. **Ethanol synthesis coefficient $\alpha$**

Generally, ethanol production is closely related to the yeast growth rate. Therefore, we could use a related Luedeking-Piret model to describe the ethanol synthesis trend. The equation is written as follows:

$$P = P_0 - \alpha X_0 + \frac{\alpha X_0 X \exp(\mu t)}{X_0 - \frac{\mu}{\alpha} X_0 \exp(\mu t)}$$

where, $t$ is culture time (h) $P$ is the current production of ethanol (g/L) $P_0$ is the initial production of ethanol (g/L) $\alpha$ is ethanol synthesis coefficient (g ethanol/g dry weight/h) related to the growth of *Saccharomyces Cerevisiae*.

The ethanol synthesis coefficient was obtained by calculating the linear regression constants between $P - P_0$ and $X_0 \left(\frac{X \exp(\mu t)}{X_0 - \frac{\mu}{\alpha} X_0 \exp(\mu t)} - 1\right)$

2.6. **Illumina transcriptome sequencing**

Two groups of *Saccharomyces Cerevisiae*, one treated with the optimal sonication conditions and the other untreated as a control group, were used to perform Illumina transcriptome sequencing. Each group consists of three parallel samples with the same treatment. Total RNA of the samples was extracted and digested with DNase to remove DNA. *Saccharomyces Cerevisiae* mRNA was enriched by magnetic beads with oligo (dT) and then sheared into short segments. The sheared mRNA was used as a template to synthesize the first cDNA using random primers of six bases. The second cDNA was synthesized simultaneously using dUTP to replace dTTP and linked with another adapter. Then the cDNA containing dUTP was digested with uracil-N-glycosylase (UNG) enzyme to give cDNA with the adapters. The remaining cDNA was then purified and repaired in its end bases. After adding 3′ Adenosines and linking with sequencing adapters, they were chosen according to their size for Polymerase chain reaction (PCR) amplification. The constructed library for the following Illumina transcriptome sequencing was checked by an Agilent 2100 Bioanalyzer. Illumina HiSeqTM 2500 was used to perform the transcriptome sequencing. Raw data were studied using the program FASTQC [15]. Differential gene expression analysis was based on the negative binomial distribution. P-value was used to evaluate differentially expressed genes between the sonication treatment and control groups [16]. P-values $<0.05$ were treated as significant differences. Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed for revealing the mechanism of ultrasound-treatment stimulated microbial fermentation.

2.7. **Real-time quantitative reverse transcriptase-polymerase chain reaction analysis**

Total RNA was isolated from the ultrasound-treated or untreated yeasts using mirVanaTM RNA Isolation Kit (Ambion, America). The SuperScript First-Strand Synthesis System (Invitrogen Life Technologies, Beijing, China) was used to generate the first strand cDNA according to the manufacturer’s protocol. The PCR was performed using a real-time PCR instrument (LightCycler® 480 II, Roche, Swiss) according to the following protocol: 10% (V: V) of cDNA mixed with 50% (V: V) of 2 × ChamQ SYBR qPCR Master Mix ((Vazyme, America), 36% (V: V) of the manufacturer’s protocol. The PCR was performed using a real-time PCR instrument (LightCycler® 480 II, Roche, Swiss) according to the following protocol: 10% (V: V) of cDNA mixed with 50% (V: V) of 2 × ChamQ SYBR qPCR Master Mix ((Vazyme, America), 36% (V: V) of nuclease-free water 4% of one of the following gene-specific oligonucleotide primer pairs: the hexose transporters 1 ($HXT1$), forward: 5′-TTCACGGTCCAAAGGCTC-3′ and reverse: 5′-TTGCGCTGGGCTTATTGA-3′; the alcohol dehydrogenase 5 ($ADH5$), forward: 5′-TTCAGTTCGTGGAGGAGGGAAGG-3′ and reverse: 5′-AGAAGCCATATGACCG-3′; the pyruvate decarboxylase 1 ($PDC1$), forward: 5′-TTCCAGGTCCAAAGGCTC-3′ and reverse: 5′-TGGAAGCTTCTGTTTCAGCTTA-3′; the pyruvate decarboxylase 5 ($PDC5$), forward: 5′-ATCCGAGATCGAAGAGGCG-3′ and reverse: 5′-CATTCTCAAGAGGTAATGACCG-3′; the pyruvate dehydrogenase 4 (ADH4), forward: 5′-TGCTCAAGAGAAGCCATGGCAAAG-3′ and reverse: 5′-TGGAAGCTTCTGTTTCAGCTTA-3′; the alcohol dehydrogenase 5 (ADH5), forward: 5′-TACATCAAGATCGAAGAGGCG-3′ and reverse: 5′-CATTCTCAAGAGGTAATGACCG-3′; the pyruvate decarboxylase 1 ($PDC1$), forward: 5′-TTCCAGGTCCAAAGGCTC-3′ and reverse: 5′-TGGAAGCTTCTGTTTCAGCTTA-3′; the pyruvate decarboxylase 5 ($PDC5$), forward: 5′-ATCCGAGATCGAAGAGGCG-3′ and reverse: 5′-CATTCTCAAGAGGTAATGACCG-3′; the pyruvate dehydrogenase 4 (ADH4), forward: 5′-TGCTCAAGAGAAGCCATGGCAAAG-3′ and reverse: 5′-TGGAAGCTTCTGTTTCAGCTTA-3′; the alcohol dehydrogenase 5 (ADH5),
forward: 5′-CATGCTTACTGCAATTCG-3′ and reverse: 5′-CCTCCCTTGATCATGCTCTA-3′; the acetaldehyde dehydrogenase 2 (ALDH2), forward: 5′-CTAAAGTGAAATGTTAGCAG-3′ and reverse: 5′-AGGTATACACACCCACCTTG-3′; the acetaldehyde dehydrogenase 3 (ALDH3), forward: 5′-ACATCTTTGACACCAAGTG-3′ and reverse: 5′-GAAGGGTATCCAGCCTG-3′. The PCR was then performed at 94 °C for 30 s, followed by 40 cycles of 94 °C for 5 s, and 60 °C for 30 s after adding 10 μL of the PCR reaction mixture into an optical plate (384-well, Roche, Swiss). The qRT-PCR experiments were performed in triplicate for all the above genes. The mRNA expression levels normalized to 18S were calculated using the 2^ΔΔCt method [17].

2.8. LC-MS analysis

Two groups of *Saccharomyces Cerevisiae*, with and without sonication treatment, were used to perform liquid chromatography-mass spectrometry (LC-MS) analysis. Each group contained eight samples with the same treatment. A high-performance liquid chromatography (HPLC) system (AB SCIEX, AB EXIONLC, USA) coupled with a high-resolution mass spectrometer (HRMS) (AB SCIEX, AB TripleTOF 6600 plus, USA) was used to analyze the metabolic profiling in both ESI positive and ESI negative ion modes. An ACQUITY UPLC HSS T3 (1.8 μm, 100 × 2.1 mm) was employed in liquid chromatography system. Column temperature was 45°C. Liquid phase conditions: mobile phase A (water containing 0.1% formic acid), B (acetonitrile containing 0.1% formic acid). Injection volume was 2 μL. Flow rate was 0.35 mL/min. Elution gradient was showed in Table 1. Electrospray ionization (ESI) was used as ion source. The signal acquisition of sample used positive and negative ion scanning mode. Mass spectrometry parameters were showed in Table 2. The acquired LC-MS raw data were processed using progenesis QI software (Waters Corporation, Milford, USA) for baseline filtration, peak identification, peak area integration, retention time correction, peak alignment, and normalization. The Excel file was obtained with three-dimension data sets, including the peak number, sample name, and normalized peak area. The internal standard was used for data QC (reproducibility). Metabolites were identified by progenesis QI (Waters Corporation, Milford, USA) Data Processing Software, based on public databases such as http://www.hmdb.ca/, http://www.lipidmaps.org/, and self-built databases. The combined positive and negative data were imported into R rposl package. Principle component analysis (PCA) and (orthogonal) partial least-squares-discriminant analysis (OPLS-DA) were carried out to visualize the metabolic alterations among experimental groups after mean centering (Ctr) and Pareto variance (Par) scaling, respectively. The differential metabolites were selected based on the combination of a statistically significant threshold of variable influence on projection (VIP) values obtained from the OPLS-DA model and P-values from a two-tailed Student’s t-test on the normalized peak areas. Metabolites with VIP values > 1.0 and P-values <= 0.05 were considered as differential metabolites.

2.9. Statistical analysis

All the experiments were performed at least three times. The data were presented as the mean value ± standard deviation (SD). Significant difference was analyzed with SPSS 19.0 software using one-way analysis of variance (ANOVA). The statistical level of significance was set at P-values < 0.05.

### Table 1

| Time(min) | A% | B% |
|-----------|----|----|
| 0.01      | 95 | 5  |
| 2         | 95 | 5  |
| 4         | 70 | 30 |
| 8         | 50 | 50 |
| 10        | 20 | 80 |
| 14        | 0  | 100|
| 15        | 0  | 100|
| 15.1      | 95 | 5  |
| 16        | 95 | 5  |

### Table 2

| Parameters                      | Positive ion | Negative ion |
|---------------------------------|--------------|--------------|
| Nebulizer Gas (GS1, PSI)        | 55           | 55           |
| Auxiliary Gas (GS2, PSI)        | 55           | 55           |
| Curtain Gas (CUR, PSI)          | 35           | 35           |
| Ion Source Temperature (°C)     | 550          | 550          |
| Ion Spray Voltage (V)           | 5500         | –4500        |
| Declustering Potential (DP, V)  | 80           | –80          |
| Mass Scan Range (TOF MS scan)   | 100 – 1000   | 100 – 1000   |
| Collision Energy (TOF MS scan, eV)| 10          | –10          |
| Mass Scan Range (Product Ion scan) | 40 – 1000     | 40 – 1000   |
| Collision Energy (Product Ion scan, eV) | 35          | –35          |
| Interface Heater Temperature (°C)| 550         | 550          |

3. Results and discussions

3.1. Single-factor optimization of ultrasonic frequency

Investigations demonstrated that frequency plays an important role in sonolytic processes [18]. As the irradiation frequency is varied, the sonochemical effects depend on several factors, for instance temperature inside a bubble, cavitation threshold, bubble population and lifetime of the bubbles. As frequency increases, the maximum temperature attained during bubble collapse decreases [19], the cavitation threshold increases [20], the bubble population increases and the resonant radius of acoustic bubbles decreases and, correspondingly, collapse times decrease as well [18]. The effect of ultrasonic frequencies at 0, 20, 23, 25, 28, 33, and 40 kHz on biomass and ethanol yield in *Saccharomyces Cerevisiae* after treatment with the ultrasound were tested. The results from Table 3 showed that all the frequencies had an increasing quantity of biomass addition. Further, all the frequencies had increased the ethanol yield except the frequency of 20 kHz (Fig. 2a). It demonstrated that sonication was able to increase yeast activity and improve ethanol synthesis ability. Because 28 kHz had an encouraging effect on the ethanol yield and reached the highest yield (9.94 ± 0.13%) compared to other frequencies, 28 kHz was chosen as the optimal frequency. We also found that both maximum specific growth rate (0.2675) and ethanol synthesis coefficient (0.8136) under the frequency of 28 kHz were significantly greater than other frequencies (< 0.05). These results might be because cavitation caused by sonication enabled faint and repairable damage to the cell membrane, resulting in improved cell membrane permeability, material transport promotion, and increased cell growth ability. Besides, sonication could change the sensitivity of enzymes and substrates [21,22]. The enzyme activities involved in ethanol synthesis tend to be improved. This might be the reason why ethanol production increased after ultrasonic treatment.

3.2. Single-factor optimization of power density

Next, we investigated the effect of ultrasonic treatment on the biomass, ethanol yield, maximum specific growth rate, and ethanol synthesis coefficient of *Saccharomyces Cerevisiae* under different power densities. From Table 3, the ethanol yield reached its peak when the power density was 180 W/L. The power densities of 60 and 300 W/L inhibited the ethanol production of the yeast (Fig. 2b). Such an occurrence might be that with the continuous increase of power density, the yeast cells were damaged, slowing down the cell proliferation and thus decreasing the yield of ethanol. For very low power density, material transportation was hindered, resulting in its inability to promote cell proliferation and thus weakening the ability of ethanol synthesis.

### Table 2

| Mass spectrometry parameters. | Positive ion | Negative ion |
|------------------------------|--------------|--------------|
| Nebulizer Gas (GS1, PSI)     | 55           | 55           |
| Auxiliary Gas (GS2, PSI)     | 55           | 55           |
| Curtain Gas (CUR, PSI)       | 35           | 35           |
| Ion Source Temperature (°C)  | 550          | 550          |
| Ion Spray Voltage (V)        | 5500         | –4500        |
| Declustering Potential (DP, V)| 80           | –80          |
| Mass Scan Range (TOF MS scan)| 100 – 1000   | 100 – 1000   |
| Collision Energy (TOF MS scan, eV)| 10          | –10          |
| Mass Scan Range (Product Ion scan) | 40 – 1000     | 40 – 1000   |
| Collision Energy (Product Ion scan, eV) | 35          | –35          |
| Interface Heater Temperature (°C)| 550         | 550          |
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Furthermore, the maximum specific growth rate and ethanol synthesis coefficient of 180 W/L power density were significantly higher than other power densities at the same conditions. Therefore, the optimal ultrasonic power density was 180 W/L.

3.3. Single-factor optimisation of treatment time

The effect of ultrasonic treatment with time on the ethanol production in Saccharomyces Cerevisiae is displayed in Table 3. Ultrasonic treatment was able to enhance ethanol yield, notwithstanding the duration. The ethanol yield in Saccharomyces Cerevisiae reached a peak when the treatment time was 24 h. However, biomass and ethanol yield decreased with the upsurge of ultrasonic application time (Fig. 2c). This, on one side, might be due to the shear force caused by sonication cavitation that damages the cell structure making it unconducive for cell survival in extended ultrasonic treatment. Conversely, protracted sonication treatment could decrease the dissolved oxygen in media, affecting yeast metabolic activity, inhibiting cell proliferation, and thus decreasing the biomass and ethanol yield. In addition, the 24 h treatment time gave the best specific growth rate and ethanol synthesis coefficient than other times. Hence, the optimal ultrasonic treatment time was 24 h.

According to the above results, the optimal conditions of ultrasonic treatment were determined as follows: the frequency is 28 kHz, the power density is 180 W/L, and the ultrasonic treatment time is 24 h.

Fig. 2a. Effects of ultrasonic frequency on the ethanol addition (Left) and biomass addition (Right) of Saccharomyces cerevisiae.

Table 3
Effect of ultrasonic frequency, power density and treatment time on biomass, ethanol yield, maximum specific growth rate and ethanol synthesis coefficient.

| Treatment            | Biomass (min) | Biomass (max) | Ethanol yield (max) | μ (max) | σ |
|----------------------|---------------|---------------|---------------------|---------|---|
| Ultrasonic frequency |               |               |                     |         |   |
| Control (0 kHz)      | 0.39 ± 0.04 g/L | 8.55 ± 0.12 g/L | 7.37 ± 0.02%       | 0.2359  | 0.6534 |
| 20 kHz               | 0.42 ± 0.02 g/L | 10.14 ± 0.16 g/L | 7.13 ± 0.14%       | 0.2109  | 0.6529 |
| 23 kHz               | 0.45 ± 0.05 g/L | 10.75 ± 0.47 g/L | 7.86 ± 0.46%       | 0.2112  | 0.7170 |
| 25 kHz               | 0.42 ± 0.03 g/L | 11.47 ± 0.27 g/L | 7.84 ± 0.43%       | 0.2587  | 0.6470 |
| 28 kHz               | 0.33 ± 0.04 g/L | 11.89 ± 0.28 g/L | 9.94 ± 0.13%       | 0.3679  | 0.8136 |
| 33 kHz               | 0.39 ± 0.04 g/L | 10.34 ± 0.42 g/L | 7.86 ± 0.47%       | 0.2273  | 0.6736 |
| 40 kHz               | 0.42 ± 0.01 g/L | 9.95 ± 0.11 g/L  | 8.72 ± 0.04%       | 0.2458  | 0.8053 |

| Power density        |               |               |                     |         |   |
|----------------------|---------------|---------------|---------------------|---------|---|
| Control (0 W/L)      | 0.39 ± 0.04 g/L | 8.55 ± 0.12 g/L | 7.37 ± 0.02%       | 0.2359  | 0.6534 |
| 60 W/L               | 0.37 ± 0.03 g/L | 8.14 ± 0.13 g/L | 6.93 ± 0.07%       | 0.2407  | 0.6365 |
| 100 W/L              | 0.43 ± 0.07 g/L | 9.46 ± 0.21 g/L | 8.25 ± 0.15%       | 0.2405  | 0.6743 |
| 140 W/L              | 0.35 ± 0.02 g/L | 10.69 ± 0.17 g/L | 8.84 ± 0.29%      | 0.2554  | 0.7281 |
| 180 W/L              | 0.33 ± 0.04 g/L | 11.89 ± 0.28 g/L | 9.94 ± 0.13%      | 0.3675  | 0.8136 |
| 220 W/L              | 0.46 ± 0.08 g/L | 10.55 ± 0.29 g/L | 9.41 ± 0.29%      | 0.3423  | 0.7970 |
| 260 W/L              | 0.38 ± 0.07 g/L | 8.64 ± 0.19 g/L  | 7.81 ± 0.06%      | 0.2420  | 0.7036 |
| 300 W/L              | 0.43 ± 0.01 g/L | 7.93 ± 0.08 g/L  | 6.26 ± 0.02%      | 0.2324  | 0.5752 |

| Treatment time       |               |               |                     |         |   |
|----------------------|---------------|---------------|---------------------|---------|---|
| Control (0 h)        | 0.39 ± 0.04 g/L | 8.55 ± 0.12 g/L | 7.37 ± 0.02%       | 0.2359  | 0.6534 |
| 6 h                  | 0.37 ± 0.02 g/L | 9.07 ± 0.23 g/L | 7.96 ± 0.04%       | 0.2451  | 0.6718 |
| 12 h                 | 0.41 ± 0.04 g/L | 10.44 ± 0.17 g/L | 8.84 ± 0.32%      | 0.2471  | 0.6919 |
| 18 h                 | 0.45 ± 0.09 g/L | 11.21 ± 0.14 g/L | 9.43 ± 0.22%      | 0.2461  | 0.7529 |
| 24 h                 | 0.37 ± 0.04 g/L | 11.89 ± 0.28 g/L | 9.94 ± 0.13%      | 0.2675  | 0.8136 |
| 30 h                 | 0.39 ± 0.07 g/L | 10.56 ± 0.37 g/L | 9.21 ± 0.37%      | 0.2394  | 0.7174 |
| 36 h                 | 0.44 ± 0.05 g/L | 9.98 ± 0.29 g/L  | 8.33 ± 0.14%      | 0.2141  | 0.6396 |
| 42 h                 | 0.37 ± 0.01 g/L | 9.33 ± 0.34 g/L  | 8.05 ± 0.05%      | 0.2321  | 0.6584 |
| 48 h                 | 0.46 ± 0.02 g/L | 8.43 ± 0.16 g/L  | 7.63 ± 0.11%      | 0.2269  | 0.6950 |

(a, b, c, d indicated there was significant difference between groups)
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for Illumina transcriptome sequencing. Each group contained three samples with the same treatment. One group was treated with the optimal ultrasonic conditions, and the other was untreated as a control group. Fig. 3a illustrates the distribution of these differentially expressed genes in the total sequenced genes. The differentially expressed genes contained 61 genes, including 14 up-regulated genes and 47 down-regulated genes. GO enrichment analysis was carried out, and the result showed that the 44 differentially expressed genes were annotated into the 146 GO terms, including 72 biological process (BP) terms, 54 molecular function (MF) terms, and 20 cellular component (CC) terms. That result showed in Fig. 3b.

Figure 3c depicts the BP list ranked according to the enrichment scores. Among them, there are some terms related to the synthesis of ethanol, such as D-glucuronic acid metabolic process (GO: 0019521), fructose 1,6-bisphosphate metabolic process (GO: 0030388), and glucose transport (GO: 1904659). The genes GND2 and ZWF1 were annotated into D-glucuronic acid metabolic process [23]. They encoded the glucose 6-phosphatedehydrogenase whose expression increased ~ 2.33 and 2.25 times, respectively. 6-phosphatedehydrogenase regulates the key steps of pentose phosphate pathway, and it is related to the synthesis of NADPH. Overexpression of gene GND2 and ZWF1 might enhance the activity of 6-phosphatedehydrogenase and increase the content of intracellular NADPH. Recent studies have shown that the major obstacle of ethanol synthesis is primarily due to a series of by-products produced in the fermentation process [24]. These by-products could induce the accumulation of reactive oxygen species (ROS) in cells, leading to cell damage and affecting the growth of microorganisms. NADPH produced by the pentose phosphate pathway could avoid damage from ROS and improve the microbial activity of the cell [25]. The gene was FBP1 annotated into fructose 1,6-bisphosphate metabolic process. It encoded the fructose-1,6-bisphosphatase 1 (Fbp1), and its expression down-regulated ~ 2.51 times. High-activity of Fbp1p could induce cell apoptosis with a high accumulation of ROS [26]. Low expression of FBP1 might decrease the activity of Fbp1, ease the damage caused by ROS and increase the activity of yeast [27]. The essence of ethanol production by Saccharomyces cerevisiae fermentation is to convert sugar sources in culture media into ethanol. Glucose source required specific transporter protein when it entered the cell from the medium. In the model yeast Saccharomyces cerevisiae, hexose uptake is mediated exclusively by a family of facilitators (Hxt, hexose transporters) [28]. The gene HXT2 encoded the Hxt2, whose expression up-regulated ~ 3.02 times. High expression of HXT2 might enhance the activity of Hxt2, increase the rate of glucose transportation and raise the content of intracellular glucose. That means that more glucose sources were utilized to produce ethanol.

Fig. 2b. Effects of Power density on the ethanol addition (Left) and biomass addition (Right) of Saccharomyces cerevisiae.

Fig. 2c. Effects of treatment time on the ethanol addition (Left) and biomass addition (Right) of Saccharomyces cerevisiae.
Low expression of the gene PCK1 might decrease the activity of Pck1, undermining the capacity of the reverse reaction, and thus decrease the reverse consumption of pyruvate. This means more pyruvate will be involved in the synthesis of ethanol. The gene ALD2 and ALD3 down-regulated by ~ 1.89 and 2.24 times, respectively. They were annotated into the term of aldehyde dehydrogenase (NAD) activity. ALD2 and ALD3 encoded the acetaldehyde dehydrogenase 2 (Ald2), which oxidizes ethanol into acetaldehyde [30]. The expression of ALD2 and ALD3 was decreased, resulting in reduced Ald2 activity and hindering ethanol oxidation reaction. According to MF analysis, sonication treatment enhanced ethanol production by decreasing the expression of genes related to the reverse reaction of pyruvate and ethanol.

Figure 3e illustrates CC lists ranked according to the enrichment scores. The GO term related to ethanol synthesis is the integral component of membrane (GO: 0016021) and fungal-type cell wall (GO: 0009277). The gene RCK1 was annotated into the integral component of the membrane. Its expression up-regulated ~ 3.36 times. Some studies have demonstrated that RCK1 overexpression might play a role in reducing the oxidative stress caused by acetic acid. Besides, even more interesting is that overexpression of RCK1 significantly improved glucose and xylose fermentation under acetic acid stress conditions. Specifically, the RCK1-overexpressing strain had higher specific ethanol productivity than the control strain in glucose fermentation under the presence of acetic acid [31]. Gene HSP12 was involved in terms of fungal-type cell wall. The study proved that ethanol was able to induce HSP12 protein synthesis and thus increase the capacity of ethanol tolerance and increase yeast activity [32]. According to MF analysis, sonication treatment enhanced ethanol production by increasing intolerance towards acetic acid, whilst improving cell activity.

3.4.2. Kyoto Encyclopedia of genes and Genomes (KEGG) pathway enrichment analysis

KEGG enrichment analysis was performed to explore the mechanism for increased ethanol production. The results indicated that 21 differentially expressed genes were involved in 40 differential metabolism-pathways. Fig. 4 presents a bar chart for the top 20 enriched KEGG pathways ranked according to their value of -log10(P-value). The pathway related to ethanol synthesis mainly entailed carbon metabolism (ko01200), pentose phosphate pathway (ko00030), pyruvate metabolism (ko000620), glutathione metabolism (ko00480), glycolysis/glucoseoneogenesis (ko00010), and citrate cycle (TCA cycle) (ko00020). The carbon metabolism pathway is a major metabolism pathway in microorganisms encompassing the pentose phosphate pathway, glycolysis/glucoseoneogenesis, and TCA cycle (an energy channel). The pyruvate metabolism pathway is key for ethanol synthesis as pyruvate is a vital intermediate. Its content directly influences ethanol production [33]. The glutathione in the glutathione metabolism pathway was able to be accumulated into the term of aldehyde dehydrogenase pathway by the microorganism. This is one of the main rate-limiting steps for yeast to ethanol synthesis. Overexpression of PDC5 might increase Pdc2 activity, leading to accelerated accumulation of acetaldehyde and thus promoting ethanol synthesis. The genes ADH4 and ADH5 encoded the alcohol dehydrogenase 1 (Adh1) and alcohol dehydrogenase 2 (Adh2) [36] respectively. Their expression up-regulated ~ 1.27 and 1.13 times in ethanol synthesis, acetaldehyde was reduced to ethanol by alcohol dehydrogenase, which is another rate-limiting step for producing ethanol. High expression of ADH4 and ADH5 might increase alcohol dehydrogenase activity, increasing the ability of ethanol synthesis.

In addition, this study found the changes in the gene of glucose transporter except for the changes in the intracellular metabolism pathway. Lagunas confirmed that the family of sugar transporters in Saccharomyces cerevisiae consisted of Hxt transporter proteins (1 ~ 17),...
Snf3 and Rgt2 glucose sensors, and galactose transporter, which was encoded by the GAL2 gene [37]. Diderich et al. found that the proteins encoded by gene HXT1 ~ HXT4, HXT6, HXT7, and GAL2 are the main transporters responsible for transporting glucose in Saccharomyces cerevisiae by functional complementation assay [38]. Further, René et al. [39] demonstrated that growth rates in Saccharomyces cerevisiae is in inverse proportion to the expression of gene HXT5. It reflects the growth activity of cells. An increase in HXT5 expression is accompanied by a decrease in the growth rate of cells. The results of this study showed that the expression of HXT1, HXT2, HXT3, and HXT7 genes up-regulated ~ 1.26, 3.02, 1.20, and 1.17 times, respectively, after ultrasound treatment. Overexpression of these genes might increase the activity of proteins encoded by these genes, enhancing the capability of glucose transport and thus raising the level of intracellular glucose. In other words, more glucose might be involved in the synthesis of ethanol. This might be another important reason why the sonication treatment was able to enhance the production of ethanol. The expression of gene HXT5 down-regulated ~ 1.56 times, and its low expression might be due to the good cell activity. This result is consistent with the forecast by the kinetic model.

3.5. Verification of differentially expressed genes

To validate the reliability of the RNA-seq data, we performed qRT-PCR analysis for some differentially expressed genes in transcriptome sequencing. In qRT-PCR analysis, the expression of the selected gene

![Fig. 3c. Biological process list top 30 that ranked according to the enrichment scores.](image-url)

![Fig. 3d. Molecular function list top 30 that ranked according to the enrichment scores.](image-url)
including HXT1, HXT2, HXT3, HXT5, PDC1, PDC5, ADH4, ADH5, ALD2 and ALD3 were ~ 1.25, 1.52, 1.24, 0.63, 1.10, 1.60, 1.25, 1.07, 0.59 and 0.43, respectively. In transcriptome sequencing analysis, the expressions of these genes were ~ 1.26, 3.02, 1.24, 0.64, 1.08, 1.64, 1.27, 1.13, 0.45 and 0.53, respectively. Based on the qRT-PCR analysis, the expression patterns of all these ten tested genes were consistent with those in the RNA-seq data, thereby indicating that the gene expression analysis by the RNA-seq was reliable (Fig. 5).

3.6. Effect of ultrasound on Saccharomyces Cerevisiae metabolites by untargeted metabolomics

In this study, 16 Saccharomyces Cerevisiae samples were prepared for LC-MS analysis, of which eight samples had the same sonication conditions and the rest without sonication served as controls. By analyzing the LC-MS data, it was found that there were 498 differential metabolites, including 283 up-regulated metabolites and 215 down-regulated metabolites. Fig. 6a shows that the PCA model, obtained by 7-fold cross-validation and the model parameters, had a good curve fitting degree. The $R^2_X$ (cum) was 0.627 based on PCA with good pairwise discriminations between the control group. The model parameters of OPLS-DA (Fig. 6b) presented a satisfying explanation and prediction, where the $R^2_Y$ (cum) (0.987), $Q^2$ (cum) (0.836), and $R^2$ (0.894) correlated well. According to the results, the metabolic profiles of the control group subjected to the sonication treatment changed significantly.

3.6.1. Metabolic pathways enrichment analysis

KEGG pathway enrichment analysis was employed to investigate the metabolic pathway changes in yeast after ultrasound treatments. The
results showed 498 differential metabolites involved in the 105 KEGG pathways. According to the significance level value, the histogram showed the differential metabolic pathways of top19 between sonicated samples and control (Fig. 7). The differential metabolic pathways were closely related to the direct synthesis of ethanol, including pyruvate metabolism, TCA cycle, and pentose phosphate pathway. The production of ethanol was likely affected through these metabolisms directly or indirectly. These data were consistent with transcriptome analysis.

3.6.2. Differential metabolites analysis

The untargeted metabolomic analysis results and their KEGG metabolic pathway enrichment analysis showed that the strain with sonication treatment had a significant difference in its intermediate metabolites, such as up-regulated D-glucose and up-regulated NADH, from the ethanol synthesis pathways compared with the control (Metabolite name, retention time (RT), fold change (FC), KEGG ID, subclass, and up or down-regulated were shown in Table 4). Glucose content directly regulated ethanol production. The D-glucose and NADH up-regulated ~1.28 and ~1.66 times, respectively. This result indicated that more glucose might involve in the synthesis of ethanol and thus increase ethanol yield. In the ethanol synthesis pathway, acetaldehyde was reduced to ethanol under alcohol dehydrogenase and NADH catalysis, in which NADH provided the reducing power. Up-regulation of NADH might promote ethanol synthesis. D-glutamine and glutathione up-regulated ~1.33 and 1.51 times, respectively. Glutamine is

| Metabolites          | RT (min) | FC  | KEGG ID  | Sub Class                                     | Red/Blue |
|----------------------|----------|-----|----------|-----------------------------------------------|----------|
| D-glucose            | 0.863    | 1.28| O00031   | Carbohydrates and carbohydrate conjugates     | Red      |
| Glucose 6-phosphate  | 0.763    | 1.47| O00092   | Carbohydrates and carbohydrate conjugates     | Red      |
| NADH                 | 2.098    | 1.66| O00003   | –                                              | Red      |
| D-glutamine          | 0.751    | 1.33| O00064   | Amino acids, peptides, and analogues           | Red      |
| Glutathione          | 10.635   | 1.51| O02471   | Amino acids, peptides, and analogues           | Red      |
| Proline              | 4.953    | 1.76| O00763   | Amino acids, peptides, and analogues           | Red      |
| Glycine              | 4.170    | 1.41| –        | –                                              | Red      |
| Acetic acid          | 2.010    | 0.854| O0164    | Short-chain keto acids and derivatives         | Blue     |
| Indoleacetic acid    | 5.394    | 0.816| O00954   | Indolyl carboxylic acids and derivatives       | Blue     |
| L-lactic acid        | 6.564    | 0.582| –        | –                                              | Blue     |
| β-lactic acid        | 5.922    | 0.674| –        | –                                              | Blue     |
the crucial limiting amino acid for intracellular glutathione synthesis. Up-regulation of glutamine is suitable for synthesizing glutathione. As an important antioxidant, glutathione takes part in many physiological processes. Up-regulation of glutathione might increase cell activity, promote cell growth and metabolism. These are beneficial for ethanol synthesis.

Moreover, the contents of proline and glycine were up-regulated ~1.76 and 1.41 times, respectively. Some studies have shown that proline and glycine play a key role in yeast-ethanol tolerance [40,41]. Generally, when ethanol concentration reached 10% (V/V) in the fermentation broth, the capacity of yeast to synthesize ethanol would be inhibited [42]. That is because the high level of ethanol can inhibit the growth and survival of the cell. Up-regulation of proline and glycine might increase ethanol tolerance, promote ethanol synthesis and thus enhance ethanol yield. In addition, metabolomics data showed that the content of acetic acid and its derivative, indoleacetic acid, in yeast down-regulated by ~1.17 and 1.23 times, respectively. Besides, L-lactic acid and β-lactic acid down-regulated ~1.72 and 1.48 times, respectively. Some studies had shown that organic acids such as acetic acid and lactic acid could inhibit the growth and reproduction of cells [43]. The accumulation of organic acids is not conducive to the synthesis of ethanol [44]. Low expression of acetic acid and lactic acid might decrease damage to yeast cells. This is beneficial for ethanol synthesis.

3.7. Conjoint analysis of transcriptomic and metabolomic

To analyze the effect of ultrasound on ethanol production more intuitively, the ethanol synthesis pathway was drawn according to the transcriptomic and metabolomic data exhibited in Fig. 8. The previous study of our group found that the cell membrane permeability increased after sonication treatment [45]. Therefore, the cell surface will form many repairable micropores due to the cavitation effect of ultrasound. These micropores might allow extracellular glucose to enter into intracellular areas without extra transporters and thus increase intracellular glucose content.

Besides that, ultrasound treatment increased the gene expression, which encodes the glucose transporter family. These changes will increase the activity of glucose transporters and enhance the content of intracellular glucose. The metabolomic data also demonstrated that the intracellular glucose content was increased. That is to say, more glucose was involved in ethanol synthesis to increase the final ethanol product. On the other side, the transcriptomic data showed that the ADH4 and ADHS genes encoded the alcohol dehydrogenase family, improving their expression. The changes might promote ethanol synthesis due to the increased activity of alcohol dehydrogenase. According to the metabolomic data, the results indicated that the content of NADH was enhanced. NADH provided reducing power in the pathway of ethanol synthesis. Alcohol dehydrogenase combined with NADH to complete the conversion of acetaldehyde to ethanol. A high level of NADH and high alcohol dehydrogenase activity were keys to increasing the ethanol content in yeast. Without a doubt, acetaldehyde is not only converted to ethanol by alcohol dehydrogenase catalysis but also to acetic acid by acetaldehyde dehydrogenase in the pathway of ethanol synthesis. The results of transcriptomic showed that the ALD2 and ALD3 genes down-regulated. These genes encoded the acetaldehyde dehydrogenase enzyme and regulated the conversion reaction of acetaldehyde to acetic acid. Low expression of ALD2 and ALD3 might decrease the acetaldehyde dehydrogenase activity and the acetic acid content. The data of metabolomic also found that the content of acetate and its derivatives were reduced. Ultrasonic treatment might change acetic acid synthesis and decrease the acetate content attributed to decreasing the acetaldehyde dehydrogenase activity. By this, more acetaldehyde is converted to ethanol, increasing overall ethanol yield.

4. Conclusion

This study described a phenotype of ethanol yield enhancement in Saccharomyces cerevisiae after ultrasonic treatment. The ethanol yield increased by 34.87% under the optimum ultrasonic conditions of fixed frequency mode, 28 kHz frequency, 180 W/L power density, and 24 h processing time. The results of non-linear fitting in the kinetic model showed remarkable improvements in cell growth activity and ethanol yield.
synthesis. Further investigations, including gene expression profiles and characterization of related metabolites treated at optimum conditions, were performed to explain ethanol yield enhancement mechanisms. The results showed that higher ethanol yield was obtained by diverse effects of ultrasonic treatment on yeast fermentation. These included increased glucone transporter activity, enhanced intracellular glucose content, increased alcohol dehydrogenase activity, accelerated ethanol synthesis, decreased acetaldehyde dehydrogenase activity, and lessened pyruvate to acetic acid conversion. In addition, ultrasonic treatments increase the intracellular reducing power, for instance, NADH, NADPH, and glutathione for resisting oxidative stress. To increase the cell activity and promote cell growth contributed to ethanol synthesis. These changes in gene expression profiles and metabolites might explain why the ultrasound increases the ethanol yield in Saccharomyces Cerevisiae.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] A. Dutta, Are global ethanol markets a ‘one great pool’?, Biomass Bioenergy 132 (2020) 105436.105436-105436.105436.
[2] L. Yan, Z. Wei, C. Li, K. Sakakibara, S. Tanaka, H. Kong, Factors affecting ethanol fermentation using Saccharomyces cerevisiae BV4742, Biomass Bioenergy 47 (2012) 395–401.
[3] S.C. Galbraith, H. Bhatia, H. Liu, S. Yoon, Media formulation optimization: current and future opportunities, Curr. Opin. Chem. Eng. 22 (2018) 42–47.
[4] Y. Tao, F. Yu, Y. Han, F. Chemat, D. Li, P.L. Show, Insight into mass transfer during ultrasound-enhanced adsorption/desorption of blueberry anthocyanins on macroporous resin by numerical simulation considering ultrasonic influence on resin properties, Chem. Eng. J. 380 (2020), 125230.
[5] Y. Tao, D. Li, W.S. Chai, P.L. Show, X. Yang, S. Manickam, G. Xie, Y. Han, Comparison between airborne ultrasound and contact ultrasound to intensify air drying of blackberry: heat and mass transfer simulation, energy consumption and quality evaluation, Ultrason. Sonochem. 72 (2021), 105410.
[6] Z.L. Zhang, F. Xiong, R.H. He, Fermentation of Saccharomyces cerevisiae in a one liter flask coupled with an external circulation ultrasonic irradiation slot: influence of ultrasonic mode and frequency on the bacterial growth and metabolism yield, Ultrason. Sonochem. 54 (2019) 39–47.
[7] Y. Chisti, Sonobioreactors: using ultrasound for enhanced microbial productivity, Trends Biotechnol. 21 (2003) 89–93.
[8] B.D. Dahroud, R.R. Mokarram, M.S. Khajam, H. Hamishehkar, A.Z. Bialvaei, Comparison between airborne ultrasound and contact ultrasound to intensify air drying of blackberry: heat and mass transfer simulation, energy consumption and quality evaluation, Ultrason. Sonochem. 72 (2021), 105410.
[9] E.J. Ok, N. Wei, S. Kwak, H. Kim, Y.S. Jin, Optimization of HCl improves acetic acid tolerance in Saccharomyces cerevisiae, J. Biotechnol. 292 (2021) 1–4.
[10] K. Sales, W. Brandt, E. Rumbak, G. Lindsey, The LEA-like protein HSP 12 in Arabidopsis thaliana enhances growth of plants under osmotic stress, J. Biophys. Biochem. Cytol. 164 (2004).
[11] G. Huang, S. Chen, Effects of ultrasound on microbial growth and enzyme activity, Ultrason. Sonochem. 37 (2017) 144–149.
[12] M. Kang, S. Han, The effect of ultrasound on the growth of Lactobacillus casei subsp. casei 431 and its metabolites, Biotechnol. Bioprocess Eng. 13 (2008) 615–621.
[13] V.M. Williamson, C.E. Paquin, Homology of Saccharomyces cerevisiae ADH4 to an ethanol dehydrogenase from Schizosaccharomyces pombe, J. Bacteriol. 177 (1995) 90–96.
[14] H.U. Chun-Keng, Protein amino acid composition of plasma membranes affects glucose transport in Saccharomyces cerevisiae, Ultrason. Sonochem. 36 (2017) 191–197.
[15] J. Boonstra, HXT5 expression is determined by growth rates in Saccharomyces cerevisiae, J. Biol. Chem. 274 (1999) 15350–15356.
[16] W.H. Park, Enhanced cell death effects of MAP kinase inhibitors in propyl gallate-treated cancer cells, Toxicol. In Vitro 24 (2010) 1172–1178.
[17] B. Meng, D. Li, Z. Li, Y. Chen, L. Zhang, Z. Ye, H. Li, R. Li, X. Lu, B. Zhang, S. Liu, NADPH oxidase inhibitor, diphenylethlenodinitrile prevents necroptosis in HK-2 cells, Biomed. Rep. 7 (2017).
[18] H. Boelens, M.D. Mattos, K.V. Dam, Glucose uptake kinetics and transcription of ZWF1, GND1, RPE1, and TKL1 in Saccharomyces cerevisiae, Appl. Microbiol. Biotechnol. 61 (2003) 339–349.
[19] T.G. Leighton, R.E. Apfel, The acoustic bubble, J. Acoust. Soc. Am. 96 (4) (1994) 2029–2039.
[20] J. Yilmaz, V. Gnanaraj, M. Junge, C. Melzer, C. Sander, C. Rohde, The role of the heat shock protein HSP26 in the response to osmotic stress in Saccharomyces cerevisiae, J. Microbiol. 61 (2003) 130–137.
[21] Y. Tao, F. Yu, Y. Han, F. Chemat, D. Li, P.L. Show, Insight into mass transfer during ultrasound-enhanced adsorption/desorption of blueberry anthocyanins on macroporous resin by numerical simulation considering ultrasonic influence on resin properties, Chem. Eng. J. 380 (2020), 125230.
[22] G. Huang, S. Chen, Effects of ultrasound on microbial growth and enzyme activity, Ultrason. Sonochem. 37 (2017) 144–149.
[23] B. Meng, D. Li, Z. Li, Y. Chen, L. Zhang, Z. Ye, H. Li, R. Li, L. Xu, B. Zhang, S. Liu, NADPH oxidase inhibitor, diphenylethlenodinitrile prevents necroptosis in HK-2 cells, Biomed. Rep. 7 (2017).
[24] E. Herker, H. Jungwirth, K.A. Lehtmann, C. Maldener, Chronological aging leads to apoptosis in yeast, J. Cell Mol. Biochem. 87 (2004) 35–52.
[25] J. Yilmaz, V. Gnanaraj, M. Junge, C. Melzer, C. Sander, C. Rohde, The role of the heat shock protein HSP26 in the response to osmotic stress in Saccharomyces cerevisiae, J. Microbiol. 61 (2003) 130–137.
[43] J. Zaldivar, L.O. Ingram, Effect of organic acids on the growth and fermentation of ethanologenic Escherichia coli LY01, Biotechnol. Bioeng. 66 (2010) 203–210.

[44] E.P. Almqvist, H. Grage, N.Q. Meinander, Main and interaction effects of acetic acid, furfural, and p-hydroxybenzoic acid on growth and ethanol productivity of yeasts, Biotechnol. Bioeng. 63 (1999) 46–55.

[45] R. He, W. Ren, J. Xiang, M. Dabbour, B.K. Mintah, Y. Li, H. Ma, Fermentation of Saccharomyces cerevisiae in a 7.5 liter ultrasound-enhanced fermenter: Effect of sonication conditions on ethanol production, intracellular Ca2+ concentration and key regulating enzyme activity in glycolysis, Ultrason. Sonochem. (2021), 105624.