Effect of sound exposure on the growth and intracellular macromolecular synthesis of *E. coli* k-12 (#7855)

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Effect of sound exposure on the growth and intracellular macromolecular synthesis of *E. coli* k-12

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Title Page

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Abstract

The sound wave (20-20000 Hz) is an important environment factors in natural word. However, the study of sound exposure on living organisms has been mostly focused on infrasound and ultrasound, and there exist relatively few studies describing the influence of audible sound waves. In this paper, we studied the biological effects of sound exposure on the growth of *E. coli* K-12 with different acoustic parameters to reveal a preliminary sound exposure dose-efficacy relationship. In addition, we also examined the intracellular macromolecular synthesis and cellular morphology of *E. coli* K-12 under sound exposure. Experimental results indicated that *E. coli* K-12 exposed to sound waves can harvest a higher biomass and a faster specific growth rate compared to the control group. Especially the maximum biomass and maximum specific growth rate was about 172.7% and 245.6% higher than the control group respectively, when *E. coli* K-12 was exposed to sound frequency 8KHz, intensity 80dB and power 61dB. We also found that *E. coli* K-12 responded rapidly to sound stress at both the transcriptional and posttranscriptional levels by promoting the synthesis of intracellular RNA and protein. And the average length of *E. coli* K-12 cells increased more than 27.3% under sound exposure compared to the control group. Therefore, our results provide a way to understand the biological effects of sound waves on microorganism growth and metabolism.

Keywords sound exposure; *E.coli* K-12; biomass; cellular morphology; intracellular macromolecular.

Introduction

The sound wave is a key component of environmental factors (Levin, 1995). It was roughly classified into three regimes by its frequency: infrasound (10^{-4}-20Hz), audible sound (20-2 × 10^4Hz) and ultrasound (2 × 10^4-10^{12}Hz). The study of sound exposure on living organisms has been mostly focused on infrasound and ultrasound (Leighton, 2007; Leventhall, 2007). Moreover, infrasound and ultrasound have already been successfully applied in medicine (Rokhina, Lens & Virkutyte, 2009; Anastassiades & Petounis, 1976). Little research concentrates on audible sound induced biological effects and its possible mechanism.

Recently the biological effects induced by audible sound waves in multicellular organisms were investigated. For example, sound stimulation could greatly enhance the germination rate of *Echinacea angustifolia* seeds (Duan et al., 2003), increase the activity of roots and the content of soluble protein of chrysanthemum (Jia et al., 2003), affect the physical state and metabolism of membrane lipid (Zhao et al., 2002). Cai et al. (2014) also showed that significant increase of growth was found in mung bean under treatments of sound wave intensity around 90dB and frequency around 2000Hz. Karippen, Dayou & Chong (2009) observed that the frequencies of 5 KHz, 10 KHz and 15 KHz showed inhibition on the growth of Aspergillus Spp. However, it brought great difficulties to reveal the mechanism of biological effects induced by sound waves, due to the complexity of multicellular organisms. Recently some work has done to study the mechanism of the sound waves biological effects using single-cell microbes. Cai et al. (2013) noted that sound waves increased the biomass of algae at 2200Hz. Ying, Dayou & Chong KP (2009) revealed that sound waves could markedly promote the growth of *E. coli* at 5 kHz. Reguera (2011) put forward that intercellular communication model in bacteria by sonic wave based on previous researches. However, so far the proper mechanism of sound effects on microorganisms is still unknown.

In this paper, we focused on the biological effects of sound waves on *E. coli* K-12 to investigate preliminary sound exposure dose-efficacy relationship on growth with different parameters under laboratory condition. Forthermore, intracellular macromolecular synthesis in *E. coli* K-12 exposed to sound waves stress was discussed. Cellular morphology of *E. coli* K-12 exposed to sound waves was also observed for the first time. This work would provide a solid basis for investigating the underlying mechanism of sound waves biological effects.

Materials and methods

E. coli strain

E. coli K-12 (4401, from The Coli Genetic Stock Center) was first cultured in LB slant agar medium at 37 ℃ for 24 h. Then cells expanded in a 250 ml conical flask containing 100 ml of

LB liquid medium with agitation of 180 rpm on a rotary shaking incubator at 37 ℃ for 10 h.

Sound exposure experiments

Sound exposure test were performed in the experimental installations (Fig. 1). It was same with our previous equipment (Gu et al., 2010) except that the speakers were put in nutrient solution and the rotating sample holder was replaced by magnetic stirrer. *E.coli* K-12 were exposed to three different conditions: sound frequency (250-16000 Hz), recording sound intensity 80dB and sound power 55dB; sound intensity (0-100 dB), recording sound frequency 8KHz and sound power 55dB; sound power (55-63 dB), recording 8 KHz and 80 dB. Control conditions included only the mechanical noise of the incubator room at approx 50 dB. Samples without sound exposure served as a control group. The temperature within the sound waves load apparatus was maintained at 37℃. The sound exposure were performed continuously in the whole experiment, and the magnetic stirrer was internal of 15 min, strring for 5 min.

Measurement of biomass and maximum specific growth rate (μ_{max})

The biomass of *E. coli* K-12 was represented by maximum optical density. Optical density of culture broth was measured at 600 nm using a spectrophotometer (UV754N, Shanghai Aucy Scientific Instrument). Cell dry weight was performed according to the method of being dried for six hours at 70℃, and the specific growth rate μ was calculated as follow:

$$\mu = \frac{\Delta m}{m\Delta t}$$

Where m is the whole dry cell weight, μ is the specific growth rate, Δm is the addition of dry cell weight in Δt hours.

Measurement of *E. coli* K-12 intracellular protein and RNA

The culture was sampled every 6 h, and then concentrated or diluted to 1 (OD_{600}). Protein was extracted using Bacterial Protein Extraction Kit (BS596, Sangon Biotech (Shanghai) Co, Ltd, China) and quantified by Modified BCA Protein Assay Kit (SK3051, Sangon Biotech (Shanghai) Co, Ltd, China). Total RNA was extracted by Hipure Bacterial RNA Kit (R4181-01, Magen, China) and quantified by a spectrophotometer （DS-11, DENOVIX, USA）.

Morphologic observation of *E. coli* K-12

Under sound wave frequency 8KHz and power 61dB, *E. coli* K-12 was exposed to intensity 80dB and 100dB respectively. cells were sampled at 48h, centrifuged, washed with distilled water, dehydrated using graded ethanol (20%, 50%, 80%, 100%), and then dissoved in distilled water. Samples were dried on glass slides, and then a layer of metal film was plated on the surface of glass slide in an vacuum evaporator. Morphology observation of *E. coli* K-12 was performed by scanning electron microscope (SEM) (JSM-5610LV, JEOL, Japan). Size of twenty cells were measured randomly.

Statistical analysis.

All the experiments were performed in triplicate, and measurements are reported as mean ± standard deviation (SD). Statistical analysis was performed by applying variance (ANOVA) multiple comparison (single factor) in SPSS. Treatment effects were considered to be significant at $P< 0.05$.

Results

Effect of different acoustic parameters on the growth of *E. coli* K-12

Effect of sound frequency on *E. coli* K-12

The biomass and μ_{max} of *E. coli* K-12 was measured in all the groups treated with different frequency sound waves. As shown in Fig 2, the biomass of *E. coli* K-12 was increased after audible sound treatments. Significant differences ($P<0.001$) in biomass were observed when *E. coli* K-12 exposed to sound frequency 2 KHz and 8 KHz, which were about 121.0% and 127.1% higher than the control group respectively. Meanwhile, exposure of *E. coli* K-12 to 2 KHz and 8KHz sound waves also led to an increase of the μ_{max}, reflecting a faster growth of the treated group than the control group. The μ_{max} of the treated *E. coli* K-12 with 2 KHz and 8 KHz were 1.951 h^{-1} and 1.961 h^{-1}, about 124.9% and 125.5% higher compared to the control group (1.562 h^{-1}) respectively. The behavior of the treated *E. coli* K-12 strongly suggested that sound waves accelerateed the *E. coli* K-12 growth and the biological effects induced by sound waves had a non-linear relationship with frequency, and showed obvious frequency peculiarities.

Effect of sound intensity on *E. coli* K-12

Under sound frequency 8 KHz and power 55 dB, *E. coli* K-12 was exposed to sound waves with different intensity. We found that the biomass of the *E. coli* K-12 were significantly higher in the treated group with sound intensity 80dB compared to the control group. A rapid increase of biomass in the treated group was observed reaching a maximum of 1.371 (OD[illegible]) with sound intensity 80 dB, about 27[illegible]% high in the treated group as compared with the control group, and then it decreased sharply (Fig. 3). And the μ_{max} of *E. coli* K-12 increased sharply and reached the peak value at sound intensity 80 dB, and then enhanced slowly (Fig. 3). Particularly when the sound intensity was 100 dB, the μ_{max} (2.151 h^{-1}) was 137.7% higher than the control group (1.562 h^{-1}). Moreover, we also found that the ogar[illegible]mic phase in the experimental group exposed to sound intensity 80 dB was 121.1% longer than sound intensity 100dB (data was shown in Figure S1), and it explained the reason why the μ_{max} enhanced slowly but the biomass redued sharply, when the sound intensity was increased from 80dB to 100dB.

Effect of sound power on *E. coli* K-12

From fig. 4, we observed the growth of *E. coli* K-12 exposed to different sound power increased greatly. The biomass presented an approximate linear growth with the increase of sound power and reached the peak value at 59 dB, while it displayed slowly increasing from sound power 59 dB to 61 dB and then reduced sharply. The maximum biomass of *E. coli* K-12 treated with sound power 61 dB was 1.863 (OD600), about 172.7% higher than the control group (OD600 1.079). The μ_{max} of *E. coli* K-12 was elevated rapaidly to the peak at 61 dB, then it fell sharply. Particularly when *E. coli* K-12 were exposed to sound power 61 dB, the μ_{max} (3.837 h^{-1}) was 245.6% higher than the control group (1.562 h^{-1}). While the sound power exceeded 61dB, both biomass and μ_{max} began to reduce, which could reflect that excess sound exposure might had a negative effect on the growth of *E. coli* K-12.

Effect of sound exposure on intracellular macromolecular synthesis in *E. coli* k-12

As shown in fig. 5, we studied the effects of sound waves on the intracellular macromolecular of *E. coli* K-12 with frequency 8 KHz, intensity 80 dB and power 61 dB and found that certain sound exposure significantly affected the intracellular protein and RNA in *E. coli* k-12. The intracellular protein and RNA both in the treated group and the contol group reduced slowly with time on. Under sound exposure, the concentration of intracellular protein presented a significant increase in the treated group at 6 h, and the value of intracellular protein in the treated gtoup reached 566.4 mg/g, 110.8% higher than the control group (511.1 mg/g). The concentration of the intracellular RNA of *E. coli* K-12 also increased singnificantly in the treated group at 6 h. When *E. coli* K-12 were continuously exposed to sound waves for 6 h, the intracellular RNA (113.0 mg/g) in treated group was 125.4% higher than the control group (90.1 mg/g). So we concluded that sound exposure can significantly promote synthesis of the intracellular protein and RNA of *E. coli* K-12 in the early treatment stages, which was in favor of cell division.

Morphological change of *E. coli* K-12 cell exposed to sound waves

E. coli K-12 cellular morphology was observed at 48h in treated group and control group (Fig.6). The length and width of *E. coli* K-12 cell was measured with the software carried by SEM. It was found that the average length of *E. coli* K-12 thallus reached 2.060±[illegible]85 (80 dB) and 2.395±0.904 (100 dB) respectively compared to the control group (1.882±0.375), and its length increases more than 27.3% under sound intensity 100dB compared to the control group. But difference was not observed in width.

Discussion

From the results, we noted that the sound waves with frequency 2 KHz and 8 KHz were the best to promote the growth of *E. coli* K-12. The results suggested that the action of sound waves showed obvious frequency peculiarities. Chen (2013) showed that sound waves with main frequency such as 2 KHz in environment of wild plants had better effects on plant growth than other kinds of audible sound. Cai et al. (2014) reported that the germination period of mung bean was reduced after audible sound treatments with 1.0-2.5 KHz, and the PO algae under exposure of sound waves with frequency of 2200 Hz had greatly significant increase in dry biomass (Cai et al., 2013). We also found better growth appeared at 2 KHz and 8 KHz. Sarvaiya & Kothari

(2015) reported that all the bacteria and yeasts used as test organisms were found to register better (3.15–40.37% higher) growth under the influence of music, except *Serratia marcescens.* However, all sonic stimuli tested reduced biomass production of the yeast cells by 14% (Aggio, Obolonkin & Villas-Bôas, 2012). So it suggested that different organisms might respond to the same sound with identical frequency differently.

Sound wave (20 - 20000Hz) is a mechanical wave (Gu et al., 2010), if it is moving through *E. coli*, then cells will be displaced both rightward and leftward as the energy of sound wave passes through them resulted in biological effects. The investigations of sound waves exposed to different intensity and power showed that sound stimulation at a certain strength can promote the growth of *E. coli* K-12. Sun & Cai (1999) and Shen et al. (1999) also found that sound stimulation can benefit the absorb of nutriment and synthesis of DNA in S period of tobacco cells, as well as synchronized the cell cycle, and promote the fluidity of membrane wall and membrane lipid. However, we also found that *E. coli* K-12 suffered a obvious decrease in growth with sound power at 63 dB as compared with 61 dB or sound intensity at 100 dB as compared with 80 dB. Li et al. (2001) noted that when the sound intensity increase from 100 dB to 110 dB, the number of tobacco cells in S period reduced greatly. Consequently, excess sound exposure might had a negative effect on the growth of *E. coli* K-12.

As shown in fig. 5, sound exposure could significantly promote synthesis of the intracellular protein and RNA in the early treatment stages. The value of intracellular protein and RNA at 6 h reached 566.4 mg/g and 113.0 mg/g in the treated group, 110.8% and 125.4% higher than the control group respectively. It was also reported that sound stimulation can promote the synthesis of intracellular molecules such as protein (Yang, 2013), RNA (Wang et al., 2003) and DNA (Li et al., 2001) in plant. So the results indicated that living organisms could respond the sound stress rapidly to modulate gene expression at both the transcriptional and posttranscriptional levels. Furthermore, Schaechter, Maaloe & Kjeldgaard (1958) concluded that not only cell mass, but also nucleic acid and protein content were a function of growth rate rather than the composition of the medium used to achieve that growth rate. So our results that the increase of the intracellular protein and RNA of *E. coli* K-12 was consisitent with the promotion of μ_{max} under sound exposure.

We also found that the average length of *E. coli* K-12 exposed to sound intensity 100 dB increased more than 27.3% compared to the control group, but difference was not observed in width. It was reported that *E. coli* had control mechanism on its cell size (Taheri-Araghi et al., 2014). Vadia & Levin (2015) used a picture to suggest that cell size was a complex phenomenon and had a linear function of nutrient availability and growth rate. So the increase of the average length may be another reason for the benefit on growth of *E. coli* K-12 under sound exposure.

Results of this study indicated that sound while travelling through microbial suspensions created a kind of mechanical stress, which can be sensed by a growth vessel inside cells, and living organisms including microbes can rapidly respond to the stress at both the transcriptional and posttranscriptional levels. However, the mechanism of sound exposure on microorganism growth is still unkown. As we all know, bacteria dominated the increasing cell-population density by quorum sensing (Taj et al., 2014), and *E. coli* had a whole quorum sensing system with AI-2 as signal molecule (Ting X, 2009). From the results in this paper, we can see that the cell-population density of *E. coli* K-12 incressed greatly without addition of any nutriments compared to the control group. It suggested that sound exposure may help *E. coli* K-12 break down the density threshold. Besides, our previous research revealed that sound waves treated culture of *E. coli* registered an increase in intracellular calcium (Gu et al., 2012). It is well kown that Ca^{2+}, as a second messenger, plays an important role in life activities of microbes (Ren et al., 2009). So Further work on the production of AI-2 and the concentration of intracellular calcium in *E. coli* K-12 that occurs in response to sound waves will certainly give new insights into the interaction of microbes with sound exposure in general.

Funding

This work was supported by National Natural Science Foundation of China (Grant No. U1304307) and the Young Core Instructor Foundation from the Education Commission of Henan Province, China (Grant No. 2014GGJS-056).

Conflict of interest statement

The authors declare that they have no competing interests.

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Figure legends

Fig. 1 Schematic of sound waves load apparatus: a-sound waves source; b-sound waves transmission conductor; c-speaker; d-ultraviolet light; e-beaker; f-metal case; g-sound-absorbing material; h-magnetic stirrer

Fig. 2 Effect of sound frequency on the growth of *E. coli* K-12. All experiments were exposed to sound intensity 80 dB and power 55 dB. Asterisks indicate significance: *** $p < 0.001$, ** $0.001 < p < 0.01$, * $0.01 < p < 0.05$.. Vertical bars represent means ± SD.

Fig. 3 Effect of sound intensity on the growth of *E. coli* K-12. All experiments were exposed to sound frequency 8 KHz and power 55 dB. Asterisks indicate significance: *** $p < 0.001$, ** $0.001 < p < 0.01$, * $0.01 < p < 0.05$. Vertical bars represent means ± SD.

Fig. 4 Effect of sound power on the growth of *E. coli* K-12. All experiments were exposed to sound frequency 8 KHz and intensity 80 dB. Asterisks indicate significance: *** $p < 0.001$, ** $0.001 < p < 0.01$. Vertical bars represent means ± SD.

Fig. 5 The total intracellular protein and RNA of *E. coli* K-12 exposed to sound wave at different time. (a) The total intracellular protein. (b) The total intracellular RNA. All experiments were exposed to sound frequency 8 KHz, intensity 80 dB and power 61dB. Asterisks indicate significance: *** $p < 0.001$, ** $0.001 < p < 0.01$.Vertical bars represent means ± SD.

Fig. 6 The pictures of *E. coli* K-12 cell of SCE. (a) The cells in the control group. (b) The cells exposed to sound frequency 8 KHz, intensity 80 dB and power 61 dB. (c) The cells exposed to sound frequency 8 KHz, intensity 100 dB and power 61 dB. Cells were sampled at 48 h.

1

Fig. 1 Schematic of sound waves load apparatus

a-sound waves source; b-sound waves transmission conductor; c-speaker; d-ultraviolet light; e-beaker; f-metal case; g-sound-absorbing material; h-magnetic stirrer

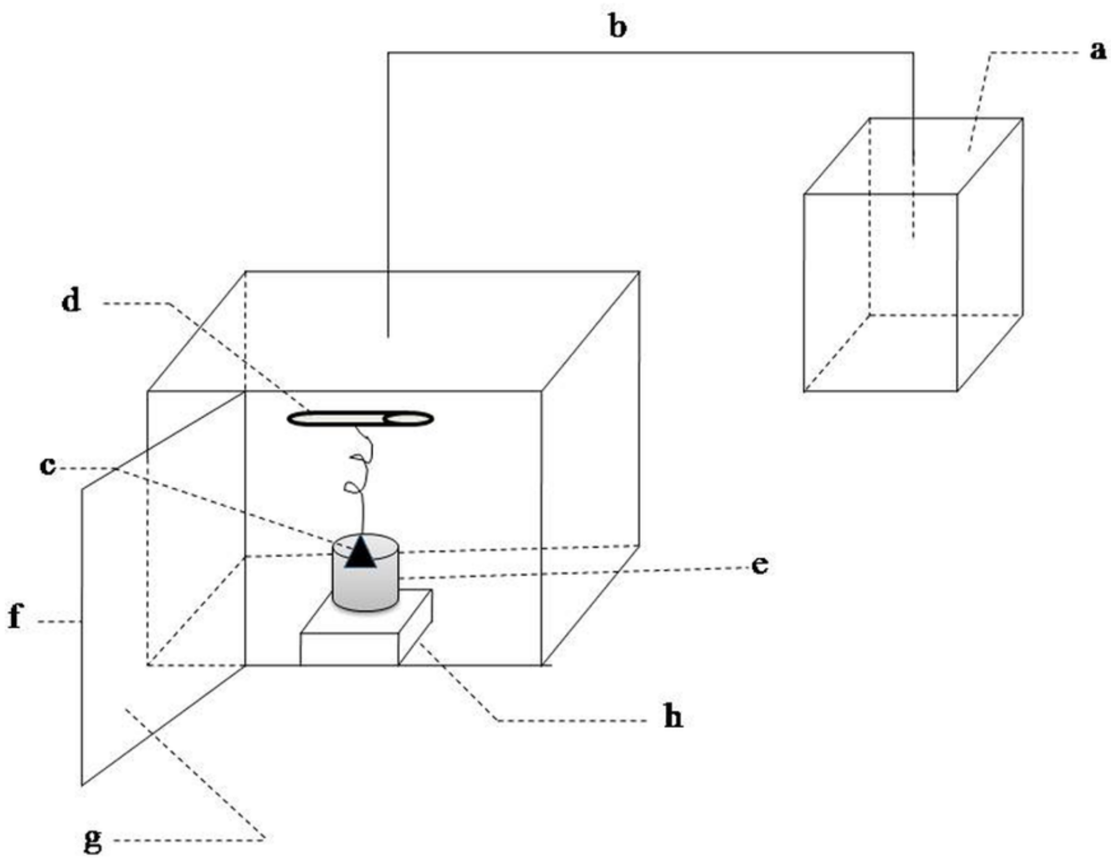

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Fig. 2 Effect of sound frequency on the growth of *E. coli* K-12

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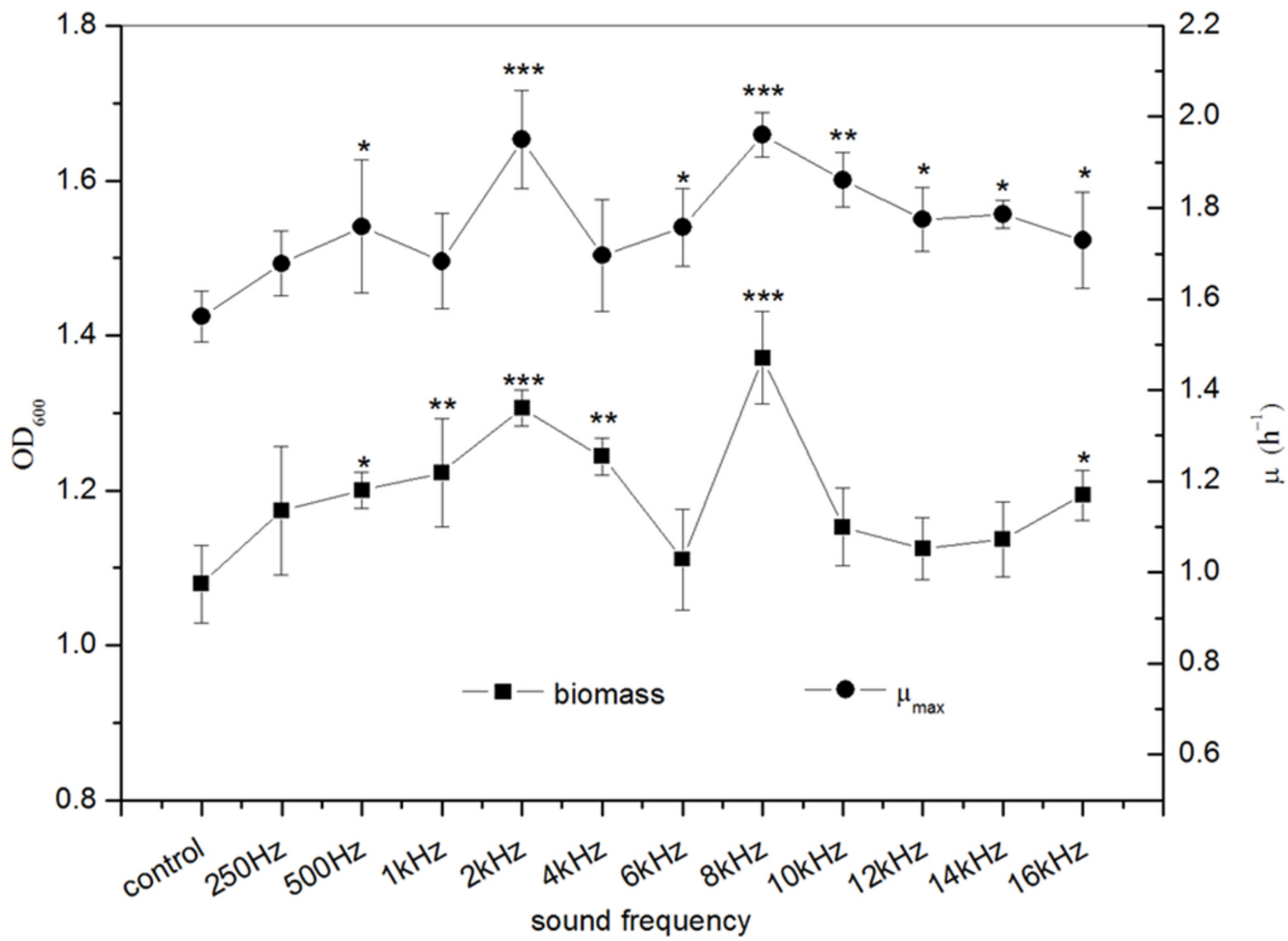

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Fig. 3 Effect of sound intensity on the growth of *E. coli* K-12.

Effect of sound intensity on the growth of *E. coli* K-12. All experiments were exposed to sound frequency 8 KHz and power 55 dB. Asterisks indicate significance: *** $p < 0.001$, ** $0.001 < p < 0.01$, * $0.01 < p < 0.05$. Vertical bars represent means ± SD.

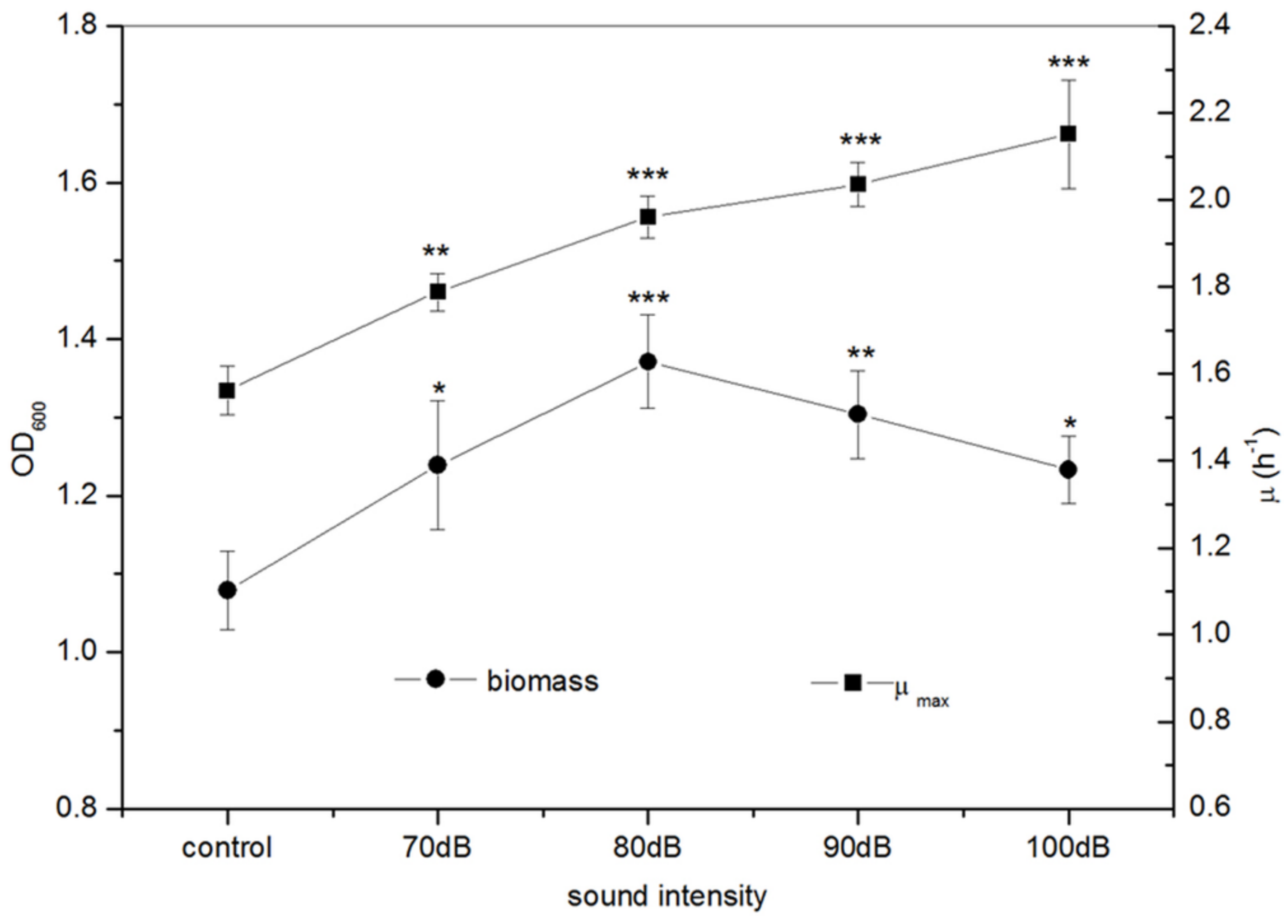

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Fig. 4 Effect of sound power on the growth of *E. coli* K-12.

Effect of sound power on the growth of *E. coli* K-12. All experiments were exposed to sound frequency 8 KHz and intensity 80 dB. Asterisks indicate significance: *** p < 0.001, ** 0.001 < p < 0.01. Vertical bars represent means ± SD.

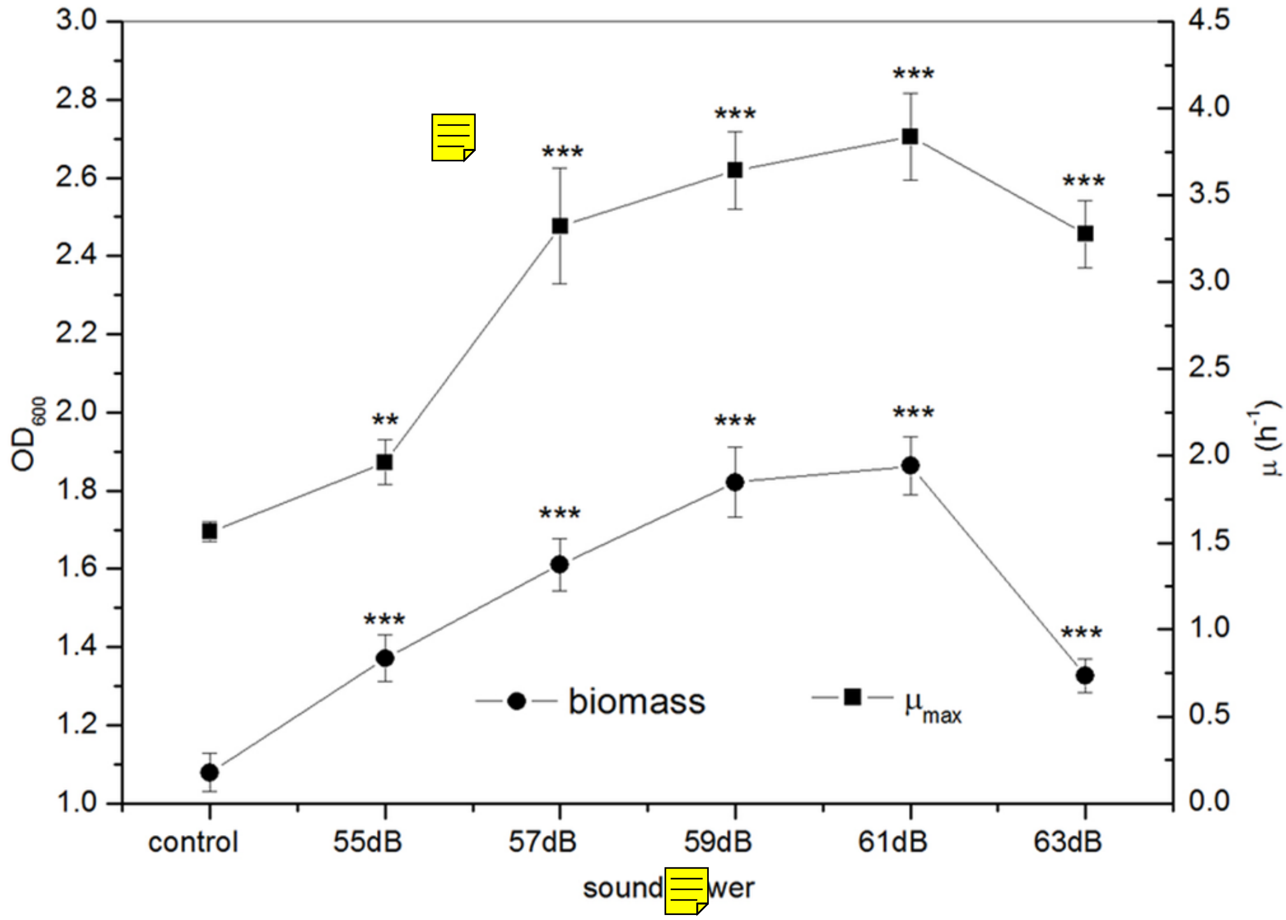

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Fig. 5 The total intracellular protein and RNA of *E. coli* K-12 exposed to sound wave at different time.

The total intracellular protein and RNA of *E. coli* K-12 exposed to sound wave at different time. (a) The total intracellular protein. (b) The total intracellular RNA. All experiments were exposed to sound frequency 8 KHz, intensity 80 dB and power 61dB. Asterisks indicate significance: *** p < 0.001, ** 0.001 < p < 0.01.Vertical bars represent means ± SD.

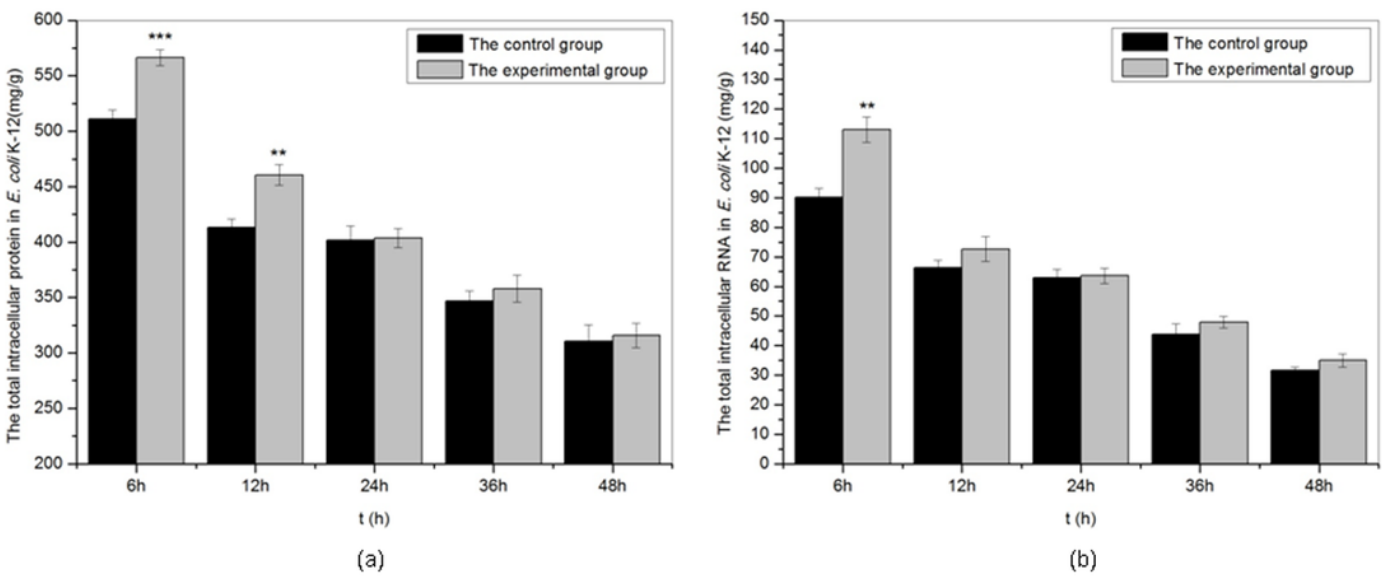

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Fig. 6 The pictures of *E. coli* K-12 cell of SCE.

(a) The cells in the control group. (b) The cells exposed to sound frequency 8 KHz, intensity 80 dB and power 61 dB. (c) The cells exposed to sound frequency 8 KHz, intensity 100 dB and power 61 dB. Cells were sampled at 48 h.

Note: Auto Gamma Correction was used for the image. This only affects the reviewing manuscript. See original source image if needed for review.