HAYATI Journal of Biosciences 24 (2017) 149–155

Contents lists available at ScienceDirect

HAYATI Journal of Biosciences

journal homepage: http://www.journals.elsevier.com/hayati-journal-of-biosciences

The Effects of Audible Sound for Enhancing the Growth Rate of Microalgae *Haematococcus pluvialis* in Vegetative Stage

Marcelinus Christwardana,[1,2*] H. Hadiyanto[1,3,4**]

[1] *Center of Biomass and Renewable Energy (C-BIORE), Department of Chemical Engineering, Diponegoro University, Jl Prof. H. Soedarto, SH, Tembalang, Semarang 50275, Indonesia.*
[2] *Graduate School of Energy and Environment, Department of New Energy Engineering, Seoul National University of Science and Technology, 232 Gongneung-ro, Nowon-gu, Seoul 01811, Republic of Korea.*
[3] *Department of Chemical Engineering, Diponegoro University, Jl Prof. H. Soedarto, SH, Tembalang, Semarang 50275, Indonesia.*
[4] *Master Program of Environmental Science, School of Postgraduate Studies, Diponegoro University, Jl Imam Bardjo, SH No 3, Semarang 50241, Indonesia.*

ARTICLE INFO

Article history:
Received 28 April 2017
Received in revised form
22 August 2017
Accepted 23 August 2017
Available online 14 September 2017

KEYWORDS:
audible sound,
biomass productivity,
growth rate,
Haematococcus pluvialis

ABSTRACT

Physico-stimulant like audible sound is one of the new promising methods for enhancing microalgae growth rate. Here, microalgae *Haematococcus pluvialis* was cultivated with the addition of audible sound with titles “Blues for Elle” and “Far and Wide.” The objective of this research was to evaluate the effect of audible sound to the growth and productivity of microalgae. The experiment has been conducted by exposing the audible sound for 8 h in 22 days to microalgae cultivation. The result showed that microalgae *H. pluvialis* treated by the music “Blues for Elle” shows the highest growth rate (0.03 per day), and 58% higher than the one without audible sound. The average number of cells in stationary phase is 0.76×10^4 cells/mL culture and the productivity is 3.467×10^2 cells/mL/day. The pH of microalgae medium slightly decreases because of proton production during photosynthesis process. The kinetic rate constant (k_{app}) is 0.078 per day, reaction half-life ($t_{1/2}$) is 8.89 days, and catalytic surface (K_{surf}) is 1.66×10^{-5}/day/cm^2. In conclusion, this audible sound is very useful to stimulate microalgae growth rate, especially *H. pluvialis*.

1. Introduction

Microalgae are unicellular microorganisms, which can be used as feedstock for producing biofuel or other value-added products (Choksi *et al.*, 2015; Hu *et al.*, 2008; Shah *et al.*, 2016) such as docosahexaenoic and eicosapentaenoic (Grima *et al.*, 2003), omega-3 (Barclay *et al.*, 1994), and protein (Hadiyanto *et al.*, 2012). Most of them contains protein, lipid, inorganic elements, polysaccharides, and also pigments such as chlorophyll, xanthophyll, zeaxanthin, canthaxanthin, astaxanthin, and β-carotene.

In food or pharmaceutical area, microalgae pigments, vitamin, and functional bioactive compound are collected as functional food or drugs because most of them has antioxidant properties, which is needed by living organisms, especially human and animals (Hadiyanto *et al.*, 2013). Microalgae also have been used as biofuel feedstock. Some microalgae can accumulate fatty acid which can be extracted to be bio-oil (Nur and Hadiyanto, 2015). In addition, microalgae have been used for wastewater treatment because they can capture carbon and nutrition from wastewater and also remove CO_2 from flue gas (Christenson and Sims, 2011; Olaizola, 2003). That is why microalgae are very useful for food, energy, and wastewater treatment area.

One of microalgae is *Haematococcus pluvialis*. These microalgae have four cycles of life; vegetative cell growth, encystment, maturation, and germination (Kobayashi *et al.*, 1997), but generally consist only of vegetative (green stage) and maturation (red stage) (Park *et al.*, 2014). *H. pluvialis* has a valuable pigment which can be used as an antioxidant, called astaxanthin (3,3′-dihydroxy-β-carotene-4,4′-dione) (Lorenz and Cysewski, 2000; Ranga Rao *et al.*, 2010). For human body, astaxanthin is 54 times more powerful than β-carotene, 65 times more powerful than vitamin C, 100 times more effective than tocopherol (Borowitzka, 2013; Koller *et al.*, 2014; Miki, 1991; Pérez-López *et al.*, 2014), and good for eye health, central nervous system, immune system, anti-aging, and fertility. Astaxanthin is not only produced by *H. pluvialis*, but also by yeast, salmon, trout, krill, shrimp, crayfish, and crustaceans

* Corresponding author.
** Corresponding author.
E-mail addresses: marcelinus@seoultech.ac.kr (M. Christwardana), hadiyanto@live.undip.ac.id (H. Hadiyanto).

Peer review under responsibility of Institut Pertanian Bogor.

http://dx.doi.org/10.1016/j.hjb.2017.08.009

(Higuera-Ciapara *et al.*, 2006; Ranga Rao *et al.*, 2014). Astaxanthin is resulted from the secretion process of *H. pluvialis* during maturation stage, whereas chlorophyll was still produced in vegetative stage.

In previous research, effect of physico-stimulation such as addition of audible sound in plant growth has been performed by several researchers (Cai *et al.*, 2014; Creath and Schwartz, 2004; Gu *et al.*, 2013; Hassanien *et al.*, 2014; Hou and Mooneyham, 1999; Hou *et al.*, 2009). For microalgae experiment, Jiang *et al.* (2012) conducted the audible sound effect experiment on chlorella and Cai *et al.* (2016) conducted on *Picochlorum oklahomensis*. But, none exploring the type of sound which was used and only focusing on the frequency of sound.

Audible sound is very important in this experiment. Larsen and Gilbert (2013) have been arranged the music called "microbial bebop" from microbial deoxyribose nucleic acid (DNA) genes sequence. That music consists of "Blues for Elle," "Bloom," "Far and Wide," and "Fifty Degrees North, Four Degrees West," where each music has different function if it is used for microbe. Other experiment about arranging notation based on live creature's DNA genes sequences had been performed by Sousa *et al.* (2005).

In this experiment, we used specific songs called "Blues for Elle" and "Far and Wide" as audible sound because that music was created to stimulate photosynthesis, nutrient consumption, and temperature resistance for microbe or microalgae. On the other hand, "Bloom" and "Fifty Degrees North, Four Degrees West" were not used as audible sound because they were created especially for cyanobacteria and pseudomonas, which is avoided in *H. pluvialis* growth (Larsen and Gilbert, 2013). Microalgae growth rate would be determined by measuring optical density (OD) and manual cell counting method using hemocytometer.

2. Materials and Methods

2.1. Materials

H. pluvialis strain (UTEX #2505) was used in this experiment and was cultured in 1 L flask with the addition of optimal haematococcus medium (OHM) (Fábregas *et al.*, 2000), which consists of (in g/L) KNO_3 0.41, Na_2HPO_4 0.03, $MgSO_4.7H_2O$ 0.246, $CaCl_2.2H_2O$ 0.11, (in mg/L) Fe(III)citrate.H_2O 2.62, $CoCl_2.6H_2O$ 0.011, $CuSO_4.5H_2O$ 0.012, Cr_2O_3 0.075, $MnCl_2.4H_2O$ 0.98, $Na_2MoO_4.2H_2O$ 0.12, SeO_2 0.005, and (in μg/L) biotin 25, thiamine 17.5, and B_{12} 15 (in g/L), then placed in a chamber. After 3 days or if the cell concentration was 0.4×10^4 cells/mL of culture, that culture was transferred into acrylic photobioreactor which contains 60 L distilled water (include OHM). The temperature of environment was set to 25°C and checked every day using Infrared Thermometer NUB8380H (Nubee, Guangdong, China). Some cool white LED lamps were placed beside photobioreactor which resulted in photosynthetic photon flux of 20.3 μmol/m^2/s. Culture was left for 22 days with dark:light cycle was 12:12, and OHM would be injected into the culture every 2 days. Aeration with the rate of 100 mL/min was fed into the photobioreactor without any additional CO_2.

2.2. Audible sound treatment

Previously, Cai *et al.* (2015) reported that 2000 Hz is the major frequency component in most audible sound in nature. Based on that, music or audio with power 60 dB was set as audible sound and average frequency of the audible sound was measured by using Audacity software (Audacity Team, New York, USA). Audible sound was played from music player HCB-811 (Hyundai, Seoul, South Korea) and placed between the two photobioreactors as shown in Figure 1 (approximately 20 cm). The audible sound was continuously generated for microalgae growth, and played 8 h per day from 10 AM to 6 PM. The power of sound was controlled by controller in music player. For control variable, culture with no addition of audible sound was set with the same parameter of temperature, light, aeration rate, and pH.

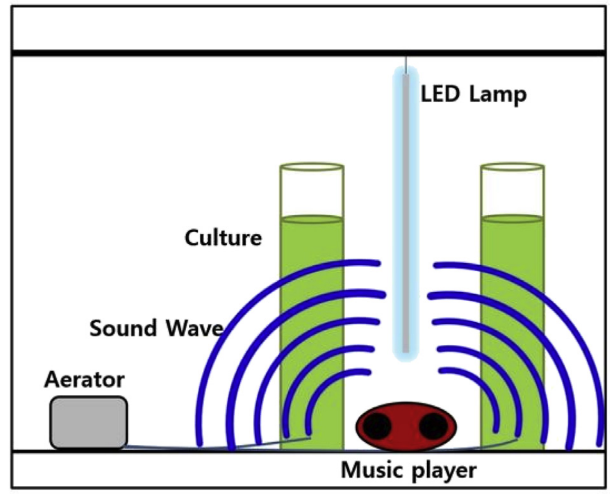

Figure 1. Schematic of *Haematococcus pluvialis* cultivation set-up.

2.3. Biomass measurement

Most studies said that the maximum absorbance for microalgae was observed at 680 nm as the maximum peak of chlorophyll to estimate biomass concentration. Eq. 1 was followed to determine microalgae growth rate. The OD was monitored using bench spectrophotometer UV-1800 (Angstrom Advanced, Massachusetts, USA). Around 20 mL of culture was collected into the sample bottle every day during growth period and used for growth rate and pH measurement. The pH of culture itself was checked by using pH meter Fisher Scientific S90525 (Fisher Scientific, Shanghai, China).

$$\mu = \frac{OD_2 - OD_1}{t_2 - t_1} \quad (1)$$

where, μ is microalgae growth rate, OD is at 680 nm, and t is time (day).

The second method to measure growth rate was using hemocytometer. In this method, 1 mL of culture was injected to hemocytometer. *H. pluvialis* was observed by using microscope BA310 (Motic, Xiamen, China), total cells number in eight areas was counted manually and then divided by eight. Then the resulted number was multiplied by 10^4. Eq. 2 was used to measure microalgae growth rate.

$$\mu = \frac{N_2 - N_1}{t_2 - t_1} \quad (2)$$

where, μ is microalgae growth rate, *N* is cells number (cells/mL), and t is time (day).

2.4. Kinetic model measurement

Profile of pseudo–first-order kinetics of *H. pluvialis* biomass during 22 days of cultivation following Eq. 3 (Rokhina *et al.*, 2010).

$$ln\frac{c_0}{c} = k_{app} \times t \quad (3)$$

where, C_0 is the initial number of *H. pluvialis* cells, C is the final number of *H. pluvialis* cells, k_{app} is apparent rate constant (per day), and t is time (day).

The slope of a linear plot of ln (C_0/C) versus time gives the apparent increasing rate constant. Also, there is a correlation between the reaction rate constant and the reaction half-life in the pseudo–first-order reactions that can be expressed as following Eq. 4 (Rokhina et al, 2010).

$$t_{\frac{1}{2}} = \frac{\ln 2}{k_{app}} \tag{4}$$

where, $t_{\frac{1}{2}}$ is half-life reaction and k_{app} is apparent rate constant (per day).

The novel catalytic parameter (K_{surf}) was reported by Sakkas *et al.* (2004), which describes the participation of the surface area in the process. K_{surf} is derived by the partition of the apparent kinetic rate, estimated for the studied process with the value of the surface area of the catalyst. As audible sound achieves the microalgae by propagating through bioreactor wall, we can say that the surface area of the bioreactor wall is the surface area of physico-stimulant following Eq. 5.

$$K_{surf} = \frac{k_{app}}{S} \tag{5}$$

where, K_{surf} is catalytic surface, k_{app} is apparent rate constant (per day), and S is surface area of the catalyst (m^2/g)

3. Results

3.1. Characterization of audible sound

It is important to characterize the audible sound before used as physico-stimulant for enhancing the growth rate of *H. pluvialis*. For that, frequency at 60 dB and octave analysis of background sound from different audible sound "Blues for Elle" and "Far and Wide" were measured. For doing that, we measured the average frequency, frequency level pattern, and octave analysis of background sound of two kinds of audible sound. From the result as shown in Figure 2A, "Far and Wide" had octave analysis of background sound more stable than "Blues for Elle," although both was set at sound level 60 dB. While the pattern of frequency level from "Far and Wide" and "Blues for Elle" is shown in Figure 2B. From that figure, intensity of frequency level from "Far and Wide" is also more stable than "Blues for Elle," compatible with phenomena in Figure 2A, where the average frequency of "Far and Wide" and "Blues for Elle" audible sound are 0.24 and 0.28 kHz, respectively.

3.2. Morphology of *H. pluvialis*

Morphology of *H. pluvialis* before and after the cultivation time was investigated using digital microscope, and they are shown in Figure 3. From that figure, the color of *H. pluvialis* in 0 day was light green, which represented the chlorophyll inside the cells (Figure 3A). Another thing is young *H. pluvialis* did not have layered cell wall, which made that cell easy to burst. In after green stage cultivation, the layered cell wall has been made and the cell wall of *H. pluvialis* become thick. That thick layered cell wall was used to protect microalgae from burst by environment during astaxanthin accumulation (Montsant *et al.*, 2001). At this time, the color of *H. pluvialis* was reddish green, where red color represented the starting accumulation of astaxanthin (Figure 3B). It means, that culture was ready to transfer to the next stage (maturation stage).

3.3. Correlation between OD and number of cells

It is meaningful to measure the accurate microalgae cell. For that, we correlated between OD and manual cell counting as a method to determine the number of microalgae cell in large scale application. Figure 4 shows the correlation between OD at 680 nm and manual cells counting using hemocytometer with the value is y = 4.858× or 1 OD = 4.858 × 10^4 cells/mL culture.

3.4. Effect of different frequency on the growth rate of *H. pluvialis*

It is very important to determine the growth rate of *H. pluvialis*. For doing that, we tried to measure the growth rate by using the first method – correlation between OD and cultivation time. From Figure 5, the following two things can be noticed: (1) the culture which was treated by "Blues for Elle" had the highest growth rate than other variable with the value 0.03 per day (growth rate values for treatment by "Far and Wide" and control were 0.015 and 0.011 per day, respectively) and (2) the OD_{680} of *H. pluvialis* which was treated by "Blues for Elle" had average value of 0.158 or 52% higher than control, whereas *H. pluvialis* which was treated by "Far and Wide" only increase up to 26%.

3.5. Effect of different frequency on biomass productivity of *H. pluvialis*

The other important thing to be considered is *H. pluvialis* biomass productivity measurement. There are some factors which can affect the productivity, such as lag phase time, growth rate, and average OD at stationary phase as shown in Table 1. From Table 1, we can see that the lag phase time of control (no additional sound)

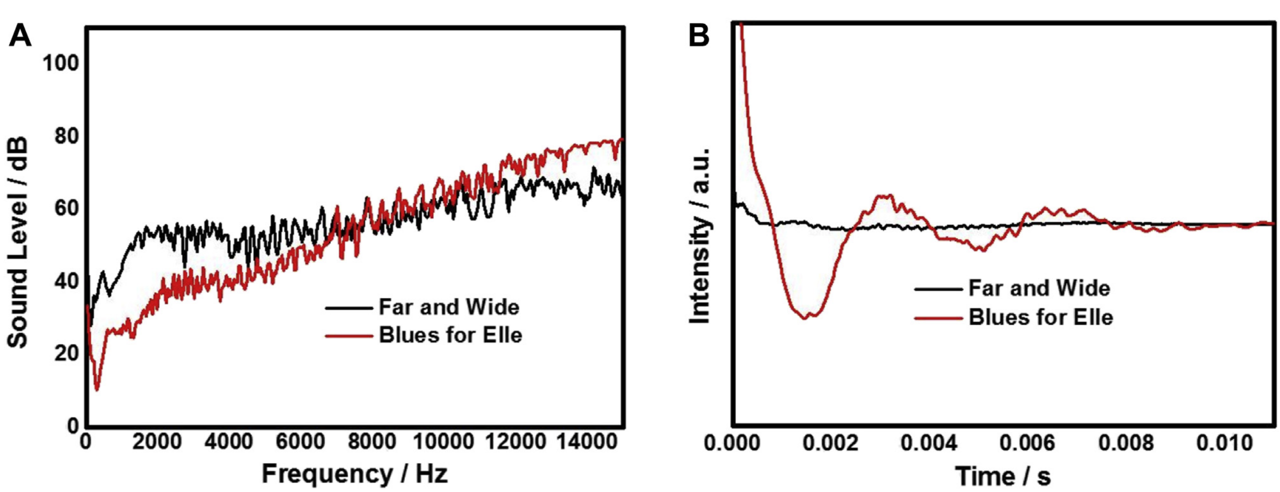

Figure 2. (A) Octave analysis of background sound and (B) level of frequency intensity from "Far and Wide" and "Blues for Elle" audible sound.

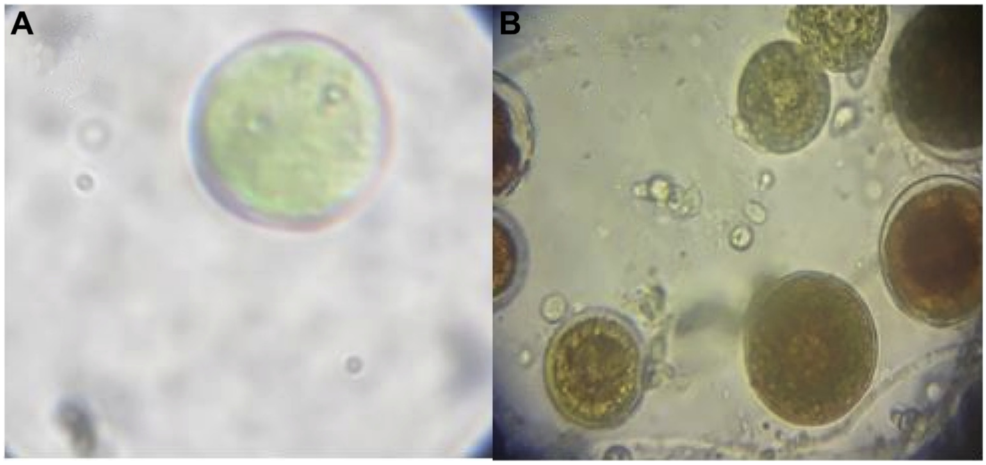

Figure 3. Microscopic images of *Haematococcus pluvialis* at (A) day 0 and (B) day 22.

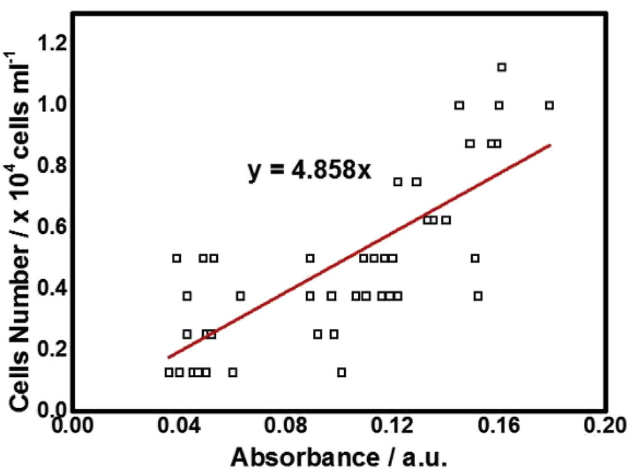

Figure 4. Calibration curve between absorbance (OD_{680}) and microalgae cells number.

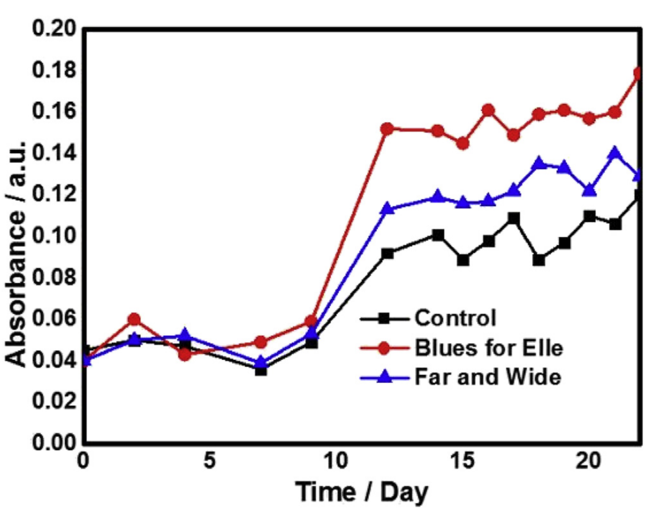

Figure 5. Growth of *Haematococcus pluvialis* in different types of audible sound based on their optical density.

and culture which was treated by “Far and Wide” are 5 days from day 7 to day 12 with growth rate 0.011 and 0.015 per day, respectively, whereas culture which was treated by the music “Blues for Elle” has lag phase time only 3 days from day 9 to day 12 with growth rate 0.03 per day.

Table 1. Lag phase time, growth rate, average maximal OD, and biomass productivity of *Haematococcus pluvialis*

Audible sound	Lag time (day)	Growth rate (per day)	Average max OD	Biomass productivity ($\times$ 10^2 cells/mL/day)
Control (no music)	5	0.011	0.104	2.297
Blues for Elle	3	0.030	0.158	3.467
Far and Wide	5	0.015	0.125	2.760

OD = optical density.

Average maximal OD for control, treated by “Blues for Elle” and “Far and Wide” are 0.104, 0.158, and 0.125 or 5.052, 7.627, and 6.073 $\times$ 10^3 cells/mL. Maximal achieved OD was obtained from average OD at stationary phase from day 12 to day 22. The highest average maximal OD was culture which was treated by “Blues for Elle,” compatible with the result in Figure 5. Productivity was obtained by following Eq. 6.

$$P = \frac{X}{t} \tag{6}$$

where,

- P is productivity (cells/mL/day)
- X is average cells number at stationary phase (cells/mL)
- t is total cultivation time (day).

Regarding microalgae productivity, from that equation, the highest productivity was culture which was treated by “Blues for Elle” is 3.467 $\times$ 10^2 cells/mL/day with total cultivation time was 22 days. Productivity of control (without audible sound) and culture which was treated by “Far and Wide” are 2.297 and 2.76 $\times$ 10^2 cells/mL/day, respectively. We can say that addition of “Blues for Elle” audible sound is very effective to increase productivity up to 50.94%, which is higher compared with “Far and Wide” as only increased up to 20.16%.

3.6. pH Phenomena during *H. pluvialis* cultivation

It is important to know the pH behavior related with audible sound treatment in *H. pluvialis*. From Figure 6, initially, pH was set in 6.5–6.8, then it gradually decreased. The decreasing pH during cultivation was due to releasing proton into medium during photosynthesis process. Because this cultivation is heterotrophic activity, illumination, aeration, and nutrient would affect the microalgae growth rate.

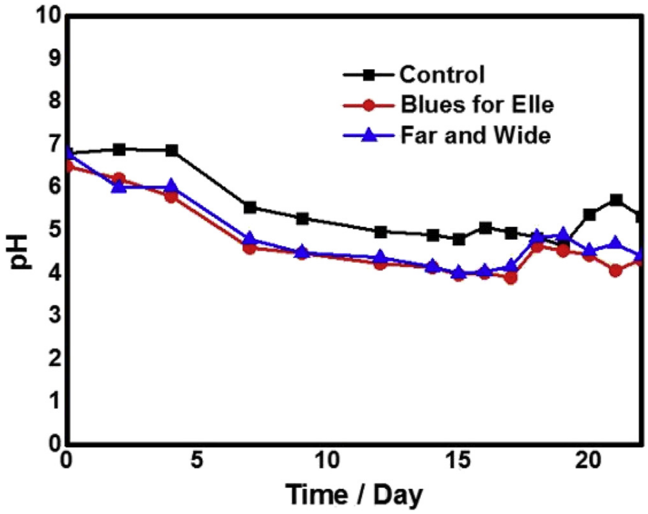

Figure 6. pH of *Haematococcus pluvialis* culture during the cultivation of vegetative phase.

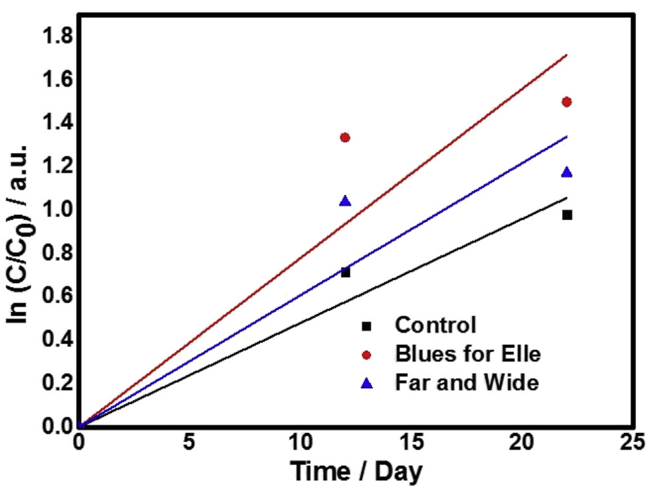

Figure 7. Pseudo–first-order of increasing biomass kinetic model.

Audible sound like "Blues for Elle" and "Far and Wide" had capability to stimulate photosynthesis process. During photosynthesis, microalgae took nutrient source from medium and released higher proton. It made the decreasing pH of *H. pluvialis*, which was treated by "Blues for Elle" and "Far and Wide" faster than control.

3.7. Kinetic study of audible sound as physico-stimulant

In previous section, we observed the strong dependence between the growth rate and the frequency of audible sound. An increase in the growth rate of *H. pluvialis* with an increase in the biomass weight or absorbance was also reported.

The Pseudo-first order kinetic model, apparent reaction rate constants (k_{app}) and correlation coefficients (R) are shown in Figure 7 and Table 2. From Table 2, *H. pluvialis* which was treated with "Blues for Elle" has k_{app} value 0.078 per day or 1.63 times higher than control (0.048 per day), whereas *H. pluvialis* which was treated with "Far and Wide" had k_{app} value 0.061 per day or 1.27 times higher than control. *H. pluvialis* which was treated with "Blues for Elle" also has $t_{1/2}$ value 8.89 day, 38.43% lower than control (the $t_{1/2}$ value of control and treated by "Far and Wide" are 14.44 and 11.36 days, respectively). Whereas, K_{surf} value of *H. pluvialis* which was treated with "Blues for Elle" was 1.66 × 10^{-5}/day/cm^2, 1.63 times higher than control (K_{surf} value of *H. pluvialis* which was treated with "Far and Wide" and control are 1.29 and 1.02 × 10^{-5}/day/cm^2, respectively.

Table 2. Relating kinetic parameters of increasing biomass of *Haematococcus pluvialis*

Audible sound	k_{app} (per day)	$t_{1/2}$ (day)	K_{surf} (× 10^{-5}/day/cm^2)	R^2
Control (no music)	0.048	14.44	1.02	0.96
Blues for Elle	0.078	8.89	1.66	0.87
Far and Wide	0.061	11.36	1.29	0.87

4. Discussion

In microbial especially microalgae, there are usually five stages or phases in their growth. That phases consist of lag or induction phase, log or exponential phase, declining relative growth phase, stationary phase, and death phase. Dry biomass, manual cell counting, and OD are usually used to determine the number of biomass in the culture. It is difficult to measure the accurate number of microalgae cells although using cell counting method by hemocytometer. For that, OD is mostly used to represent the number of cells inside culture. According to Banerjee *et al.* (1993), the most accurate indirect method for microalgae cells counting measurement was dry biomass, but its accuracy would decrease for large scale application (Pirt, 1975). By correlating the OD at 680 nm and cells number of microalgae, the accurate amount of biomass can be achieved.

Notation of "Blues for Elle" which was mapped to temperature, nutrient reactivity, and chlorophyll activity (photosynthetic) give the big impact in *H. pluvialis* photosynthesis and growth because it can increase the microalgae metabolism. In this case, "Far and Wide" only affect the photosynthetic activity and temperature. Beside that, the unstable frequency intensity level gives the impact in growing and cell dividing processes. The genre of "Blues for Elles" which has the unstable rhythm also plays an important role on this. In this experiment, frequency of edible sound is lower than other references, for instance, the experiment conducted by Jiang *et al.* (2011) and Jiang *et al.* (2012) used audible sound with frequency 0.4 kHz to grow *Chlorella pyrenoidosa.*

The "Blues for Elle" audible sound decreases thermodynamic phase transition, which illustrates the enhancement of the fluidity of the cell wall and membrane of *H. pluvialis* than "Far and Wide" (Hassanien *et al.* 2014). It means, it will enhance the *H. pluvialis* cells to grow and divide faster and easily. Generally, in plant, cell division cycle consists of four phases: G1-phase, S-phase, G2-phase, and M-phase (Depamphilis, 2003). Sound waves increase the capacity of indule-3-acetic acid metabolism and inhibit the abscisic acid metabolism during cell division process (Lovelli *et al.* 2012; Zhang *et al.* 2006), which cause the *H. pluvialis* is difficult to stress.

In the microalgae cycle, longer lag phase time is meaningless if the growth rate is low. Capability of the music "Blues for Elle" can stimulate the growth of microalgae by affecting amino acid and DNA sequences, relevant with our reference (Takahashi and Miller, 2007).

The electric potential from audible sound frequency made *H. pluvialis* cell membranes could be changed by enhancing plasmalemma and Adrenoleukodystrophy mRNA exists in the outermost part of cells which consists of membrane lipid and protein (Sun and Xi, 1999; Jia *et al.* 2003). As the effect, lipid accumulation inside *H. pluvialis* also occurred faster. The audible sound frequency also improves the cell membrane deformability which would change under external force (Wang *et al.*, 2001). It made that cells have stronger resistance to the new environment and other inhibitors such as protozoa or fungus.

The effect of audible sound on *H. pluvialis* productivity is very high. “Blues for Elle” audible sound would change the chlorophyll fluorescence as well as the content of chlorophyll, the net photosynthetic rate, the photochemical efficiency of PSII by enhancing the electron transport (Fan *et al.* 2010; Meng *et al.* 2011; Meng *et al.* 2012; Zhou *et al.* 2010). Because of the increasing photosynthetic rate, *H. pluvialis* cells are divided rapidly and then enhance biomass productivity.

Microalgae took nutrient source from medium and released higher proton into medium during photosynthesis. The higher number of protons made the decreasing pH of *H. pluvialis* which was treated by “Blues for Elle” and “Far and Wide” decrease faster than control. It is almost similar with the experiment which was conducted by Cai *et al.* (2016).

According to the kinetic model, higher k_{app} means increasing the biomass also high. *H. pluvialis* which treated with “Blues for Elle” has the short reaction half-life, which means need shorter time to achieve maximum biomass amount. While K_{surf} calculation for audible sound as physico-stimulant is more complicated because audible sound is unchanged and does not have real surface area.

In this experiment, effect of audible sound for enhancing microalgae growth has been studied. *H. pluvialis* has been cultivated for 22 days and correlation between OD_{680} and cells number is $y = 4.858\times$. Music with the title “Blues for Elle” which is blues genre had big impact in increasing biomass productivity of microalgae cells up to 50.94% or 3.467×10^2 cells/mL/day for 22 days and growth rate is higher than “Far and Wide” and control (no additional sound). The apparent kinetic rate constant (k_{app}) is 0.078 per day, reaction half-life ($t_{1/2}$) is 8.89 days, and catalytic surface (K_{surf}) is 1.66×10^{-5}/day/cm^2. In the future, sound with high frequency or high power is needed to stimulate growth of microalgae in larger scale. Decreasing medium pH during cultivation due to proton which resulted from photosynthesis process after taking nutrient sources. The effect of audible sound for enhancing astaxanthin production in maturation process is needed to be studied advance.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. Authors thank to Center of Biomass and Renewable Energy (C-BIORE), Diponegoro University for their facilities and guidance.

References

Banerjee UC, Chisti Y, Moo-Young M. 1993. Spectrophotometric determination of mycelial biomass. *Biotechnol Tech* 7:313–6.
Barclay WR, Meager KM, Abril JR. 1994. Heterotrophic production of long chain omega-3 fatty acids utilizing algae and algae-like microorganisms. *J Appl Phycol* 6:123.
Borowitzka MA. 2013. High-value products from microalgae—their development and commercialisation. *J Appl Phycol* 25:743–56.
Cai W, Dunford NT, Wang N, Zhu S, He H. 2016. Audible sound treatment of the microalgae *Picochlorum oklahomensis* for enhancing biomass productivity. *Bioresour Technol* 202:226–30.
Cai W, He H, Zhu S, Wang N. 2014. Biological effect of audible sound control on mung bean (*Vigna radiate*) sprout. *BioMed Res Int* 2014:931740.
Cai W, Zhu S, Wang N, He H, Ying B. 2015. Design of an experimental platform to investigate the effects of audible sounds on plant growth. *Int J Agric Biol Eng* 8: 162–9.
Chokshi K, Pancha I, Trivedi K, George B, Maurya R, Ghosh A, Mishra S. 2015. Biofuel potential of the newly isolated microalgae *Acutodesmus dimorphus* under temperature induced oxidative stress conditions. *Bioresour Technol* 180:162–71.
Christenson L, Sims R. 2011. Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. *Biotechnol Adv* 29:686–702.
Creath K, Schwartz GE. 2004. Measuring effects of music, noise, and healing energy using a seed germination bioassay. *J Altern Complement Med* 10:113–22.
Depamphilis ML. 2003. Review the ‘ORC cycle’: a novel pathway for regulating eukaryotic DNA replication. *Gene* 310:1–15.
Fábregas J, Domínguez A, Regueiro M, Maseda A, Otero A. 2000. Optimization of culture medium for the continuous cultivation of the microalga *Haematococcus pluvialis*. *Appl Microbiol Biotechnol* 53:530–5.
Fan R, Zhou Q, Zhao D. 2010. Effect on changes of chlorophyll fluorescence in cucumber by application of sound frequency control technology. *Acta Agric Bor Sin* 19:194–7.
Grima EM, Belarbi EH, Fernández FGA, Medina AR, Chisti Y. 2003. Recovery of microalgal biomass and metabolites: process options and economics. *Biotechnol Adv* 20:491–515.
Gu SB, Yang B, Wu Y, Li SC, Liu W, Duan XF, Li MW. 2013. Growth and physiological characteristics of *E. coli* in response to the exposure of sound field. *Pak J Biol Sci* 16:969–75.
Hadiyanto, Christwardana M, Soetrisnanto D. 2013. Phytoremediations of palm oil mill effluent (POME) using aquatic plants and microalgae for biomass production. *J Environ Sci Technol* 6:79–90.
Hadiyanto, Nur MMA, Hartanto GD. 2012. Cultivation of *Chlorella* sp. as biofuel sources in palm oil mill effluent (POME). *Int J Renew Energy Dev* 1:45–9.
Hassanien RH, Hou TZ, Li YF, Li BM. 2014. Advances in effects of sound waves on plants. *J Integr Agr* 13:335–48.
Higuera-Ciapara I, Felix-Valenzuela L, Goycoolea FM. 2006. Astaxanthin: a review of its chemistry and applications. *Crit Rev Food Sci Nutr* 46:185–96.
Hou T, Li B, Guanghui T, Zhou Q, Xiao Y, Qi L. 2009. Application of acoustic frequency technology to protected vegetable production. *Trans Chinese Soc Agric Eng* 25: 156–9.
Hou T, Mooneyham R. 1999. Applied studies of plant meridian system: I. The effect of agri-wave technology on yield and quality of tomato. *Am J Chin Med* 27: 1–10.
Hu Z, Li Y, Sommerfeld M, Chen F, Hu Q. 2008. Enhanced protection against oxidative stress in astaxanthin-overproduction Haematococcus mutant (Chlorophyceae). *Eur J Phycol* 43:365–76.
Jia Y, Wang BC, Wang XJ, Duan CR, Toyama Y, Sakanishi A. 2003. Influence of sound wave on the microstructure of plasmalemma of chrysanthemum roots. *Colloids Surf, B* 29:109–13.
Jiang S, Huang J, Han XH, Zeng X. 2011. Influence of audio frequency mixing of music and cricket voice on growth of edible mushrooms. *Trans Chinese Soc Agric Eng* 27:300–5.
Jiang S, Rao H, Chen Z, Liang M, Li L. 2012. Effects of sonic waves at different frequencies on propagation of *Chlorella pyrenoidosa*. *Agric Sci Technol Hunan* 13: 2197–201.
Kobayashi M, Kurimura Y, Tsuji Y. 1997. Light independent, astaxanthin production by the green microalga *Haematococcus pluvialis* under salt stress. *Biotechnol Lett* 19:507–9.
Koller M, Muhr A, Braunegg G. 2014. Microalgae as versatile cellular factories for valued products. *Algal Res* 6:52–63.
Larsen P, Gilbert J. 2013. Microbial Bebop: creating music from complex dynamics in microbial ecology. *PLoS One* 8:e58119.
Lorenz RT, Cysewski GR. 2000. Commercial potential for Haematococcus microalgae as a natural source of astaxanthin. *Trends Biotechnol* 18:160–7.
Lovelli S, Scop A, Perniola M, Tommaso TD, Sofo A. 2012. Abscisic acid root and leaf concentration in relation to biomass partitioning in salinized tomato plants. *J Plant Physiol* 169:226–33.
Meng QW, Zhou Q, Gao Y, Zheng SJ. 2011. Effects of acoustic frequency treatment on photosynthetic and chlorophyll fluorescence characters of tomato. *Acta Agric Jiangxi* 23:57–9.
Meng QW, Zhou Q, Gao Y, Zheng SJ, Gao Y. 2012. Effects of plant acoustic frequency technology on the growth traits, chlorophyll content and endogenous hormones of *Lycopersicon esculentum*. *Hubei Agric Sci* 51:1591–4.
Miki W. 1991. Biological functions and activities of animal carotenoids. *Pure Appl Chem* 63:141–6.
Montsant A, Zarka A, Boussiba S. 2001. Presence of a nonhydrolyzable biopolymer in the cell wall of vegetative cells and astaxanthin-rich cysts of *Haematococcus pluvialis* (Chlorophyceae). *Mar Biotechnol* 3:515–21.
Nur MMA, Hadiyanto. 2015. Enhancement of *Chlorella vulgaris* biomass cultivated in POME medium as biofuel feedstock under mixotrophic conditions. *J Eng Technol Sci* 47:487–97.
Olaizola M. 2003. Commercial development of microalgal biotechnology: from the test tube to the marketplace. *Biomol Eng* 20:459–66.
Park JC, Choi SP, Hong ME, Sim SJ. 2014. Enhanced astaxanthin production from microalga, *Haematococcus pluvialis* by two-stage perfusion culture with stepwise light irradiation. *Bioprocess Biosyst Eng* 37:2039–47.
Pérez-López P, González-García S, Jeffryes C, Agathos SN, McHugh E, Walsh D, Murray P, Moane S, Feijoo G, Moreira MT. 2014. Life-cycle assessment of the production of the red antioxidant carotenoid astaxanthin by microalgae: from lab to pilot scale. *J Clean Prod* 64:332–44.
Pirt SJ. 1975. Principles of Microbe and Cell Cultivation. Halsted Press book, first ed. NY, USA: John Wiley and Sons, NY, USA. pp. 15–7.
Ranga Rao A, Reddy AH, Aradhya SM. 2010. Antibacterial properties of *Spirulina platensis*, *Haematococcus pluvialis*, *Botryococcus braunii* microalgal extracts. *Curr Trends Biotechnol Pharm* 4:809–19.

Ranga Rao A, Siew Moi P, Ravi S, Aswathanarayana RG. 2014. Astaxanthin: sources, extraction, stability, biological activities and its commercial applications—a review. *Mar Drugs* 12:128–52.
Rokhina EV, Repo E, Nolte, Virkutyte J. 2010. Comparative kinetic analysis of silent and ultrasound-assisted catalytic wet peroxide oxidation of phenol. *Ultrason Sonochem* 17:541–6.
Sakkas VA, Arabatzis IM, Konstantinou IK, Dimou AD, Albanis TA, Falaras P. 2004. Metolachlor photocatalytic degradation using TiO_2 photocatalysts. *Appl Catal, B* 49:195–205.
Shah MMR, Liang Y, Cheng JJ, Daroch M. 2016. Astaxanthin-producing green microalga *Haematococcus pluvialis*: from single cell to high value commercial products. *Front Plant Sci* 7:531.
Sousa AS, Baquero F, Nombela C. 2005. The making of The Genoma Music. *Rev Iberoam Micol* 22:242–8.
Sun KL, Xi BS. 1999. The effects of alternative stress on the thermodynamic properties of cultured tobacco cells. *Acta Biochim Biophys Sinica* 15:579–84.
Takahashi R, Miller JH. 2007. Conversion of amino-acid sequence in proteins to classical music: search for auditory patterns. *Genome Biol* 8:405.
Wang BC, Zhao HC, Liu YY, Jia Y, Sakanishi A. 2001. The effects of alternative stress on the cell membrane deformability of chrysanthemum callus cells. *Colloids Surf B* 20:321–5.
Zhang JH, Jia WS, Yang JC, Ismail AM. 2006. Role of ABA in integrating plant responses to drought and salt stresses. *Field Crops Res* 97:111–9.
Zhou Q, Qu YH, Li BM, Hou TZ, Zhu BY, Wang D. 2010. Effects of sound frequency treatment on plant characters and chlorophyll fluorescence of the strawberry leaf. *J China Agric Univ* 15:111–5.