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Relationship between Gas Concentration Emitted from Cut Cucumber Cross Sections and Growth Axis

Osamu Takagi[*], Masamichi Sakamoto[2], Hideo Yoichi[1], Hideyuki Kokubo[1], Kimiko Kawano[1], Mikio Yamamoto[1]

[1]*Information and Research Center, International Research Institute (IRI), 1108-2 Sonno, Inage, Chiba 263-0051, Japan.*

[2]*Aquavision Academy, 1228-3 Tsubuura, Narita, Chiba 287-0236, Japan.*

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ABSTRACT

Vegetables and fruits show bio-reactions to various stimuli. As an example, it is known that by stimulating plants, gas is emitted due to communication between the stimulated plant part and its defense reaction. In this paper, we studied the gas concentration dependence on the direction of round cut cucumber cross sections. This was to obtain new knowledge on the gas emission in the bio-reaction caused by injuring the cucumber fruit. We examined the relationship between gas concentration and growth axis. We assumed the emitted gas concentration differed depending on whether the direction of the cucumber cross section was the same direction or the opposite direction to the direction of the growth axis. The experimental results demonstrated that when the direction of the cucumber cross section was opposite to the direction of the growth axis, the average gas concentration was about 7.5 ppm (about 2%) higher than in the same direction ($p=3.8\times10^{-2}$; n=1817). As a result of calculating the ratio of the gas concentration emitted from the surface with different directions of the cross section, it turned out that the ratio value changed between day and night ($p=1.5\times10^{-6}$; n=1292 as day measurements and n=525 as night measurements). As a result of the analysis, we discovered that the gas concentration ratio vibrated with a waveform synthesized by the circadian rhythm (one cycle was 24 h) and multiple periodic fluctuations (one cycle was 12 h, 4.8 h, or 1.8 h).

1. INTRODUCTION

In plants such as vegetables and fruits, bio-reactions are known to last for a long period of time even after harvesting[1]. As an example of a bio-reaction, a plant emits gas after being subjected to some stimulation or trauma. Also, it is thought that plants exchange information about coexisting with insects and bacteria by emitting gas, and they have protective reactions and immune reactions against foreign enemies[2-5]. Therefore, research on bio-reactions of vegetables and fruits after harvest is important for elucidating the unknown ability and nature of plants, and in practical terms for measures against pest control, and for measures to ensure safe distribution, storage, etc.

We have studied the gas concentration emitted from round cut cucumber fruit cross sections after harvesting. As a result, we clarified that there is a periodic fluctuation such as the circadian rhythm in the relationship between the time when the cucumber was cut for section preparation and the gas concentration emitted afterward. We discovered that the periodicity of gas concentration varied depending on the season; there was one cycle of 6 hours in summer and one cycle of 24 hours in winter[6]. As a reason for the difference in the periodic change of the gas concentration depending on the season, we considered that the cucumber may adjust its gas generation reaction according to the effective time for protection from the enemy.

The aim of this paper was to obtain new findings on gas emission, which is a bio-reaction after injuring the cucumber fruit. We focused on whether the gas concentration depended on the direction of the cucumber cross section. Specifically, we verified whether the gas concentration differed depending on whether the orientation of the cucumber cross section was the same direction as the growth axis or the opposite direction. The experimental results demonstrated that when the direction of the cucumber cross section was opposite to the direction of the growth axis, the average gas concentration was about 7.5 ppm (about 2%) higher than in the same direction ($p=3.8\times10^{-2}$; $n=1817$). When comparing summer and winter data, the same relationship between gas concentration and growth axis was confirmed irrespective of the season. Similarly, even when comparing day and night data, the same relationship between gas concentration and growth axis was also confirmed. Furthermore, as a result of calculating the ratio of the gas concentration emitted from the surface with different directions of the cross section, it turned out that the value of the ratio changed between day and night ($p=1.5\times10^{-6}$; $n=1292$ as day measurements and $n=525$ as night measurements). As a result of the analysis, we discovered that the gas concentration ratio was a synthesized cycle fluctuation of circadian rhythm (one cycle was 24 h) and multiple periodic rhythms (one cycle was 12 h, 4.8 h, or 1.8 h). The research results of this paper may be basic data necessary for further improving measurement accuracy when cucumber sections are used as a biosensor for detecting peculiar spatial characteristics.

2. MATERIALS AND METHODS

2-1. Measurement of gas concentration emitted from cucumber cross sections

In order to measure the gas concentration emitted from the cucumber cross section by the bio-reaction, samples were prepared by the Simultaneous CAlibration Technique (SCAT)[7]. This technique can correct the gas concentration measured at the experiment point by the gas concentration measured at the calibration control point. The data of the corrected experiment points are those in which deviations in measurement data generated due to variations in individual cucumbers and the difference in surrounding environmental conditions are eliminated. Therefore, at the experiment point, it was possible to detect a minute effect that has some influence on the cucumber gas generation reaction system. Using SCAT, we have succeeded in detecting a unique spatial characteristic which was difficult to detect with other existing sensors[8-11]. In this paper, however, only the cucumber samples placed at the calibration control point which is not influenced by the experiment point were studied. We tried to verify whether or not the gas concentration differed depending on whether the orientation of the cucumber cross section was the same direction as the growth axis or the opposite direction.

2-2. Preparation and placement of samples

The preparation of cucumber section samples is shown in Figs.1 (a) to (c). In one experiment unit (minimum experimental unit), 4 cucumbers and 8 petri dishes were used. We aligned the vine side and the flower side of 4 cucumbers (Fig.1 (a)). The definitions of the direction of cucumber fruit were as follows. (1) The direction of the growth axis was set as the direction from the vine side to the flower side in the cucumber fruit. (2) The direction of the cucumber cross section was considered only with respect to the cross section of the upper surface when the slice of cucumber was placed on a petri dish. Its direction was perpendicular to the petri dish and was from the lower cross section in contact with the petri dish to the direction of the upper cross section in contact with the atmosphere (Fig.1 (b)).

Cucumber section samples were prepared as follows. For cucumber A, the part (3 to 5 cm) where the thickness of the vine side was not uniform was cut off (Fig. 1 (b)). Four cross sections (A1 to A4) with a length of 2 cm were cut from the part where the thickness became uniform. The 2 cm long section A1 was cut in half and placed in a separate petri dish (pair 1). The sections A2 to A4 were also cut in half the same as A1, placed in separate petri dishes, and pair 2 to pair 4 were created. Cucumbers B to D were also cut like cucumber A and cross sections were placed on petri dishes of pair 1 to pair 4. However, in order to eliminate the influence due to the variation of the individual cucumbers and the distance from the vine side, each petri dish was similarly allocated 4 sections in total, one section from each of 4 cucumbers. Samples with the upper cucumber section surface placed on the petri dish in the same direction as the growth axis were taken as experimental samples (G_{E1} to G_{E4}) and the samples in the opposite direction were taken as control samples (G_{C1} to G_{C4}) (Fig. 1 (c)). In SCAT, two petri dishes G_{E1}, G_{E2} of the experimental samples were stacked and placed at the experiment point. The control samples G_{C1}, G_{C2} and the samples G_{E3}, G_{E4}, G_{C3}, G_{C4} were stacked two by two and placed at a calibration control point well separated from the experiment point.

In data analysis, in order to strictly compare whether or not the gas concentration emitted from the cucumber cross section depending on the direction of the cross section, the following was done. Experimental samples (G_{E1}, G_{E2}) on the experiment point and control samples (G_{C1}, G_{C2}) as pairs of experimental samples were excluded from the analysis because there was a possibility that they were affected by some external influence. We analyzed the data of pair 3 (G_{E3}, G_{C3}) and pair 4 (G_{E4}, G_{C4}) which were in exactly the same condition except for the direction of the cross section. The average value of G_{E3} and G_{E4} where the direction of the cucumber cross section was in the same direction as the growth axis was E and the average value of G_{C3} and G_{C4} which was the direction opposite to the growth axis was C (Fig. 1 (c)). The numbers of E and C data were each 1817.

All cucumbers used in this experiment were from Japan and were edible cucumber (*Cucumis sativus* 'white spine type' cucumber) which is commonly distributed in Japanese markets[12]. Generally, Japanese cucumbers have consistent quality, uniform shape and thickness. Therefore, we considered that the weights of the prepared pairs of samples were almost identical. In other words, (G_{E3}, G_{E4}) and (G_{C3}, G_{C4}) in Fig. 1 (c) were considered to be in the same state except that the direction of the cucumber cross section was different. In addition, we thought that the lower surface of the cucumber section placed on the petri dish was in close contact with the petri dish, and gas emission from the lower surface could be neglected.

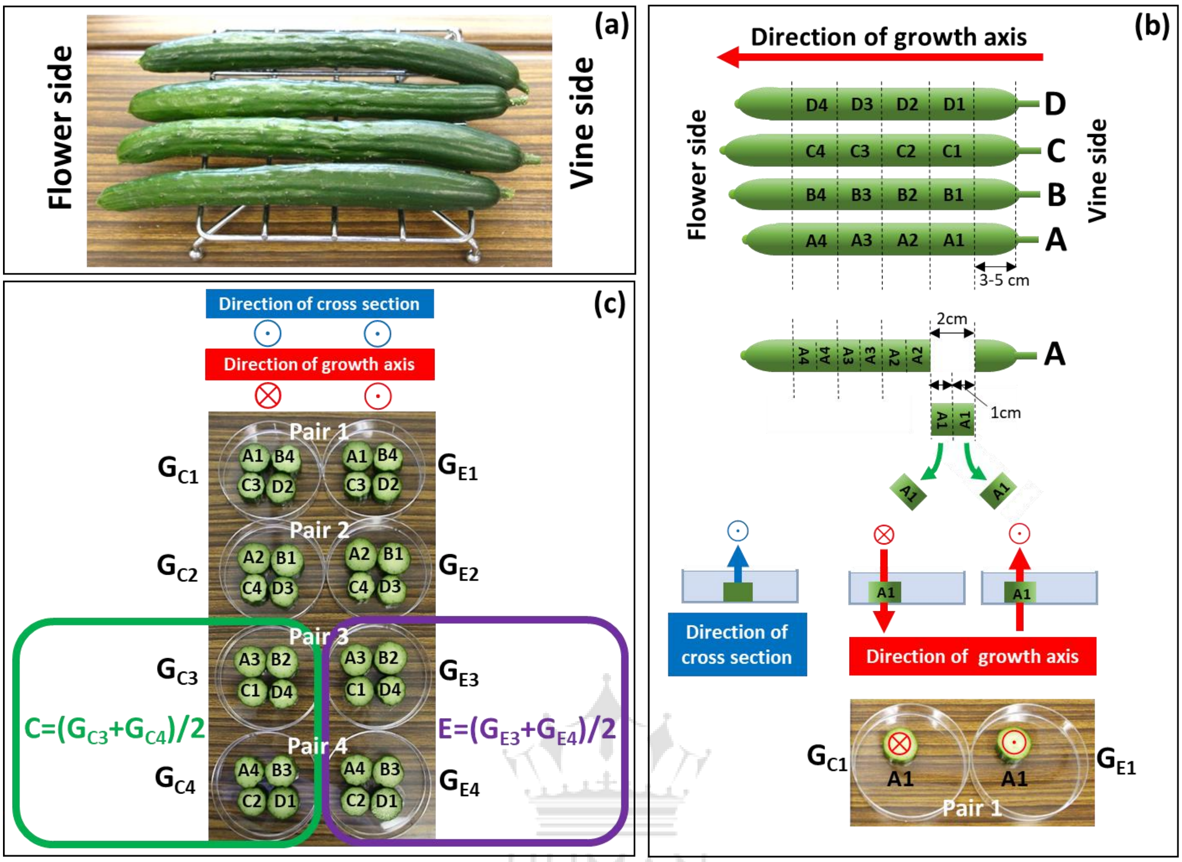

Fig.1 Preparation of experimental samples

2-3. Measurement of gas concentration

The cucumber section samples were set at the experiment point and the calibration control point for 30 minutes after preparation. After that, the lid of each petri dish was removed and the dishes containing the samples were placed in a polypropylene sealed container having a volume of 2.2 liters. Hexanol-hexanal series gas was emitted from the cucumber cross sections in the sealed container. It has been found that there are mainly 16 kinds of gases emitted from cucumber[13, 14]. After about 12 hours, the gas concentration emitted in the sealed container reached a maximum and then remained in equilibrium. The sealed containers were kept at controlled room temperature (22 to 24 degrees Celsius) without direct sunlight for more than 24 hours. After storage, the gas concentration was measured by aspirating 300 mL of gas using a gas detector (GV-100: Gas Tech, Japan) and a gas detection tube (141 L: Gas Tech, Japan).

The gas detection tube (141 L) can measure 2-hexanol gas. When 2-hexanol gas was measured, since the conversion factor is 3, the absolute value of 2-hexanol gas concentration could be determined by tripling the read value (ppm) of the detection tube. However, the 16 kinds of gases emitted as a mixture from the cucumber cross sections contain isomers of 2-hexanol and the like. In order to accurately determine the gas concentration emitted from the cucumber, the constitutional ratio of the 2-hexanol isomer and the conversion coefficient are required. But the conversion factor of the 2-hexanol isomer is unknown at present.

The purpose of this paper was not to determine the absolute value of the gas concentration emitted from cucumber but to clarify whether the gas concentration differed depending on the direction of the cross section. Therefore, in this paper, we made out analysis using the read value (ppm) of the detection tube as the gas concentration. The experiment was conducted throughout the year from 2010 to 2017. The number of experiments was 1817 in the minimum experimental unit (the number of cucumbers used was 7268). Part of the data used in this paper was previously reported[8-11].

3. RESULTS

Fig.2 shows the gas concentration measurement results. These are the results when the direction of the cucumber cross section was the same direction ($E=(G_{E3}+G_{E4})/2$) as the growth axis and in the opposite direction ($C=(G_{C3}+G_{C4})/2$). The vertical axis represents gas concentration (ppm), and the horizontal axis represents 24 hours from 0 o'clock to 24 o'clock at the time when the samples were prepared (time when the samples were placed at the measurement point). Purple squares (■) are the results of E, green rhomboids (◆) are the results of C. The number of data was n=1817 for each. Fig.2 (b) plots the average value of E and C. The error bars are 99% confidence intervals. When the direction of the cucumber cross section was opposite (C) to the direction of the growth axis, it was seen that the average gas concentration was about 7.5 ppm (about 2%) higher than in the same direction (E).

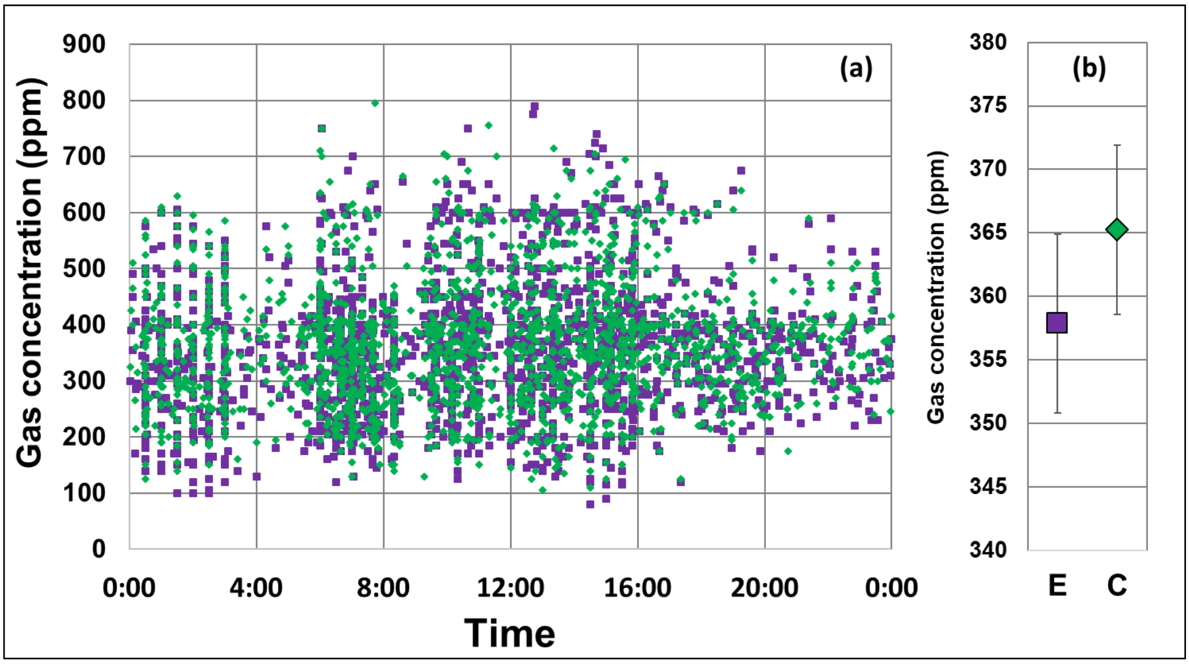

Fig. 2 Measurement results of gas concentrations for E and C

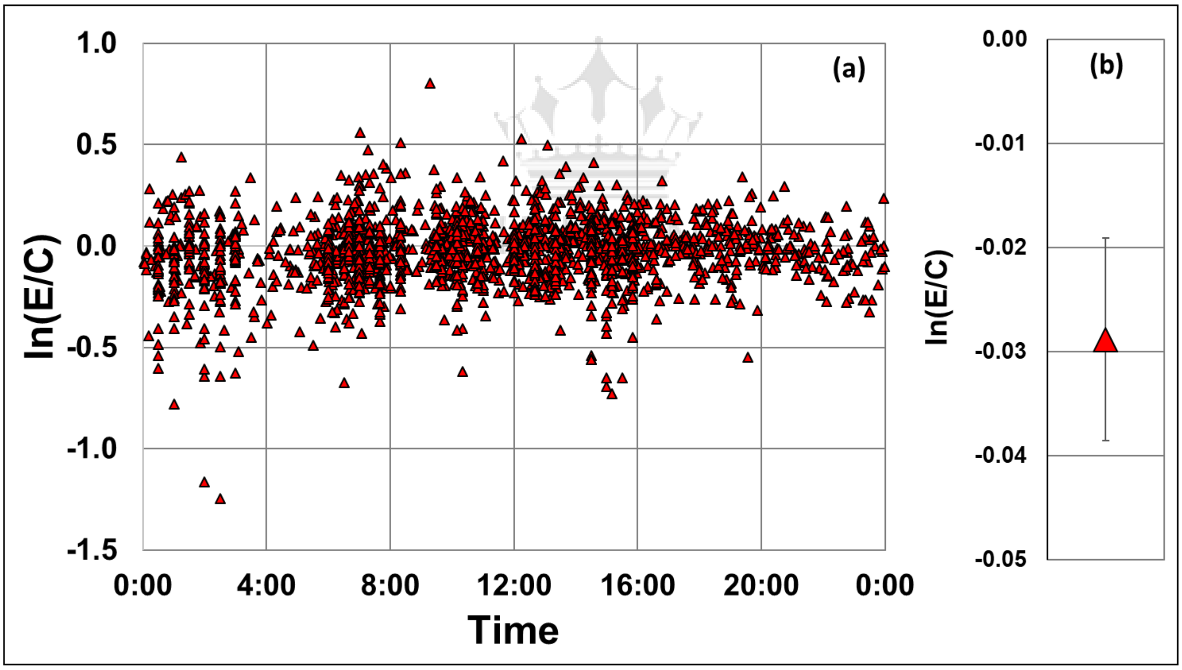

Fig. 3 Gas concentration ratio ln(E/C)

Fig.3 shows the result of the gas concentration ratio ln(E/C). The horizontal axis represents 24 hours from 0 o'clock to 24 o'clock at the time when the samples were prepared. The red triangles (▲) are the results of plotting ln(E/C). The number of data was n=1817. Fig.3 (b) plots the average value of ln(E/C). The error bars are 99% confidence intervals. It was found that the average value of ln(E/C) was significantly negative (E<C).

Figs.4 (a) to (c) show the histograms of gas concentrations E, C and ln(E/C). The value on the horizontal axis is the median. As a result of the analysis, it was found that Figs.4 (a) - (c) were neither a normal distribution nor a log-normal distribution.

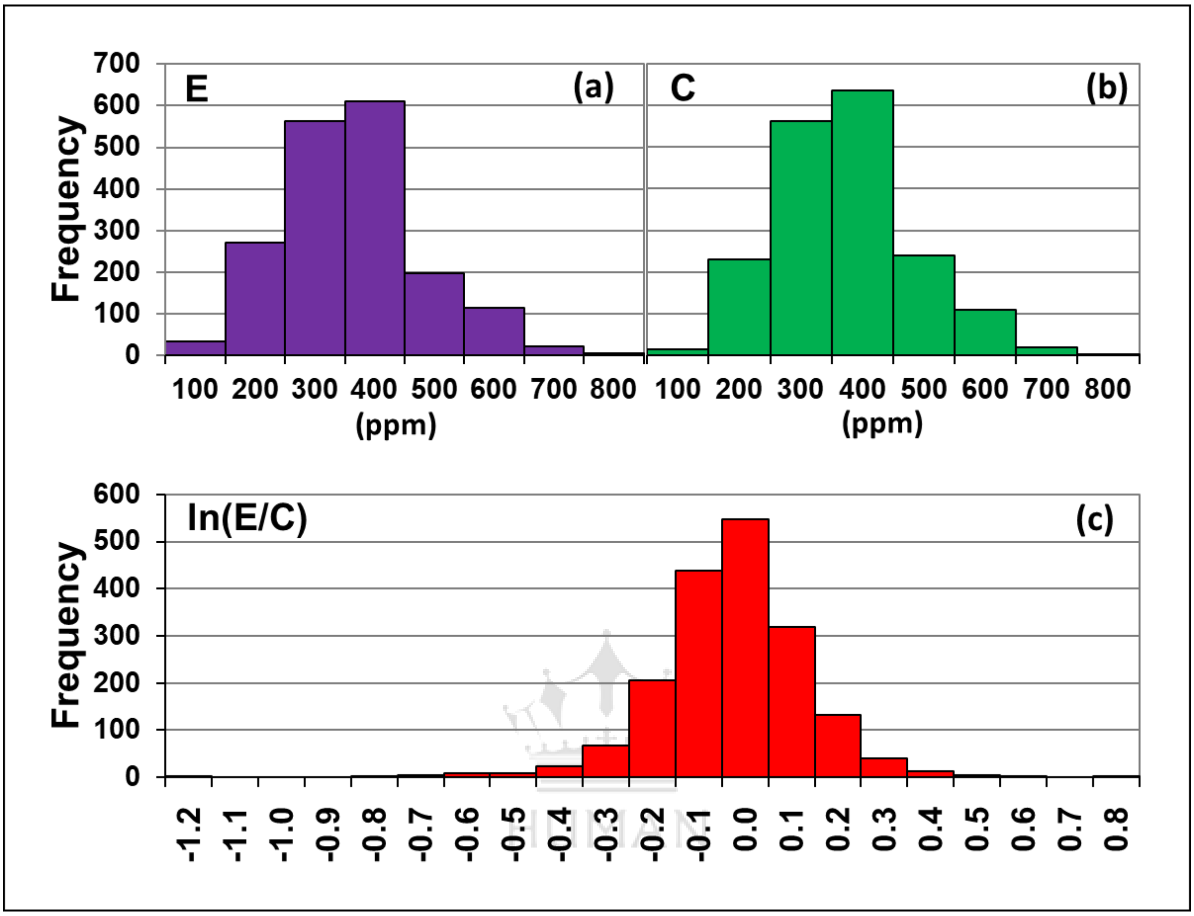

Fig. 4 Histograms of E, C and ln (E / C)

Fig.5 (a) shows the results of comparing E and C data in summer and winter. Green marks show all data (n=1817), red marks show summer data (n=998), and blue marks show winter data (n=819). The summer data were the results of the experiment conducted when the daytime was 12 hours or more, and the winter data are results when the daytime was less than 12 hours. Fig.5 (b) compares the results of E and C data for day and night. The green marks are all data (n=1817), yellow marks are the daytime data (n=1292), and black marks are the nighttime data (n=525). The day data are the experimental results obtained between 6:00 and 18:00 and the night data are the experimental results obtained from 18:00 to the next day 6:00. Fig.5 (c) shows the results after dividing ln(E/C) data into summer and winter. Fig.5 (d) shows the results after dividing ln(E/C) data into day and night. The average values of all data of E and C were 357.9 ppm and 365.3 ppm, respectively. As a result of the verification, a significant difference was detected between E and C ($p=3.8 \times 10^{-2}$; n=1817). The error bars are 99% confidence intervals. From this result, it was shown that ln(E/C) <0 was statistically significant, and it was proved that E<C. Also, a significant difference was detected between the day data and the night data ($p=1.5 \times 10^{-6}$; n=1292 as day measurements and n=525 as night measurements).

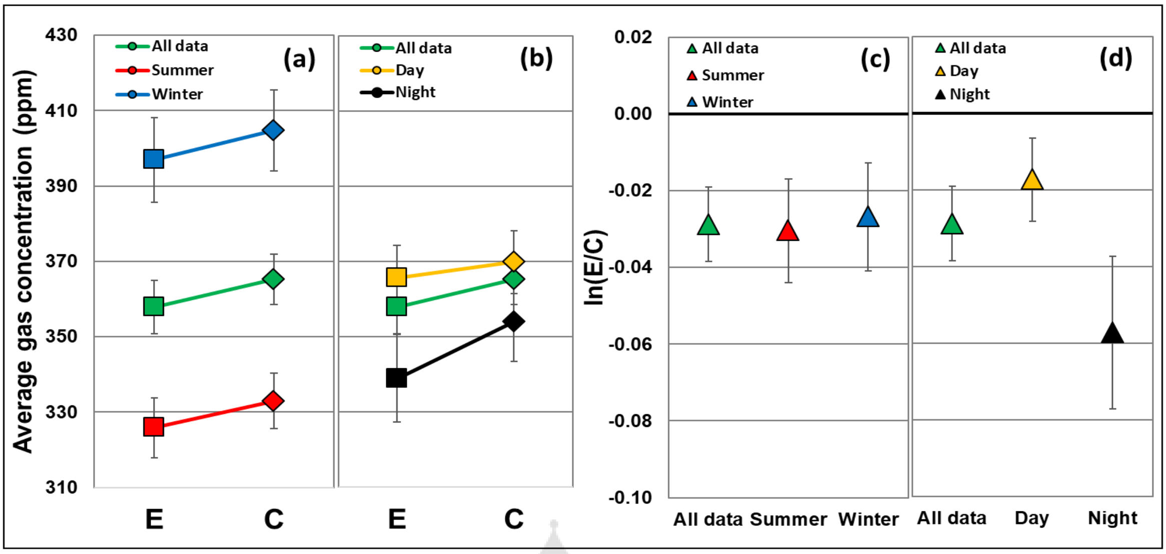

Fig. 5 Average of E, C and ln (E / C)

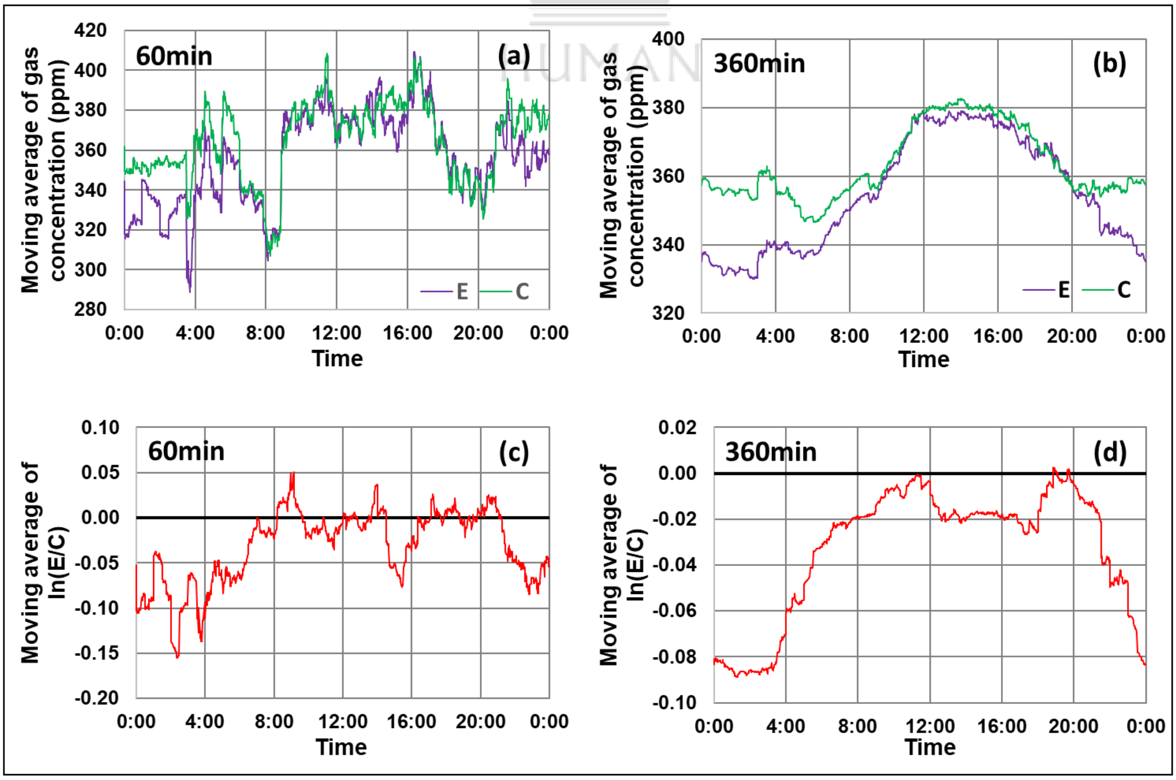

Fig. 6 Moving average of E, C and ln(E/C)

Fig.6 shows the results of moving average of E, C and ln(E/C). The moving average step is one minute. Figs.6 (a) and (b) are the results of E (blue) and C (green). Fig.6 (a) shows the moving average window length of 60 minutes, and Fig.6 (b) shows the results of the moving average window length of 360 minutes. From Fig. 6 (b) it was clear that there was a difference between E and C from 20:00 to the next day at 10:00. Figs.6 (c) and (d) are the results of ln(E/C). Fig.6 (c) shows the moving average window length of 60 minutes, and Fig.6 (d) shows the results of the moving average window length of 360 minutes. From the results in Fig. 6 (d), it became clear that the gas concentration change varied depending on the direction of the cucumber cross section. Especially, from 20:00 to the next day at 10:00, gas concentration difference due to the difference in direction appeared.

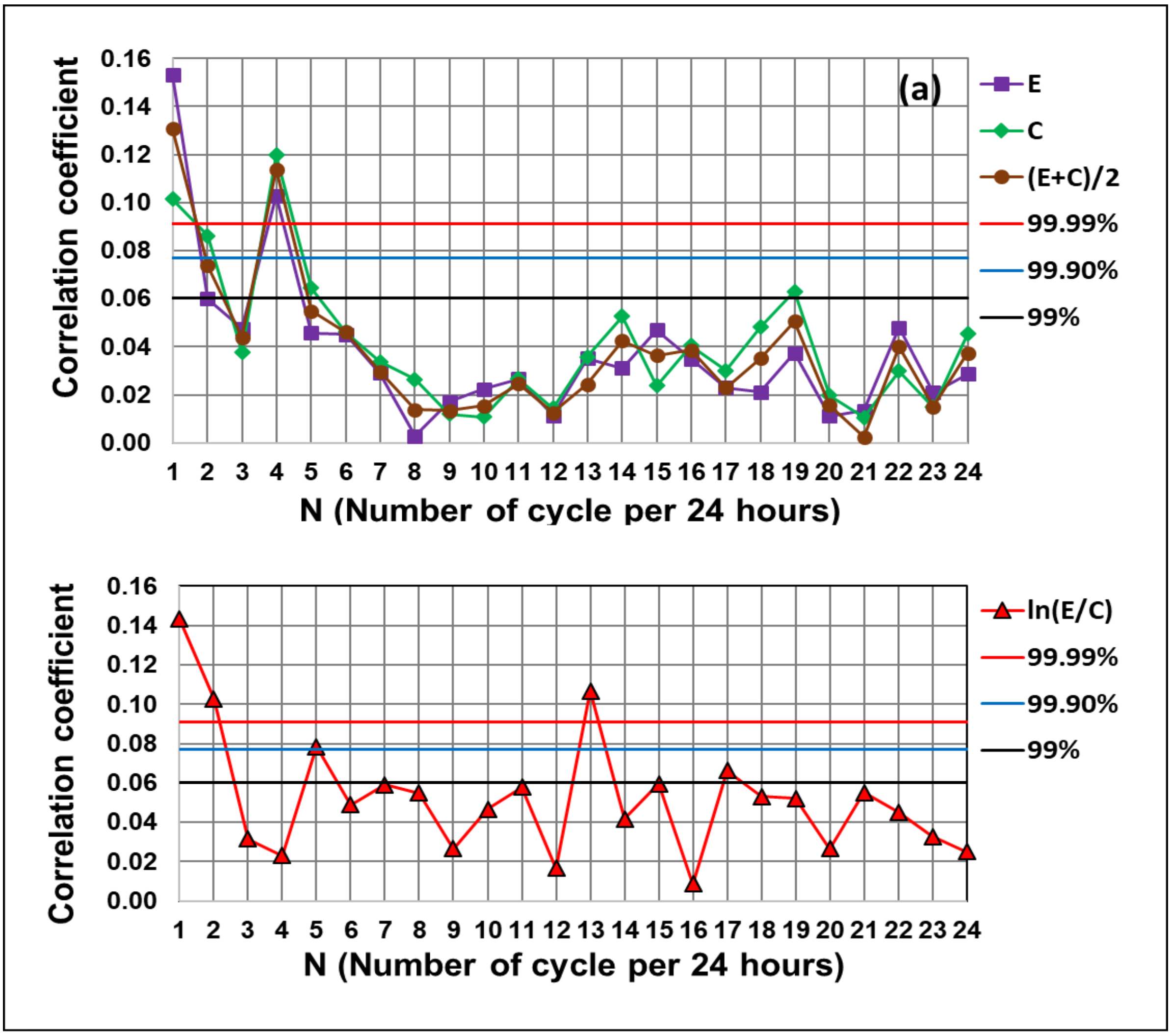

Fig. 7 E, C and ln (E/C) and the correlation coefficient of the periodic approximation curve

Fig.7 shows the correlation coefficient between E=(G_{E3}+G_{E4})/2, C=(G_{C3}+G_{C4})/2 and ln(E/C) and the periodic approximation curve. Here, we describe the periodic approximation curve. For the samples G_{E3}, G_{E4}, G_{C3}, G_{C4} placed at the calibration control point, the environmental conditions were the same from the time of preparation of the cucumber section to the measurement of the gas concentration. Therefore, we considered that the bio-reactions of G_{E3}, G_{E4}, G_{C3}, and G_{C4} are synchronized. If there is a certain rhythm in the bio-reactions, it was considered that E and C also change with the rhythm. In order to verify whether E and C were periodically changing, the periodic approximate curve shown in equation (1) was introduced.

$$y = a + b\sin(2\pi x N) + c\cos(2\pi x N) = a + \sqrt{b^2 + c^2}\sin(2\pi x N + \varphi), \quad \varphi = \arcsin\left(\frac{c}{\sqrt{b^2 + c^2}}\right). \ldots(1)$$

Here, a, b and c are constants and π is the circumference ratio. X shows the time, but the time from 00:00 to 24:00 corresponds to a numerical value in the range of 0 to 1. The reason for assuming equation (1) as a periodic approximation curve is that the cucumber is an organism and basically has a circadian rhythm (one cycle is 24 hours). Therefore, the change in the gas concentration emitted by the bio-reaction was also predicted to be in phase at every 24 hours. N is the number of periods per 24 hours and N is an integer from 1 to 24. Equation (1) represents a periodic approximation curve in which 1 cycle is 24 hours when N=1, 1 cycle is 1 hour when N=24. We calculated the periodic approximation curves of gas concentration averages for E and C with the respective values of N from 1 to 24 and fixed the constants a, b and c. Thereafter, a correlation coefficient between E, C and the periodic approximation curve was calculated. As a result, if we judge that the correlation coefficient is significant, we can conclude that the period of the periodic approximation curve is the period of the gas concentration and further the period of the bio-reaction.

Fig.7 (a) is the correlation coefficient between E, C and (E+C)/2 and the periodic approximation curve. Purple squares (■) are the results of E, green rhomboids (◆) are the results of C and brown circles (●) are the results of (E+C)/2 (n=1817). The line in the figure is a test value for judging whether or not the correlation coefficient is significant when the number of data is n=1817. The significance levels are 0.01% (red line), 0.1% (blue line) and 1% (black line), respectively. The test values are 0.0911, 0.0771 and 0.0604, respectively. From this result, we saw that E and C changed with a period of N=1 (one cycle 24 hours) and N=4 (one cycle 6 hours). This result was the same as we reported previously[6]; however, in the earlier work, the number of data (n=1056) was less than the present paper and

$(G_{C1}+G_{C2}+G_{E3}+G_{E4}+G_{C3}+G_{C4}) / 6$ was adopted as analysis data. Fig.7 (b) is the correlation coefficient between ln(E/C) and its periodic approximation curve. From this result, we considered that ln(E/C) periodically changed. The number of cycles N per 24 hours was significant in the case of N=1, 2, 5, 13 mainly. At this time, the correlation coefficient values were 0.143, 0.103, 0.078, and 0.107, respectively.

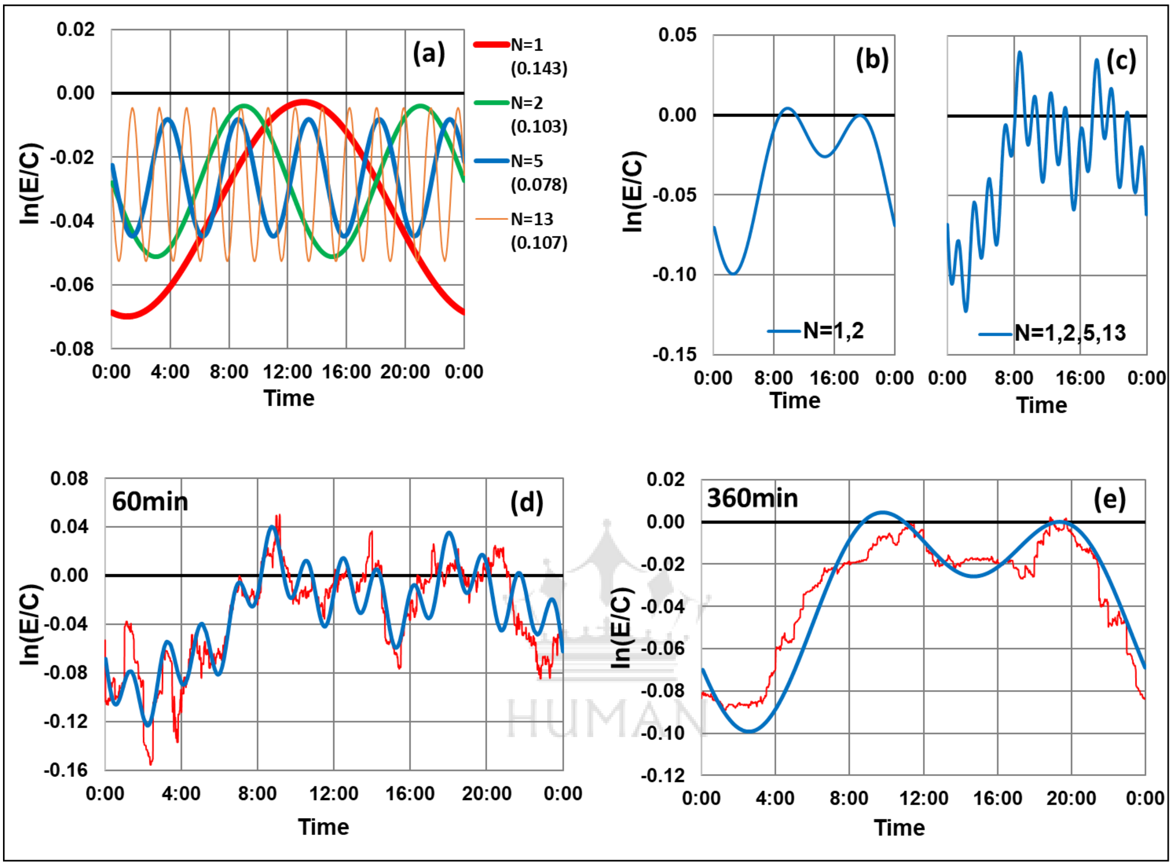

Fig. 8 Periodic approximation curve of ln(E/C)

Fig.8 (a) is a periodic approximation curve of ln(E/C). The periodic approximation curves for ln(E/C) when the correlation coefficient was significant are plotted from the results in Fig. 7 (b) (N=1 (red), 2 (green), 5 (blue), 13 (orange)). Fig.8 (b) is a synthesized periodic approximation curve of N=1, 2. Fig.8 (c) is a synthesized periodic approximation curve of N=1, 2, 5, 13. Fig.8 (d) is a plot of the 60-minute window length moving average (Fig.6(c)) and the synthesized periodic approximate curve (Fig.8(c)) with respect to ln(E/C). Fig.8 (e) is a plot of the 360-minute window length moving average (Fig.6(d)) and the synthesized periodic approximate curve (Fig.8(b)) with respect to ln(E/C). We found that the moving average and the synthesized periodic approximation curve coincided very well and we demonstrated that ln(E/C) periodically changed.

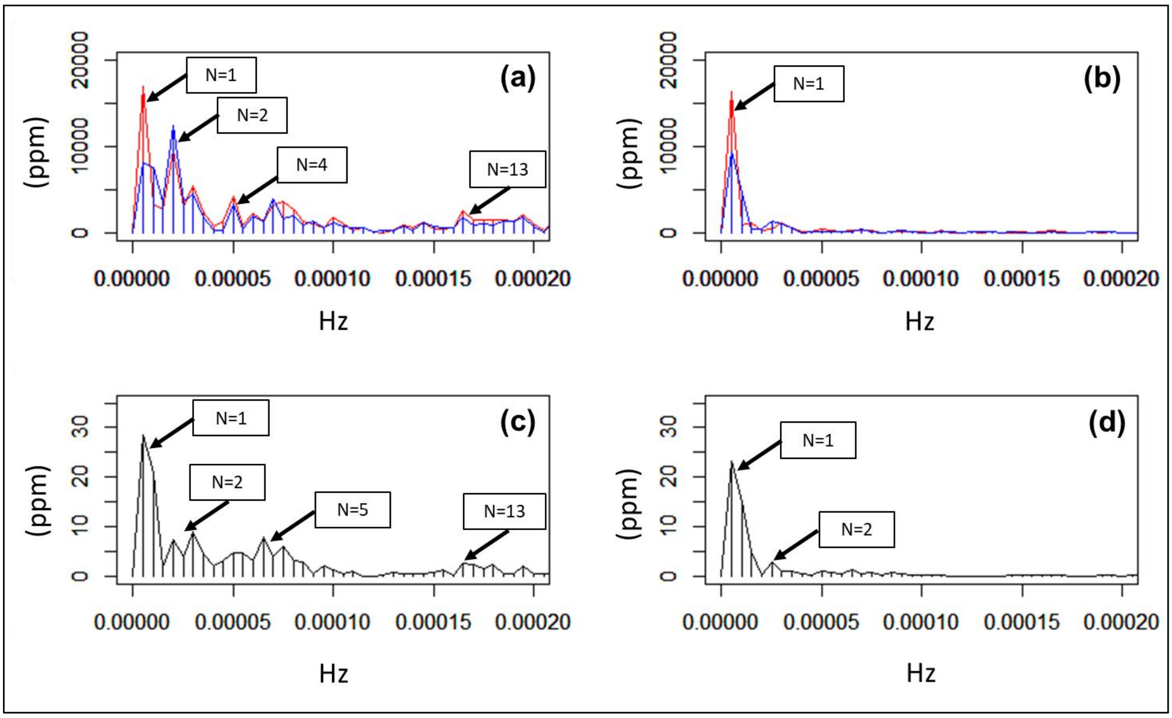

Fig.9 FFT analysis of moving average of E, C and ln (E/C)

Fig.9 shows the results of fast Fourier transform (FFT) analysis of the moving average of E, C and ln(E/C). Fig.9 (a) is the FFT analysis of the moving average of the 60-minute window length of E and C (Fig.6 (a)). The horizontal axis is Hz. Red lines are E results and blue lines are C results. For the typical peak appearing in Fig.9(a), the number of cycles N per 24 hours in Fig. 7 (a) was made to correspond (N = 1, 2, 4, 13). Fig.9 (b) is the FFT analysis of the moving average of the 360-minute window length of E and C (Fig.6 (b)). Fig.9 (c) is the FFT analysis of the moving average of the 60-minute window length of ln(E/C) (Fig.6 (c)). For the typical peak appearing in Fig.9(c) the number of cycles N per 24 hours in Fig. 7 (b) was made to correspond (N=1, 2, 5, 13). Fig.9 (d) is the FFT analysis of the moving average of the 360-minute window length of ln(E/C) (Fig.6 (d)). From the results of the FFT analysis on the moving average, it was proved that ln(E/C) periodically varied as in Fig.8.

4. DISCUSSION

We conducted a verification experiment with the hypothesis that a difference in gas concentration could be detected depending on whether the direction of the cucumber cross section was the same direction as the growth axis or the opposite direction. As a result of the experiment, the following points were found out. (1) In the analysis of all the data (n=1817), it was demonstrated that when the direction of the cucumber cross section was opposite to the direction of the growth axis, the average gas concentration was about 7.5 ppm (about 2%) higher than in the same direction ($p=3.8\times10^{-2}$; n=1817). Therefore, it was found that E<C and that the emitted gas concentration was different depending on whether the direction of the cucumber cross section was the same or opposite to the direction of the growth axis (Figs.2(b), 5(a) and 5(b)). (2) As a result of data analysis in the summer (n=998) and winter (n=819), the difference in gas concentration due to the difference in the direction of the cucumber cross section was present regardless of the season. Regardless of the season E<C was proved (Fig.5(a)). (3) As a result of data analysis in the daytime (n=1292) and nighttime (n=525), the difference in gas concentration due to the difference in the direction of the cucumber cross section was present regardless of it being day or night. E<C was proved regardless of day and night (Fig.5(b)). (4) The average of ln(E/C) did not differ significantly between summer and winter, but a significant difference was detected between day and night ($p=1.5\times10^{-6}$) (Figs.5(c) and (d)). (5) Since ln(E/C) detected a significant difference between daytime and nighttime, time change of ln(E/C) was analyzed. As a result, we discovered that ln(E/C) was periodically changing. The correlation coefficient between ln(E/C) and the periodic approximation curve was high when the number of cycles N per 24 hours was 1, 2, 5, and 13 (Fig.7(b)). It was found that ln(E/C) was a synthesized cycle fluctuation of circadian rhythm (one cycle was 24 h) and multiple periodic rhythms (one cycle was 12 h, 4.8 h, or 1.8 h). This result was also confirmed by FFT analysis (Fig. 9 (c)).

The research results of this paper revealed that the gas concentration emitted from the cucumber cross sections was related to the growth axis and that the gas concentration changed with multiple periodicity. These results may be basic data necessary for further improving measurement accuracy when cucumber sections are used as a biosensor for detecting peculiar spatial characteristics.

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