

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



# Oscillation in Bacterial Bioluminescence

Satoshi Sasaki

Tokyo University of Technology

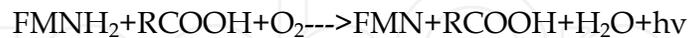
Japan

## 1. Introduction

Live cells show various dynamic characteristics, such as cell division or material production. When we consider that a cell is a chemical reactor that contains an enzyme in its structure, the rates of chemical reaction catalysed by them depend on the cell density. As the amount of enzyme within the cell differs according to the rate of expression of a specific gene, the rate of the reaction also depends on the condition of the cell. In short, chemical reactions caused by cells are nonlinearly related to the cell density; the reaction rate is not proportional to the cell density. This is one remarkable aspect of live cells. In the field of chemical analysis, bacterial cell behaviour is often used. For example, changes in respiration caused by chemical compounds that inhibit the respiratory chain (such as KCN) can be quantified, theoretically, by measuring the changes in the dissolved oxygen concentration.

Biomaterial-based devices have been reported, such as biochips or biosensors. These are not truly “bio” because they use an enzyme or antibody outside of the cell. Microbial sensors (Melidis, P.; Georgiou, D.(2002).; Kang, KH.; Jang, JK.; Pham, TH.; Moon, H.; Chang, IS. & Kim, BH. (2003).; Moon, H.; Chang, IS.; Kang, KH.; Jang, JK. & Kim, BH. (2004). ; Chang, IS.; Moon, H.; Jang, JK. & Kim, BH. (2005).; Kogure, H.; Kawasaki, S.; Nakajima, K.; Sakai, N.; Futase, K.; Inatsu, Y.; Bari, ML.; Isshiki, K. & Kawamoto, S. (2005).; Vaiopoulou, E.; Melidis, P.; Kampragou, E. & Aivasidis, A. (2004).; Yano, Y.; Numata, M.; Hachiya, H.; Ito, S.; Masadome, T.; Ohkubo, S.; Asano, Y. & Imato, T. (2001).; Kim, M.; Hyun, MS.; Gadd, GM.; Kim, GT.; Lee, SJ. & Kim, HJ. (2009). Davila, D.; Esquivel, JP.; Sabate, N. & Mas, J. (2011).), known as the analysis of devices that use live microbial cells as molecular-recognition material, are the only exception. This sensor, however, is based on a shift from one equilibrium to another. For example, a respiration inhibition-based microbial sensor measures a certain toxic compound because the dissolved oxygen concentration near the cells increases when the toxic compound exists. The main reason for the use of microorganisms is that they are more cost-effective than purified enzymes or antibodies. The dynamics of the bacterial cells are not at all used. The nonlinearity of cell behaviour has recently been studied (Wu, BM.; Subbarao, KV. & Qin, QM. (2008).; Kenkre, V. M.; & Kumar, N. (2008).; Dobrescu, R. & Purcarea, VI. (2011)). A suitable bacterium model is, therefore, needed to start a fundamental study on the nonlinearity of the cell. In our group studies, bioluminescence characteristics have been identified (Sasaki S., Mori Y., Ogawa M., Funatsuka S.,(2010)). Bioluminescent bacteria are those that emit light autonomously without the need of excitation light. The bioluminescence reaction is catalysed by bacterial

luciferase (Raushel, F. M. & T. O. Baldwin; (1989), Lee, J., Y. Y. Wang and B. G. Gibson; (1991), Hastings, J. W. (1996), Shirazy, N. H., B. Ranjbar, S. Hosseinkhani, K. Khalifeh, A. R. Madvar and H. Naderi-Manesh (2007)). The reaction requires a flavin mononucleotide (FMNH<sub>2</sub>), a long-chain aliphatic aldehyde, and O<sub>2</sub> to produce light (Balny, C. and J. W. Hastings (1975), Kurfurst, M., S. Ghisla and J. W. Hastings (1983), Tu, S. C., B. Lei, M. Liu, C. K. Tang and C. Jeffers (2000)).



This reaction is catalysed by bacterial luciferase (Karatani, H.; Izuta, T. & Hirayama, S. (2007)). This enzyme is synthesised by a process called quorum sensing, in which the synthesis occurs only after the cells recognise each other to be above a threshold in density. Two substrates, FMNH<sub>2</sub> and RCHO (linear alkyl aldehyde), of the reaction are also synthesised in the cell. The substrate with the least amount is, therefore, the rate-determining factor. The intensity of the bioluminescence has been reported primarily in connection with the oxygen concentration, but, theoretically, two other compounds might be candidates. Bacterial luminescence that has been used for environmental monitoring has been reviewed (Girott, S.; Ferri, E.N.; Fumo, M.G.; & Maiolini, E. (2008)). Recently, an oscillation in luminescence from a well-stirred bacterial suspension was reported (Sato, Y. and S. Sasaki (2008)). Here, in this chapter, the relationship between the oxygen and oscillation mode was investigated.

Changes in the luminescence spectra are also reported.

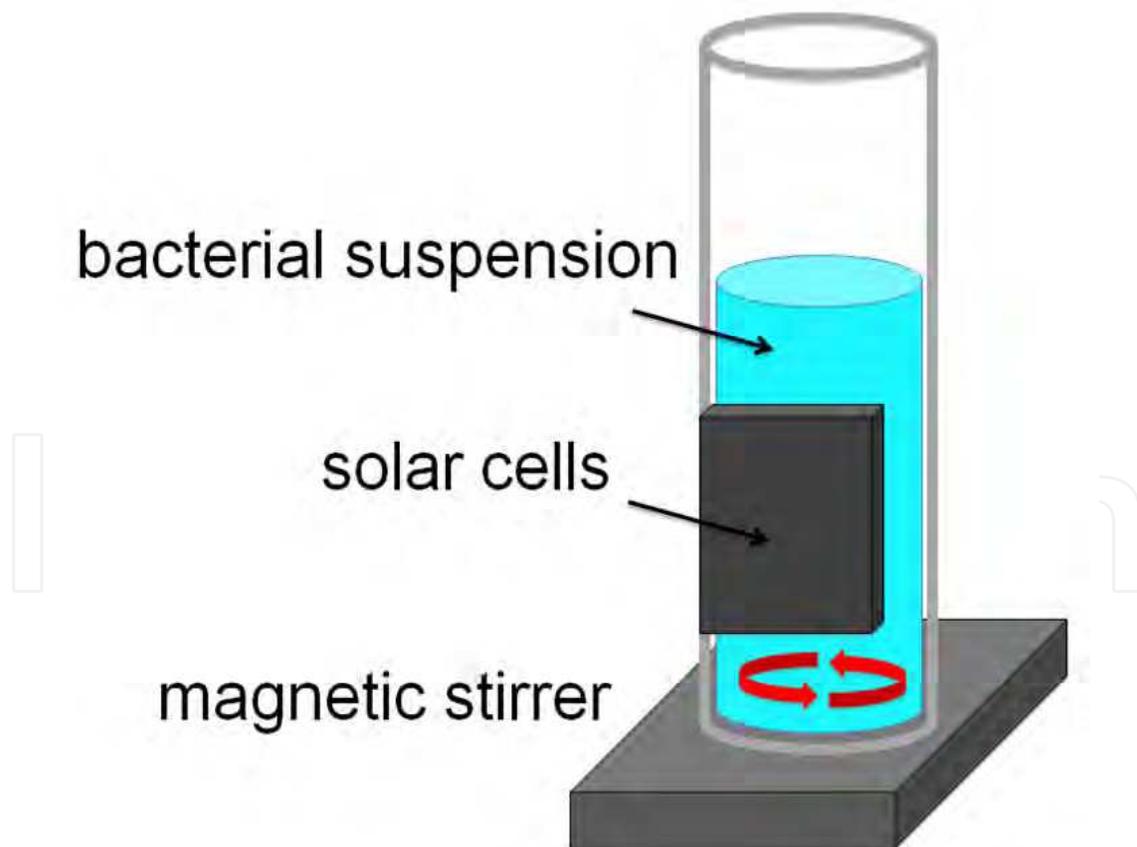
## 2. Experimentals

Bioluminescent bacteria, *Photobacterium kishitanii*, collected from the skin of a cuttlefish and *Todarodes pacificus* (for sashimi), were purified and used. In a well-stirred solution, dissolved oxygen is in equilibrium with the atmospheric oxygen. This may not be the case with a bioluminescent bacterial suspension. As reported above, the luminescent reaction consumes oxygen to produce light. Simultaneously, production of the substrate FMNH<sub>2</sub> requires energy that is produced by respiration. Karatani calculated the energy required to produce light and concluded that the bacterial bioluminescence is an extremely oxygen-consuming process. A bioluminescent bacterial suspension was, therefore, suspected to show a very low dissolved oxygen (DO) concentration. In this study, we began with the measurement of both DO and luminescent intensity through the period of oscillation.

As the luminescent reaction occurs inside the cell, the luminescent intensity is affected by the [DO] inside the cell rather than that in the suspension. Because the dynamic measurement of [DO] within a bacterium is considered to be difficult, we focused on any change in cell density during the oscillation period. The colour of bacterial bioluminescence is determined by the fluorescent protein (LumP) (Sato Y, Shimizu S, Ohtaki A, Noguchi K, Miyatake H, Dohmae N, Sasaki S, Odaka M, Yohda M., Crystal structures of the lumazine protein from *Photobacterium kishitanii* in complexes with the authentic chromophore, 6,7-dimethyl-8-(1'-D-ribityl) lumazine, and its analogues, riboflavin and flavin mononucleotide, at high resolution., *J Bacteriol.* 2010 Jan;192(1):127-33.). We then, therefore, measured the spectral change in luminescence through the oscillation period.

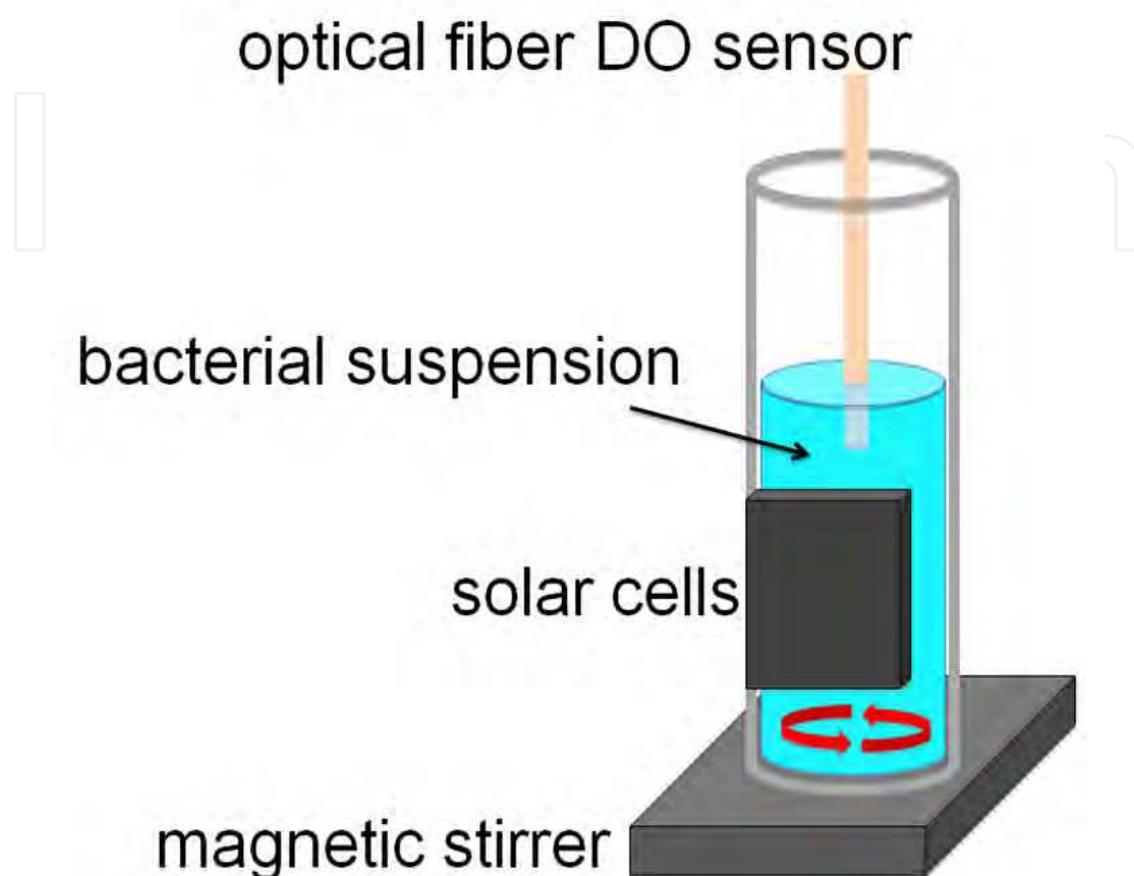
## 2.1 Relationship between the bacterial bioluminescence and dissolved oxygen concentration in a bacterial suspension

Photobacterium belongs to a family of Gram-negative, facultatively aerobic bacteria (Urbanczyk, H.; Ast, J.C. & Dunlap, P.V. (2011)). We started by measuring the oxygen effect on bioluminescence. The intensity of the bioluminescence was measured using a self-made luminescence detector (five commercially available solar cells were connected in series) or optical power meter (Model 3664, Hioki E.E. Co.). The output voltage generated by both devices was measured and recorded with an A/D converting logger (NR 250, Keyence Co.). An oscillation broth (Yeast extract 2.5 g L<sup>-1</sup>, Bacto peptone 5 g L<sup>-1</sup>, and NaCl 30 g L<sup>-1</sup>) or marine broth (Difco™ marine broth 2216, Becton, Dickinson, and Company) was prepared and filtrated using a 0.22 µm filter (Nalgene disposable filter unit, Thermo Fisher Scientific, Inc.). A glass cell with an inner diameter of 31 mm was placed over a magnetic stirrer. The schematic illustration of the measurement system is shown in Fig. 1. All the equipment was placed in an incubator (VS401, Versos Co., Ltd.) adjusted at 17°C with 10, 20, 30, and 50 mL of oscillation broth to determine the effects of the air-liquid interface area/volume. In addition, the dilution effect of the marine broth on the oscillation mode was investigated by diluting the broth 1.5 and 3 times. For the simultaneous measurement of luminescence and dissolved oxygen concentration, an optical fibre-based DO sensor (FOXY R, Ocean Optics, Inc.) was placed into the bacterial suspension (Fig. 2).



An aluminium foil cap was placed loosely on the glass tube to prevent contamination during the measurement.

Fig. 1. Schematic illustration of the bioluminescence intensity measurement.

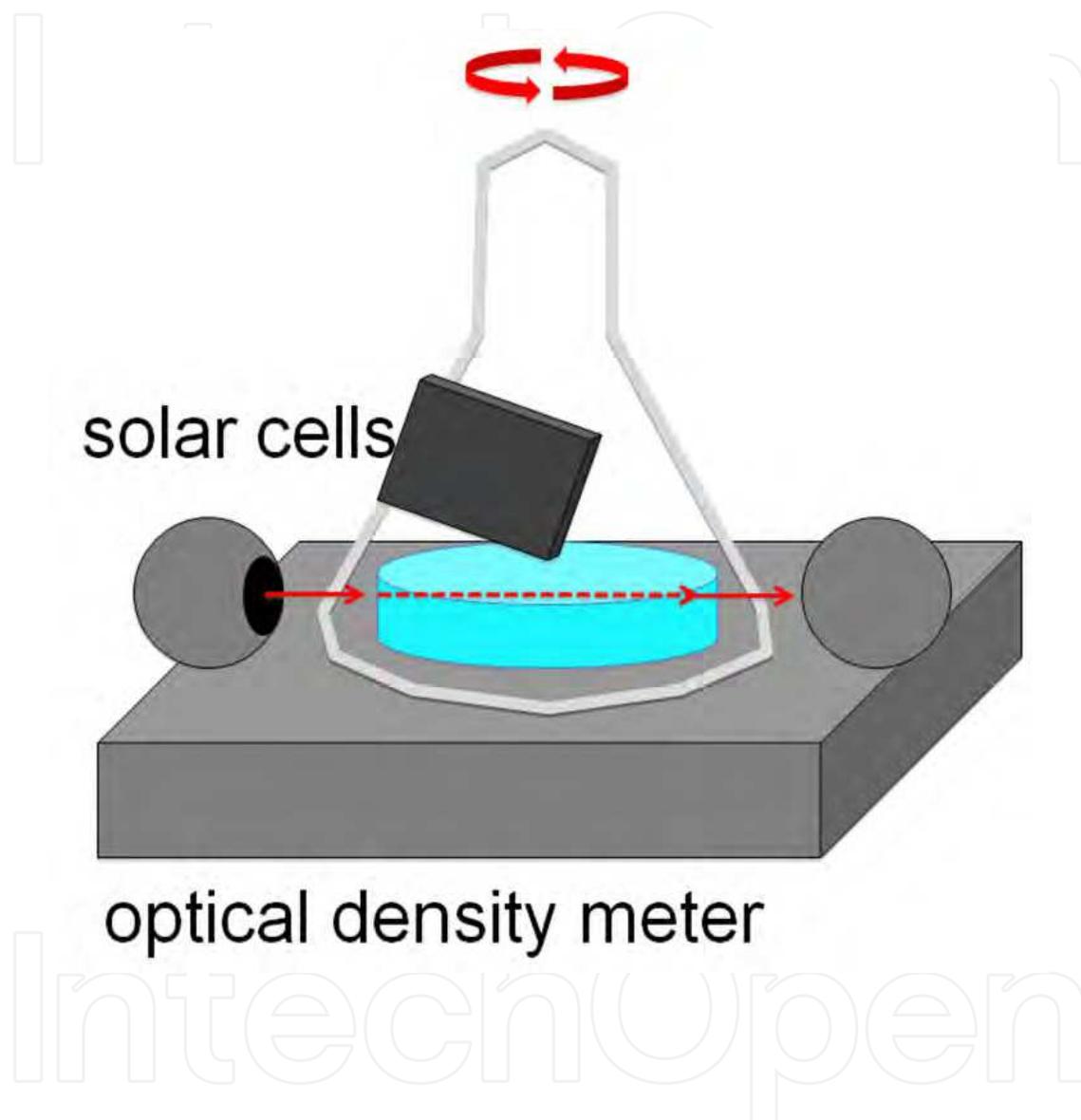


An optical fibre sensor tip was placed vertically in the middle of the bacterial suspension. An aluminium foil cap was placed loosely on the glass tube to prevent contamination during the measurement.

Fig. 2. Schematic illustration of the system for the simultaneous measurement of the luminescence intensity and dissolved oxygen concentration.

## 2.2 Simultaneous measurement of the luminescence and cell density during oscillation

Continuous measurement of the optical density (OD) of the bacterial suspension was performed using an OD meter (ODBox-A, TAITEC Co.). A 500 mL Erlenmeyer flask with 100 mL of bacterial suspension was set over a rotary shaker (NR-2, TAITEC Co.), and, on the surface of the flask, five solar cells connected in a series were attached (Fig. 3). The generated voltage was measured and recorded by the same logger as reported in 2.1. All the equipment was placed in a self-made dark box, and measurements were performed at room temperature ranging from 20 to 23°C.

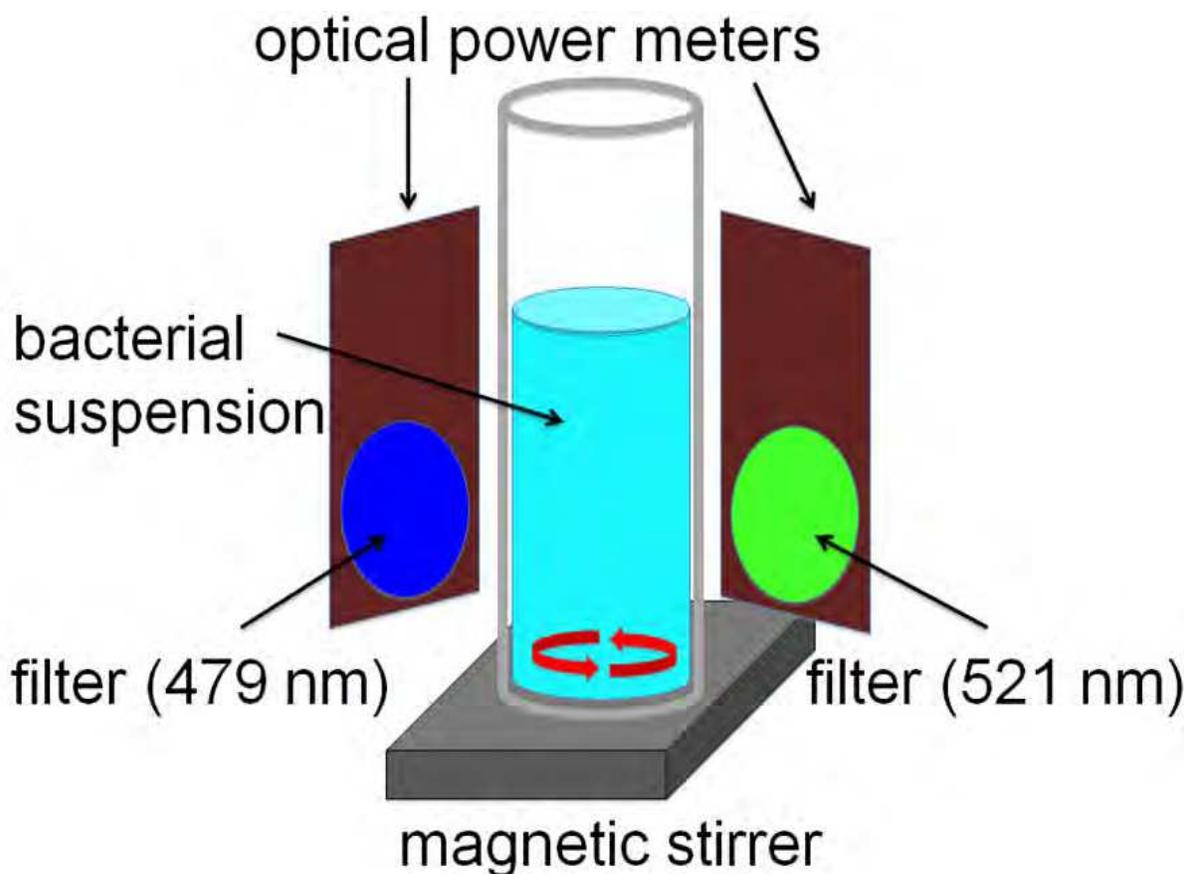


A 500 mL Erlenmeyer flask with 100 mL oscillation broth was shaken at 100 rpm. Solar cells were attached on the flask surface. All the optical setup was enclosed in a self-made dark box.

Fig. 3. Experimental setup for the simultaneous measurement of the luminescence and cell density.

### 2.3 Spectral change in the bacterial bioluminescence during oscillation

Two optical filters that transmit wavelengths of 479 and 521 nm (Optical Coatings Japan) were placed to cover the sensor windows of the optical power meter (Fig. 3).

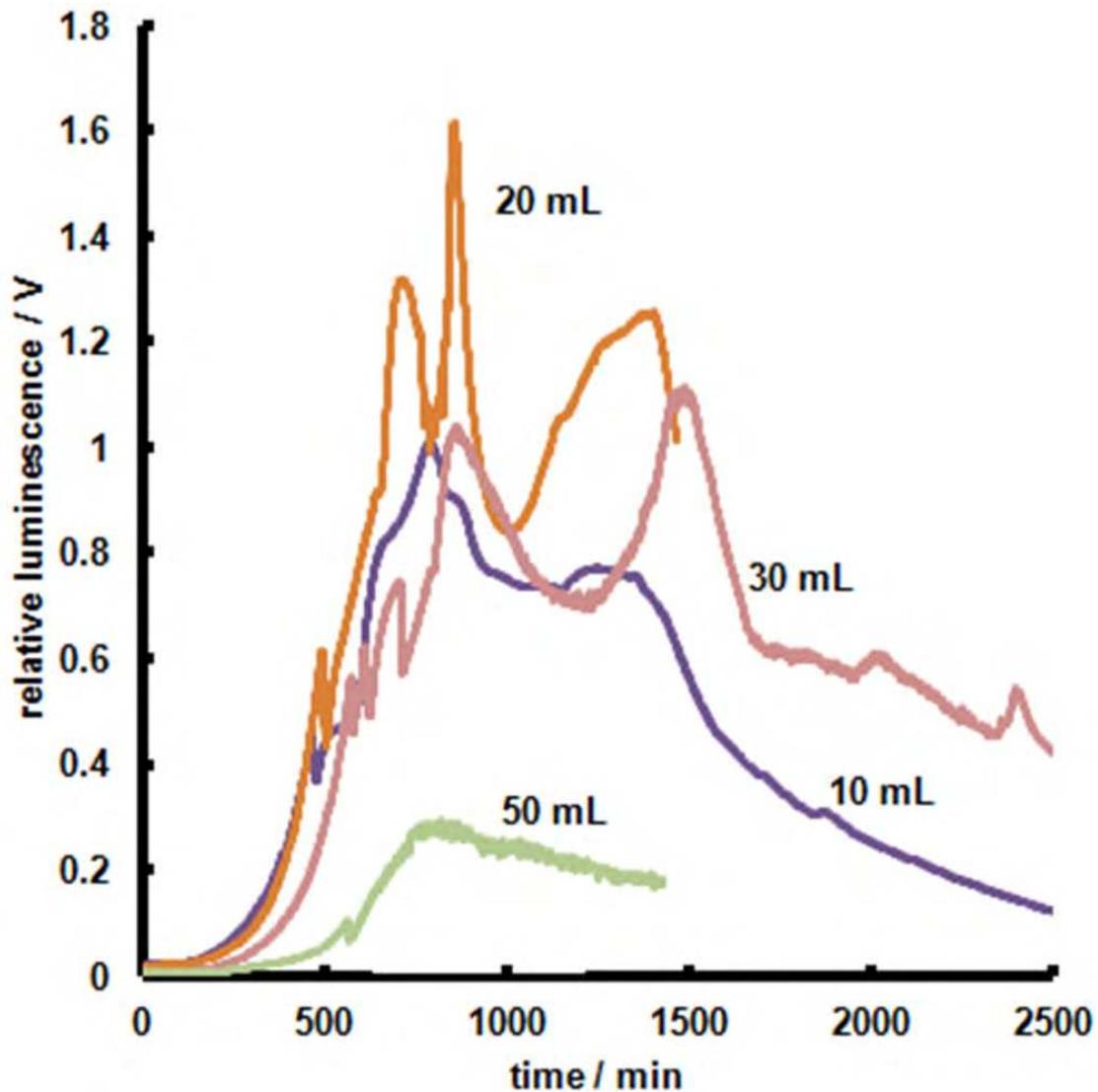


Two sensors with filters were placed at the same height so that the stirrer bar did not affect the measurement. All the optical setup, including the magnetic stirrer, was enclosed in the incubator.

Fig. 4. System setup for the measurement of spectral change.

### 3. Results and discussion

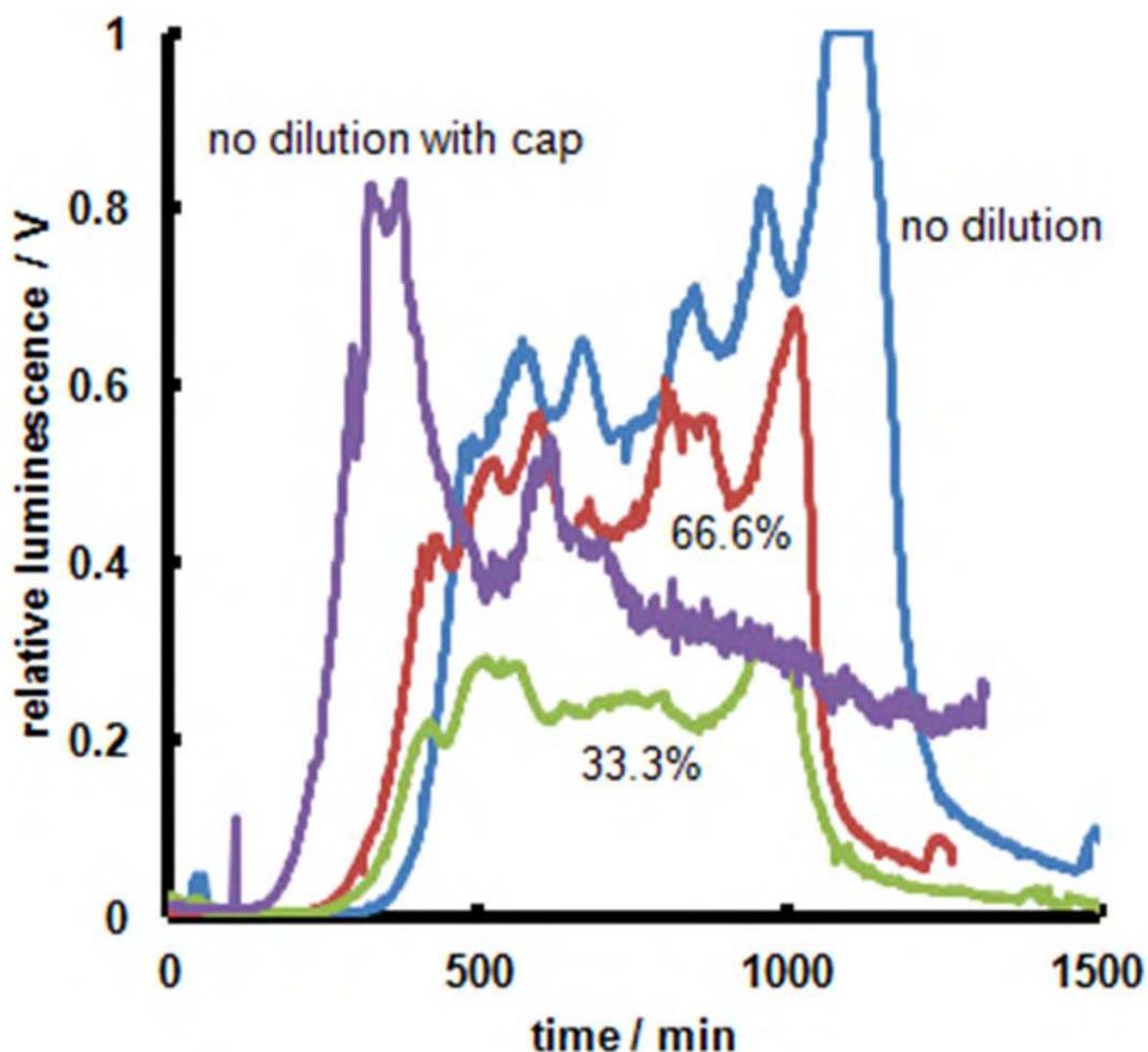
The effects of the suspension volume on the oscillation mode are shown in Fig. 5. Remarkable oscillatory waves were observed in the case of 10, 20, and 30 mL but not in the case of 50 mL. It was noteworthy that, even with the largest volume, the 50 mL suspension showed the smallest luminescent intensity. This might be due to the shortage of the oxygen supply, as the fixed liquid-air interface area could allow a fixed amount of oxygen diffusion into the suspension. In the case of 50 mL, the distributed oxygen to each cell should be smaller than in the case of other volumes. As the case of 30 mL showed the most distinct oscillation, this volume was chosen for further experiments.



*P. kishitanii* was inoculated into oscillation broth with each volume in a 22 mm diameter glass tube. The suspension was stirred using a magnetic stirrer. The temperature was maintained at 17°C.

Fig. 5. Suspension volume effect on the oscillation in bacterial bioluminescence.

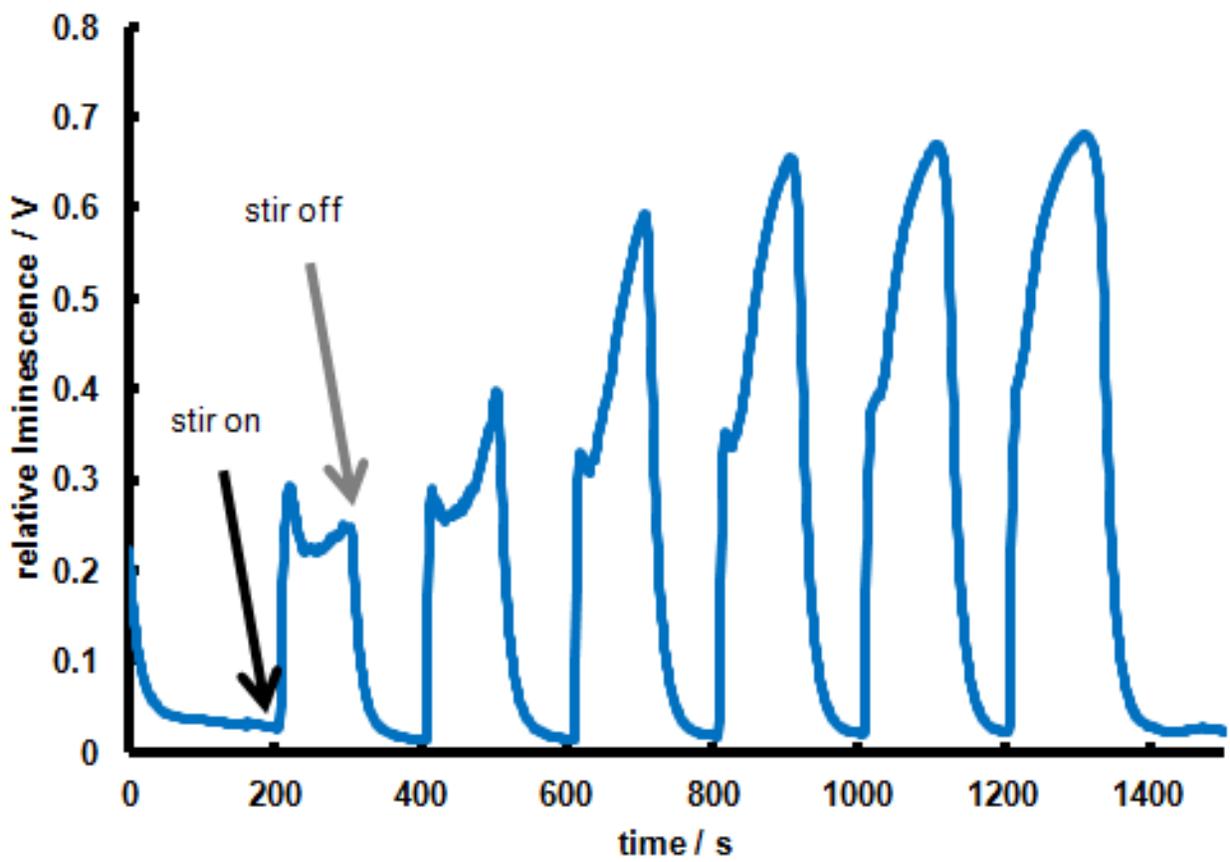
First, oscillation in bioluminescence was observed only in the case with the oscillation broth (Sato, Y. & S. Sasaki (2008)). We were interested in the use of the common marine broth and tried to determine the broth dilution effect on the mode of oscillation (whether or not it oscillated) because, in our previous report, the oscillation was thought to be the result of a lack of nutrients. Therefore, even with the marine broth, the oscillation was observed (Fig. 6). In cases of no dilution, clearer peaks were observed than in the cases with dilution. In addition, the luminescence measurement was performed with a cap on the glass tube. This case also showed, though with a different mode, an oscillatory behaviour. Through measurement with two different broths, the effect of oxygen supply into the suspension on the mode change of oscillation was strongly indicated.



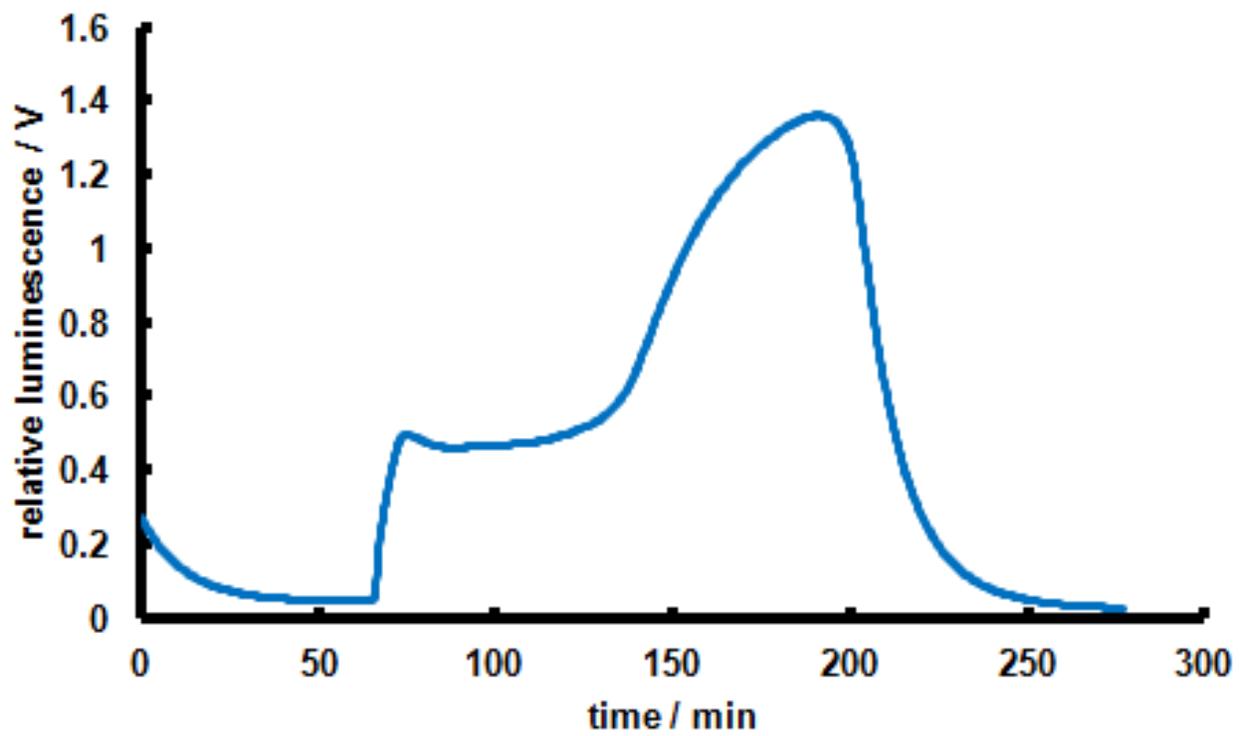
The measurement conditions were the same as those reported in Fig. 5.

Fig. 6. Broth-dilution effect on the oscillation in bacterial bioluminescence.

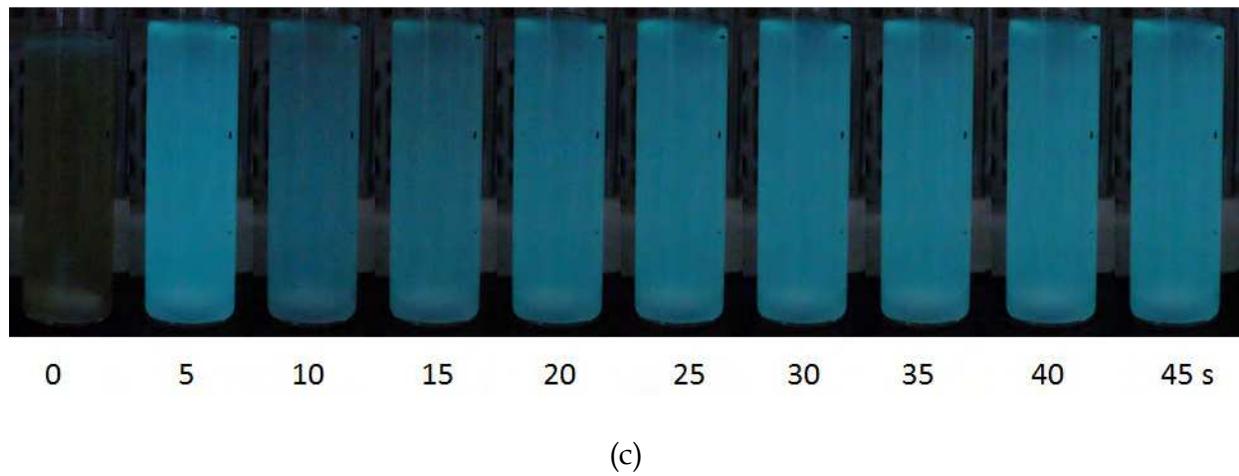
Encouraged by the above results, we tried to see the stirring effect on the bioluminescence over a shorter period because, during measurements lasting more than a day (1,440 min), the cell density effect on the luminescence could not be ignored. We, therefore, used brightly glowing suspensions (5 – 19 hours after inoculation /  $10^8$ - $10^9$  cells  $\text{mL}^{-1}$ ) and investigated the effect of stirring on the luminescence intensity. First, the dark suspension was stirred until the luminescence reached a stable intensity. The result is shown in Fig. 7 (a). The luminescence intensity was gradually increased. This might be due to the increase in the fluorescence activity of LumP. In other bioluminescent bacteria, *V. fischeri* Y1, a fluorescent protein changes the fluorescent activity in its redox states; i.e., when reduced, the fluorescence is lost, and, when oxidised, the original fluorescence is retrieved (Karatani, H.; Izuta, T.; & Hirayama, S. (2007)). LumP in *P. kishitanii* might have similar characteristics.



(a)



(b)



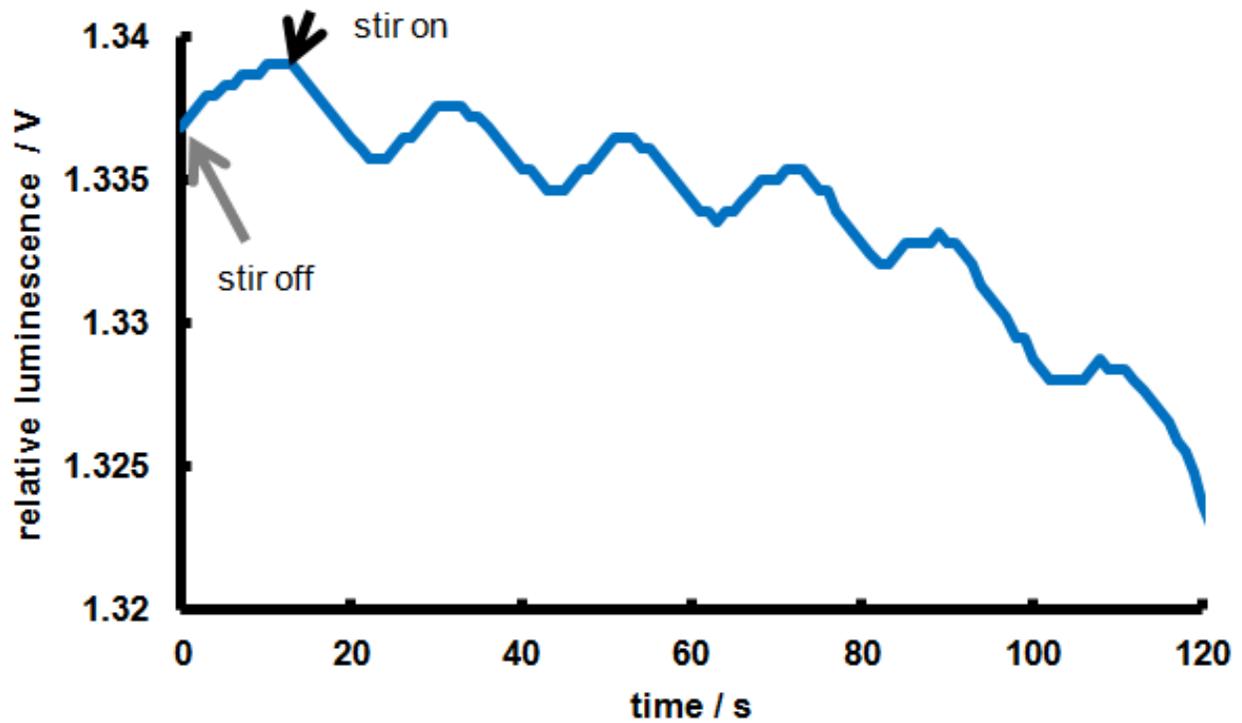
In (a), the stirrer was switched on at 200, 400, 600, 800, 1,000, and 1,200 min and off at 300, 500, 700, 900, and 1,100 min. In (b), the stirrer was switched on at 60 s and off at 180 s. The measurements in both (a) and (b) were performed at 17°C. Photographs in (c) were taken at a 5 s interval.

Luminescence from the suspension after the stirrer was switched on was measured for two minutes (Fig. 7 (b)). A local maximal luminescence was observed right after the stirring (ca. 60 s), and then, a gradual increase was observed. This characteristic might be related to the LumP fluorescence ability, but the photographs of the luminescence showed no significant colour change (Fig. 7 (c)).

Fig. 7. Time course of the luminescence from the dark suspension after repeated stirring (a), a typical luminescence curve showing two peaks of intensity (b), and interval photographs of luminescence from the suspension in experiment (b) (c).

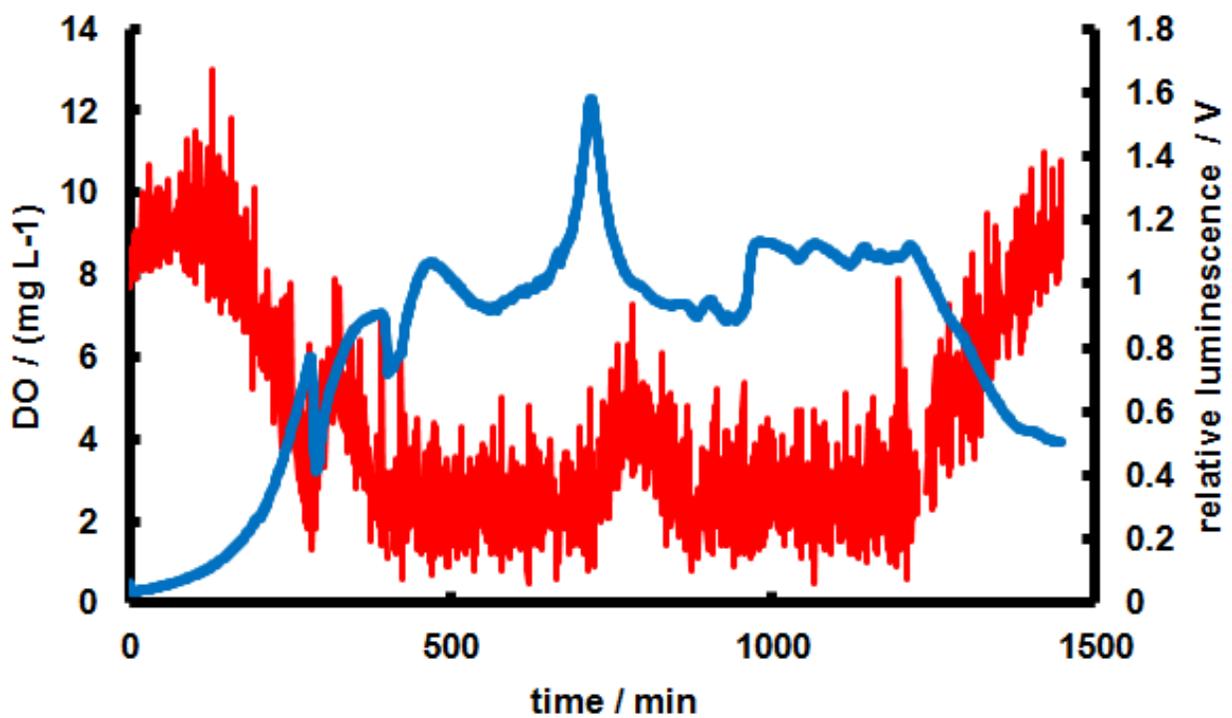
The effect of stirring on the bright (originally well-stirred) suspension luminescence resulted in different outcomes (Fig. 8). The luminescence increased after switch-off and decreased after switch-on. This tendency is the opposite of the results in Fig. 7 (a). The reason for the decreasing tendency of luminescence under the stirred condition is difficult to explain as long as we regard the suspension to be homogeneous. As is reported later, the condition of the cells in the suspension seemed to be inhomogeneous.

The suspension DO characteristic during the oscillation is shown in Fig. 9. As is evident from the figure, the DO during the oscillation was approximately zero. This result was considered to be reasonable, since the origin of bioluminescence was an oxygen-quenching mechanism. One evolutionary purpose of bioluminescence is oxygen quenching (Rees, J.F. (1998), Timmins, G.S. (2001), Szpilewska, H., Czyz, A. & Wegrzyn, G. (2003)). In a well-stirred condition, oxygen in the atmosphere diffused into the suspension, but most of it was assumed to be consumed by both the luminescence reaction and respiration. *Vibrio fischeri* was reported to perform anaerobic respiration using a certain gene regulator (Septer, A.N.; Bose, J.L.; Dunn, A.K. & Stabb, E.V. (2010)). No such report was available for the *Photobacterium* species. As a result, there was no significant relationship between the suspension DO and oscillatory waves. From this result, we recognised the importance of considering the DO within rather than outside the cell.



The stirrer was switched off at 0, 20, 40, 60, 80, and 100 s and on at 10, 30, 50, 70, 90, and 110 s. The measurement was performed at 17°C.

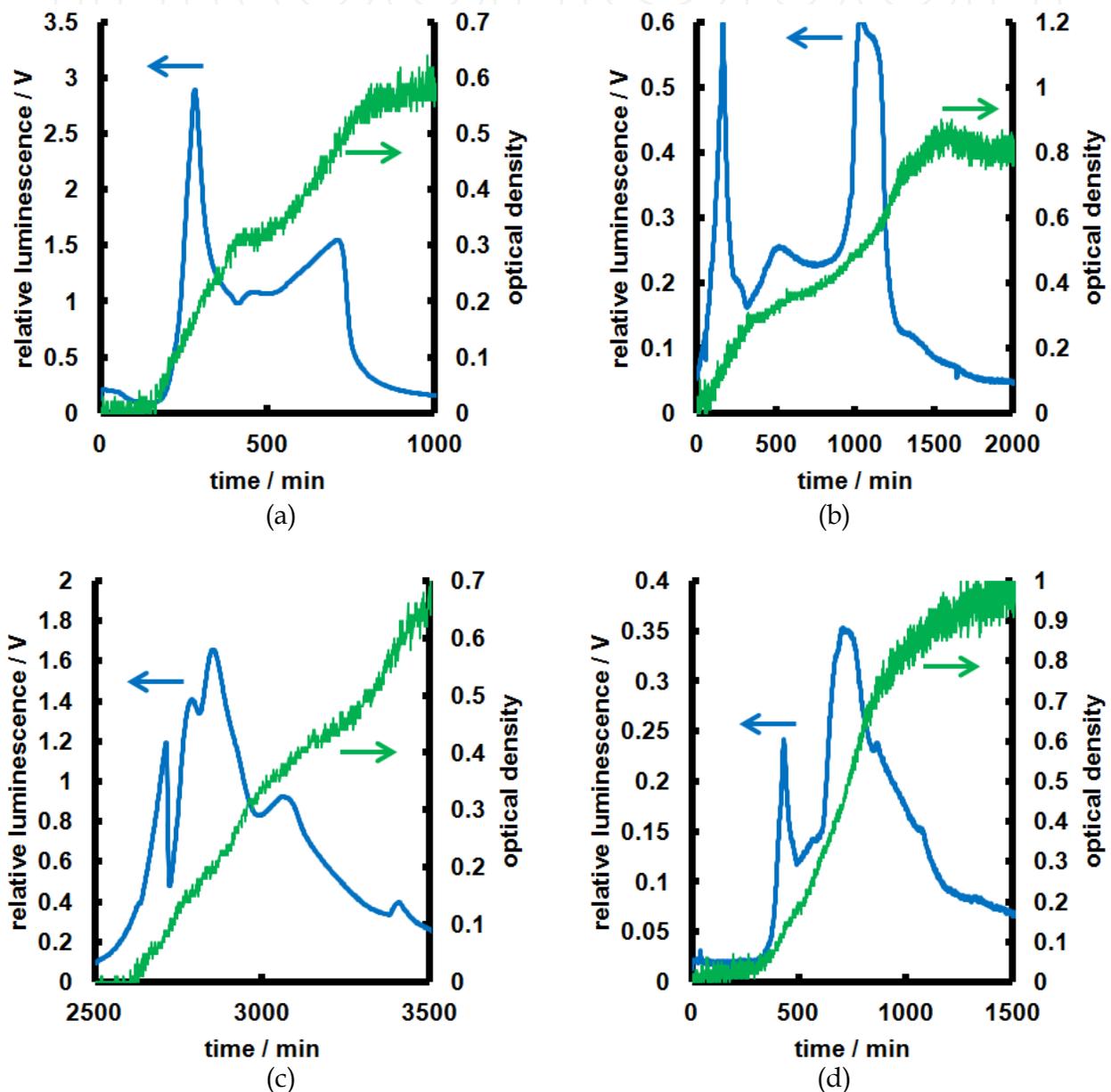
Fig. 8. Effect of stirring on the bright suspension.



Data was recorded every ten minutes.

Fig. 9. Time courses of dissolved oxygen and luminescence.

The cell density was expressed by the optical density (OD) in the measurement. OD was measured as the decrease in near-infrared light measured at the sensor (Fig. 3). This OD probe light did not affect the bioluminescence measurement using solar cells. Four results of the simultaneous measurement of DO and luminescence are shown in Fig. 10 (a) - (d). We searched for the common characteristics between the DO and luminescent curves in the four cases and found that, after the luminescence peak, a plateau in the DO curve appeared. This might be due to the decrease in DO inside the cell after the luminescence that inhibited the respiration. Lack of oxygen might have suppressed the energy production by the respiration.

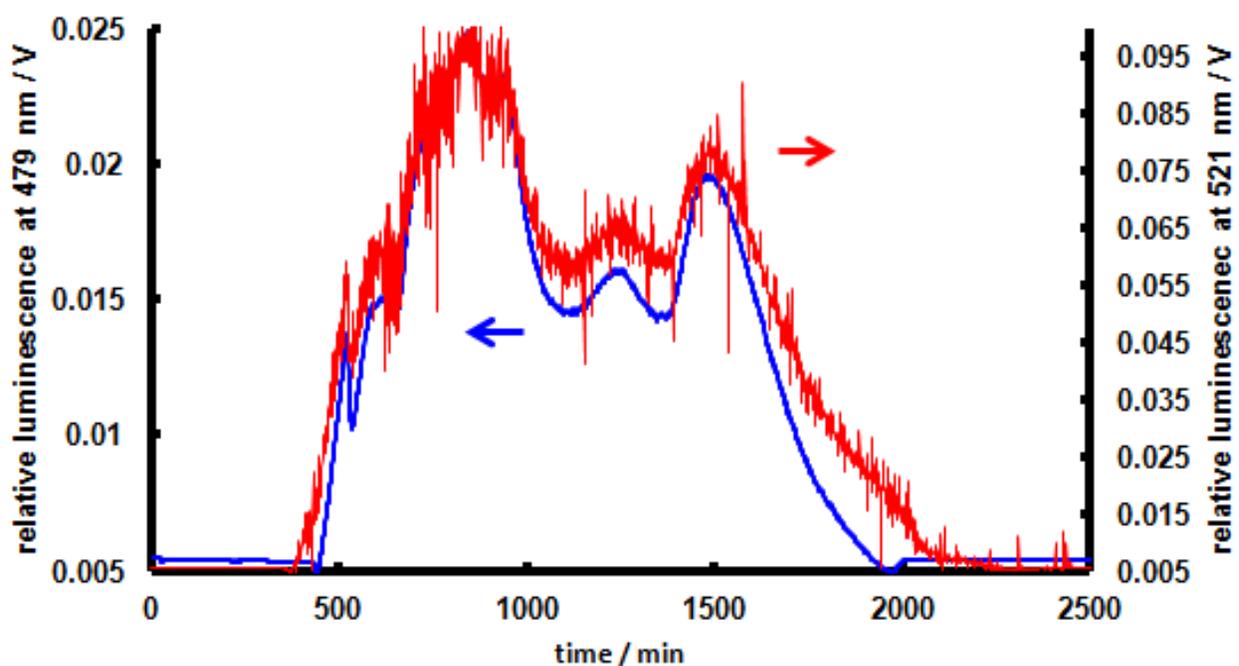


A 100 mL oscillation broth in a 500 mL Erlenmeyer flask was used for each measurement. Measurements were performed at room temperature (20-23°C).

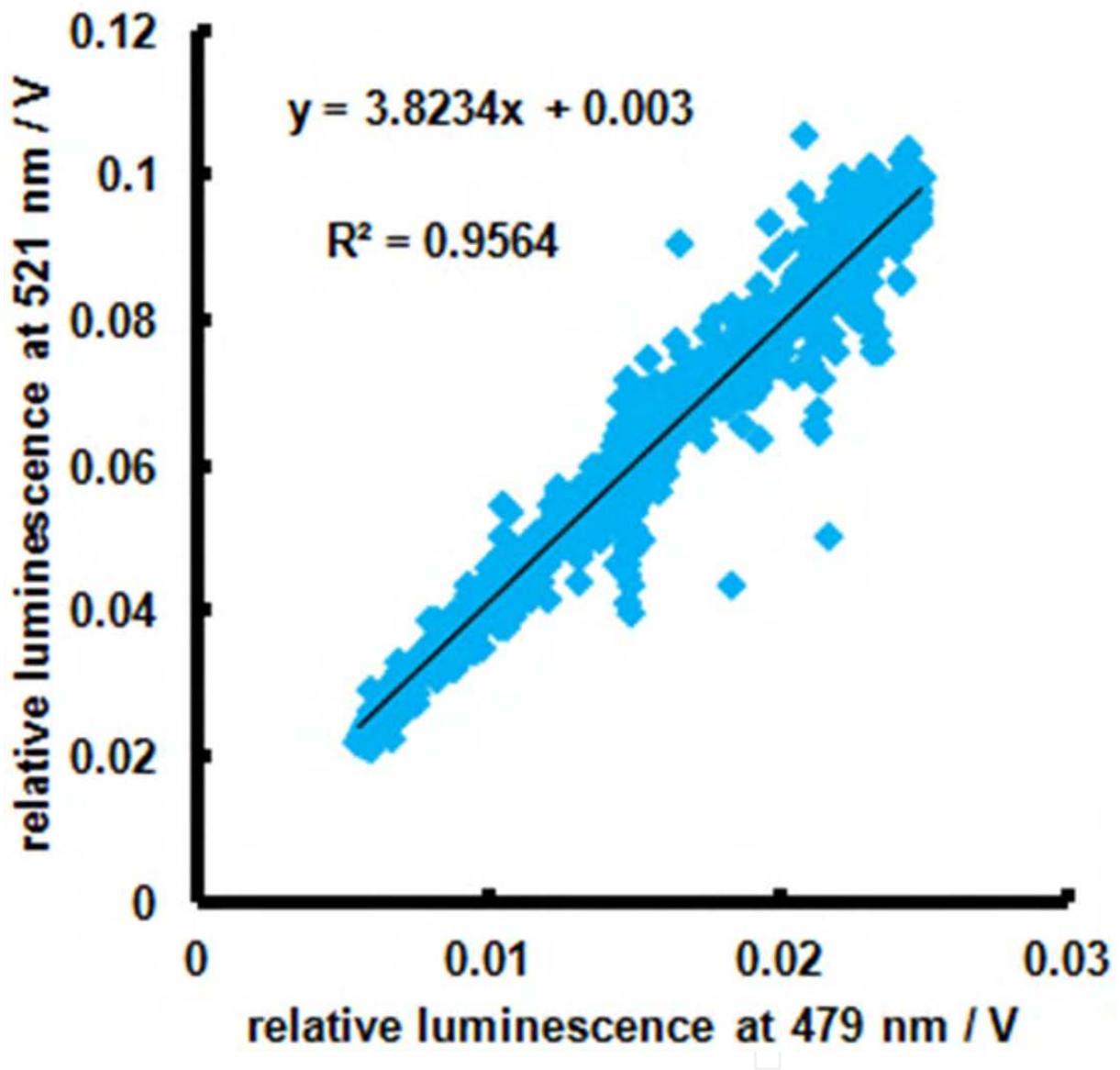
Fig. 10. Time courses of the luminescence and optical density in four experiments under the same condition.

The oscillation mode observed under the same suspension condition differed, as shown in the figures. These differences should be kept in mind for the following experiments. As reported above, the luminescence from LumP (peak wavelength: ca. 475 nm) was the main part of the observed light. The ratio of the luminescence at throughout the oscillation was estimated by the use of optical filters. The results are shown in Fig. 11 (a). A blue light with a spectral peak at 479 nm appeared ca. 1 h after a green light (521 nm) and quenched 4 h before that. This result indicated the change in the fluorescence ability at the beginning and at the end of the oscillation. When the luminescence intensity at 521 nm was plotted against that at 479 nm, the two showed a linear relationship (Fig. 11 (b)). This indicated that the LumP fluorescence ability was stable during the oscillation period.

For the first time, we found an oscillation in bioluminescence intensity. The next step would be to identify the initial reason for the oscillation. Since a definitive answer is not yet available, we propose the hypothesis explained below. Bacterial luminescence spectral change has been reported (Eckstein, JW.; Cho, KW.; Colepicolo, P.; Ghisla, S.; Hastings, JW. & Wilson, T. (1990).; Karatani, H.; Matsumoto, S.; Miyata, K.; Yoshizawa, S.; Suhama, Y. & Hirayama, S. (2006).; Karatani, H.; Yoshizawa, S. & Hirayama, S. (2004).). Under the DO-rich condition, the LumP fluorescence capacity is high, and a blue light is evident, whereas, under a DO-poor condition, luciferin-luciferase luminescence (with a peak wavelength of 540 nm) occupies the main part, and a green light is evident. When the luminescence spectra measured with and without stirring were compared, a slight difference in the peak wavelength was observed (Fig. 12). This result agreed with the above-mentioned report.



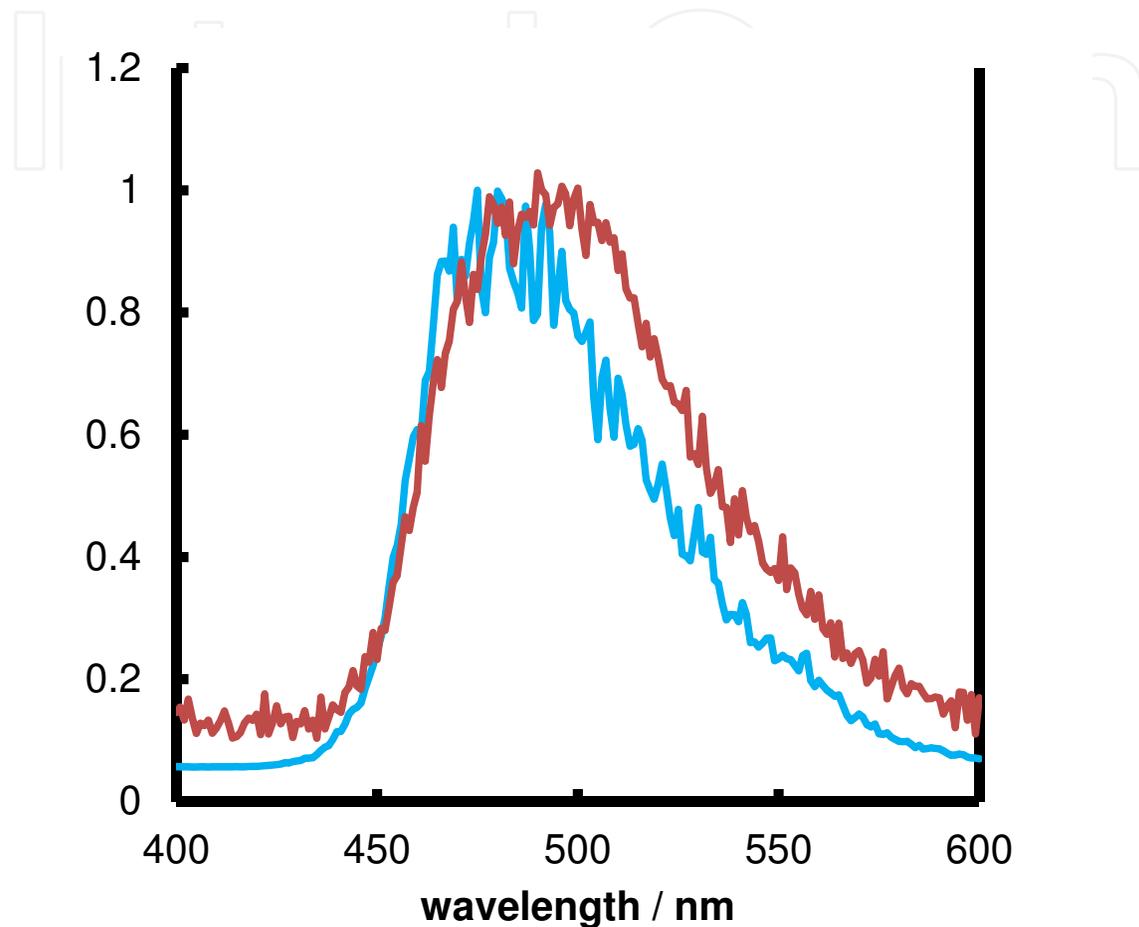
(a)



(b)

An approximation line between the two luminescences is illustrated. The coefficient of determination ( $R^2$ ) was calculated to be 0.9564.

Fig. 11. Bioluminescence oscillation observed in two colours (a) and relationship between blue (479 nm) and green (521 nm) colours (b).



The blue curve indicates the spectrum of luminescence at 479 nm, and the brown curve indicates that at 521 nm.

Fig. 12. Bioluminescence spectra with and without stirring (normalized).

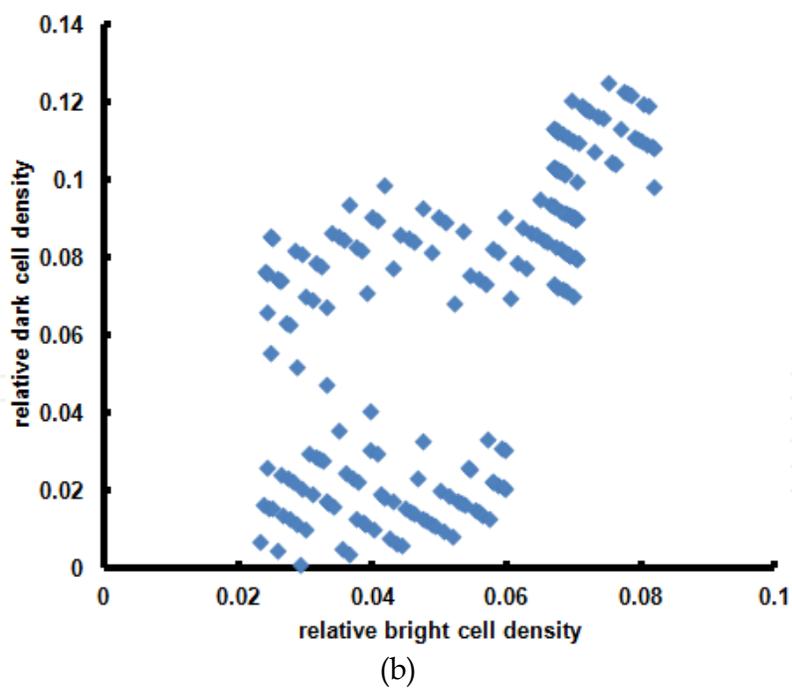
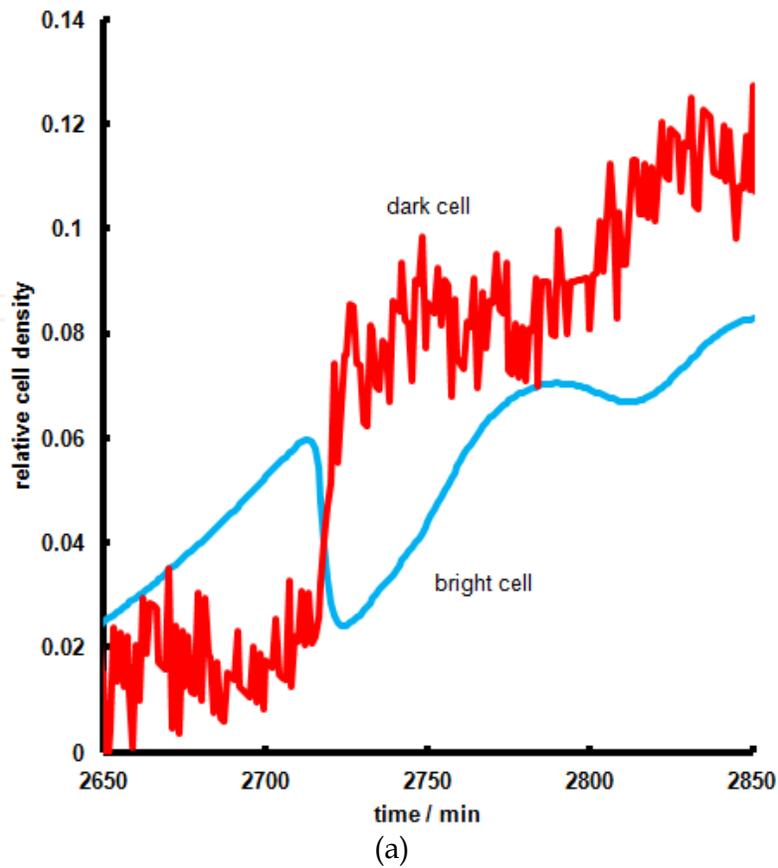
During cell cultivation, the variety of cell phases was assumed to increase with cell growth even when the inoculated cells had the same, synchronised cell phases. In the glowing suspension, the cell condition was assumed to be inhomogeneous. A photograph of the bioluminescent suspension after the stirrer was switched off is shown in Fig. 13. A slowly precipitating block of cells was glowing as brightly as the air-liquid interface part. At that moment, the DO in the middle of the suspension was zero. Unlike others, this block of cells emitted light even under the  $[DO]=0$  condition.



The image was photographed using a digital still camera (GR Digital 3, Ricoh Company, Ltd.) with exposure time of 1/20 s, ISO 1600, f/1.9. The raw image was modified to enhance the contrast using image software (ImageJ).

Fig. 13. Image of brightly glowing cell block precipitating in the suspension.

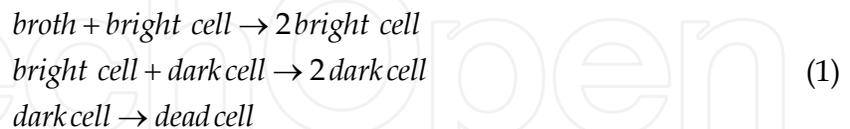
The results in Fig. 10 indicated the possibility that the luminescence affected the cell growth; i.e., an increase in luminescence caused oxygen deficiency and inhibited the respiration needed for cell growth. Cell growth was assumed to be expressed by the time derivative of the optical density. We, therefore, plotted the time courses of relative luminescence and the time derivative of OD in the same time scale (Fig. 14 (a)). The result shown in Fig. 10 (c) was used because it showed five obvious peaks in the relative luminescence curve. As is clear in Fig. 14 (a), the peaks and valleys in the luminescence curve coincided with those in the time derivative of the optical density. We then plotted the derivative against the relative luminescence (Fig. 14 (b)). The obtained curve showed that the two parameters were in the relationship with a negative Pearson product-moment correlation coefficient.



In (a), the relative bright cell density was calculated as  $0.05 \times (\text{relative luminescence})$ , whereas the relative dark cell density was calculated as  $\{\text{OD} - 0.05 \times (\text{relative luminescence})\}$ . In (b), data at 2650 - 2850 min were chosen.

Fig. 14. Time courses of bright and dark cells (a) and relative dark cell density plotted against the relative bright cell density (b).

The type of model that could describe such oscillatory behaviour should be identified. One of the best-known models is the one proposed by Alfred Lotka and, later, by Vito Volterra (Mounier, J.; Monnet, C.; Vallaey, T.; Arditi, R.; Sarthou, AS.; Helias, A. & Irlinger, F. (2008).; Varon, M. & Zeigler, BP. (1978).; Tsuchiya, HM.; Drake, JF.; Jost, JL. & Fredrickson, AG. (1972).) This model is often used to characterise predator-prey interactions. If we were to adjust the bacterial bioluminescence in the model, the following might be examples:



In these reactions, we regarded that

1. one bright cell divides into two bright cells with the supply of infinite broth;
2. one bright cell becomes a dark cell as a result of interaction with a dark cell (both cells consume oxygen as a result of respiration and become dark ones);
3. a dark cell becomes a dead cell.

If we write

A: broth, X: bright cell, Y: dark cell, P: dead cell, then the above equations can be written as



We consider A, the broth, to be infinite and not to decrease through the oscillation reaction (however, in an experiment, it does). As X and Y are the function of the time t, we can write two equations, such as,

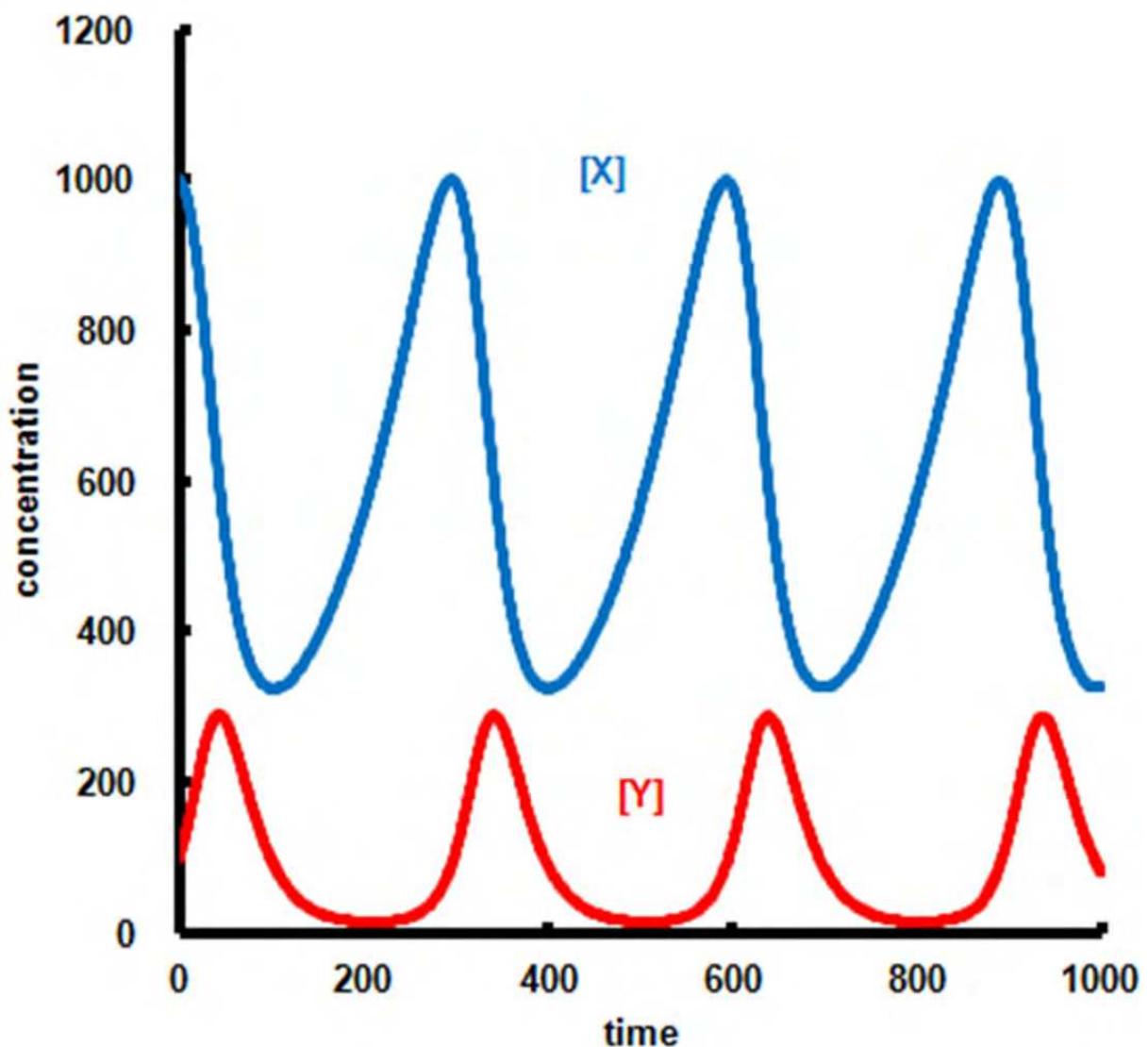
$$\begin{aligned} \frac{dX}{dt} &= k_1[A][X] - k_2[X][Y] \\ \frac{dY}{dt} &= k_2[X][Y] - k_d[Y] \end{aligned} \quad (3)$$

These are the typical equations that appear in the model. We have a numerical solution of the two equations, i.e., the time course of X and Y through the simulation using a common spreadsheet software that runs on a personal computer.

Model (1) is not proved to interpret what is going on in the oscillation, but we can approach the real image of the oscillatory reaction. By changing the parameters  $k_1$ ,  $k_2$ , and  $k_d$ , we will have curves that look like what we observe, and we should then determine the values for the three parameters and evaluate their suitability from a biochemical viewpoint.

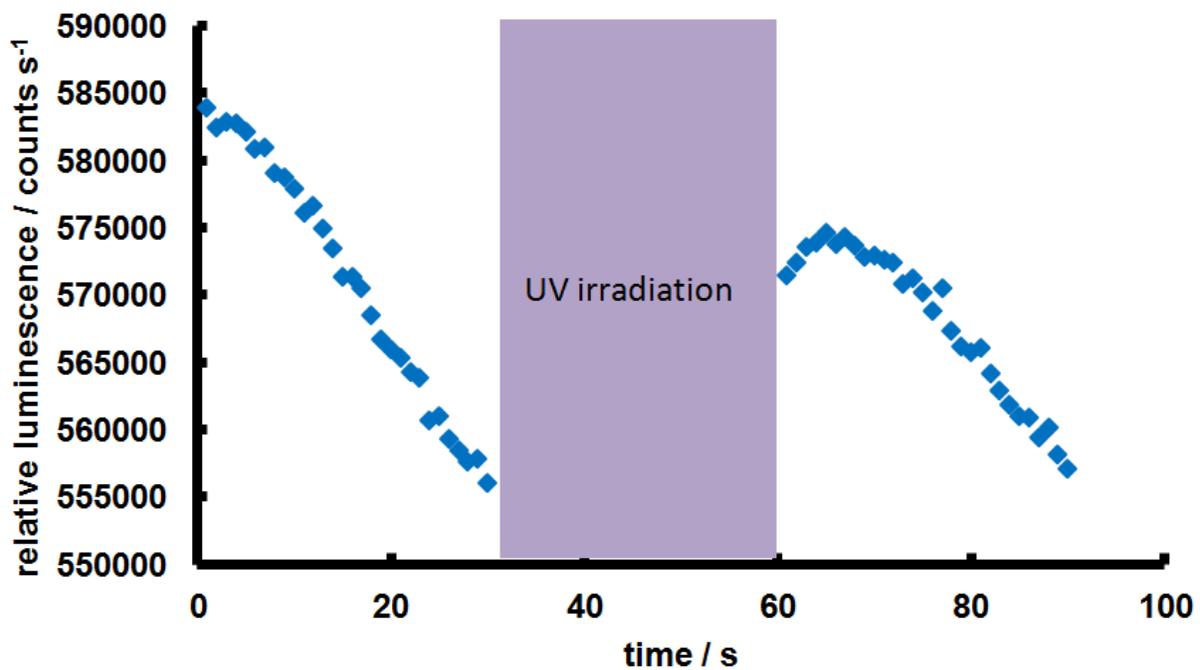
As reported in relation to Fig. 13, luminescence from the suspension with a volume of several tens – hundreds of mL might contain luminescence from cells of different conditions. Future investigation of cells with similar conditions is indicated, therefore, to be necessary. The relationship between the bacterial motility and luminescence was investigated (Sasaki,

S.; Okamoto, T. & Fujii T. (2009)). The evaluation of surface-adsorbed cells was thought to be an effective way for this purpose. The characteristics of the luminescence from ca.  $1.0 \times 10^6$  cells adsorbed on a glass surface are shown in Fig. 16. Irradiation of the cells was performed using a near-UV light (UV lamp—long wavelength, # 166-0500EDU, BIO RAD). The irradiation has the potential to cause a change in the redox state of FMN or other materials that produce an increase in luminescence. Bacterial bioluminescence from the electromagnetic viewpoint has been studied (Pooley DT. (2011)). Investigation of this luminescence from physico-chemical as well as biochemical viewpoints would be needed to explain the entire image of bacterial bioluminescence.



The initial values were  $[X]=1,000$  and  $[Y]=100$ , with constants  $k_1=0.009$ ,  $k_2=0.06$ , and  $k_d=0.0001$ . The integration time was set at 1, and the calculation was performed using Microsoft Excel 2007 running on a personal computer.

Fig. 15. Solution of Equation (2) using a numerical calculation (Runge-Kutta method)



Oil was used to prevent the bacterial environment from drying. Glass with an amino group modification (MAS coated glass slides, Matsunami Glass Ind., Ltd.) was used for the adsorption. The glass was soaked in a marine-broth-based bacterial suspension overnight. A measurement was performed using a luminescence meter (GENE LIGHT GL-200S, Microtec Nichion).

Fig. 16. Effect of irradiation to the luminescence from cells adsorbed on a glass surface.

#### 4. Conclusion

Oscillation in the bacterial bioluminescence mode is strongly dependent on the amount of oxygen supply to the solution. There is no clear relationship between the DO concentration and luminescence intensity, perhaps due to the consumption of oxygen by both the luminescence and respiration. The oscillation occurred at a very low DO concentration, and, when the time course of cell density was plotted with the same timescale as the luminescence intensity, the cell growth rate seemed to decrease after the strong luminescence. The fluorescence ability of LumP seemed constant during the oscillation period, but, at the beginning and at the end, it seemed to decrease. The characterisation of luminescence from a smaller number of cells would be necessary for further investigation of oscillation, considering that the suspension is a mixture of cell groups with a variety of cell phases.

## 5. Acknowledgments

The author thanks Dr. Hajime Karatani of the Kyoto Institute of Technology for his participation in discussions and Shoji Yamada, Kenshin Tamura, Shingo Kuriyama, Mika Mochizuki, and Hajime Kimoto for their assistance with the experiments.

## 6. References

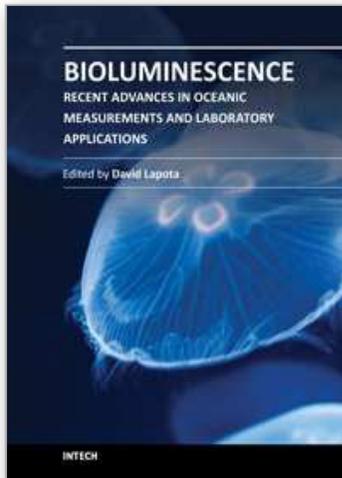
- Aivasidis, A.; Melidis, P.; Georgiou, D. (2002). Use of a microbial sensor: a new approach to the measurement of inhibitory effects on the microbial activity of activated sludge. *Bioprocess and Biosystems Engineering*, Vol. 25, No. 1, (April 2002), pp. 29-33, ISSN 1615-7591
- Balny, C. & Hastings, J. W. (1975). Fluorescence and Bioluminescence of Bacterial Luciferase Intermediates. *Biochemistry*, Vol. 14, No. 21, (October 1975), pp. 4719-4723, ISSN 0006-2960
- Chang, IS.; Moon, H.; Jang, JK. & Kim, BH. (2005). Improvement of a microbial fuel cell performance as a BOD sensor using respiratory inhibitors. *Biosensors and Bioelectronics*, Vol. 20, No. 9, (March 2005), pp. 1856-1859, ISSN 0956-5663
- Davila, D.; Esquivel, JP.; Sabate, N. & Mas, J. (2011). Silicon-based microfabricated microbial fuel cell toxicity sensor. *Biosensors and Bioelectronics*, Vol. 26, No. 5, (October 2010), pp. 2426-2430, ISSN 0956-5663
- Dobrescu, R. & Purcarea, VI. (2011). Emergence, self-organization and morphogenesis in biological structures. *Journal of medicine and life*, Vol. 4, No. 1, (February 2011), pp. 82-90, ISSN 1844-122x
- Eckstein, JW.; Cho, KW.; Colepicolo, P.; Ghisla, S.; Hastings, JW. & Wilson, T. (1990). A time-dependent bacterial bioluminescence emission spectrum in an in vitro single turnover system: energy transfer alone cannot account for the yellow emission of *Vibrio fischeri* Y-1. *Proceedings of the National Academy of Sciences U S A*, Vol. 87, No. 4, (February 1990), pp. 1466-1470, ISSN 0027-8424
- Girott, S.; Ferri, E. N.; Fumo, M. G.; & Maiolini, E. (2008). Monitoring of environmental pollutants by bioluminescent bacteria. *Analytica Chimica Acta*, Vol. 608, No. 1, (February 2008), pp. 2-29, ISSN 0003-2670
- Hastings, J. W. (1996). Chemistries and Colors of Bioluminescent Reactions: a Review. *Gene*, Vol. 173, No. 1, (July 1995), pp. 5-11, ISSN 0378-1119
- Kang, KH.; Jang, JK.; Pham, TH.; Moon, H.; Chang, IS. & Kim, BH. (2003). A microbial fuel cell with improved cathode reaction as a low biochemical oxygen demand sensor. *Biotechnology Letters*, Vol. 25, No. 16, (August 2003), pp. 1357-1361, ISSN 0141-5492
- Karatani, H.; Izuta, T. & Hirayama, S. (2007). Relationship between the redox change in yellow fluorescent protein of *Vibrio fischeri* strain Y1 and the reversible change in color of bioluminescence in vitro. *Photochemical and Photobiological Sciences*, Vol. 6, No. 5, (January 2007), pp. 566-570, ISSN 1474-905X
- Karatani, H.; Matsumoto, S.; Miyata, K.; Yoshizawa, S.; Suhama, Y. & Hirayama, S. (2006). Bioluminescence color modulation of *Vibrio fischeri* strain Y1 coupled with alterable levels of endogenous yellow fluorescent protein and its fluorescence imaging.

- Photochemistry and Photobiology*, Vol. 82, No. 2, (March 2006), pp. 587-592, ISSN 0031-8655
- Karatani, H.; Yoshizawa, S. & Hirayama, S. (2004). Oxygen triggering reversible modulation of *Vibrio fischeri* strain Y1 bioluminescence *in vivo*. *Photochemistry and Photobiology*, Vol. 79, No. 1, (January 2004), pp. 120-125, ISSN 0031-8655
- Kenkre, V. M.; & Kumar, N. (2008). Nonlinearity in bacterial population dynamics: Proposal for experiments for the observation of abrupt transitions in patches. *Proceedings of the National Academy of Sciences U S A*, Vol. 105, No. 48, (November 2008), pp. 18752-18757, ISSN 0027-8424
- Kim, M.; Hyun, MS.; Gadd, GM.; Kim, GT.; Lee, SJ. & Kim, HJ. (2009). Membrane-electrode assembly enhances performance of a microbial fuel cell type biological oxygen demand sensor. *Environmental Technology*, Vol. 30, No. 4, (April 2009), pp. 329-336, ISSN 0959-3330
- Kogure, H.; Kawasaki, S.; Nakajima, K.; Sakai, N.; Futase, K.; Inatsu, Y.; Bari, ML.; Isshiki, K. & Kawamoto, S. (2005). Development of a novel microbial sensor with baker's yeast cells for monitoring temperature control during cold food chain. *Journal of Food Protection*, Vol. 68, No. 1, (January 2005), pp. 182-186, ISSN 0362-028X
- Kurfurst, M.; Ghisla, S. & Hastings, J. W. (1983). Bioluminescence Emission from the Reaction of Luciferase-Flavin Mononucleotide Radical with  $O_2 \cdot$ . *Biochemistry*, Vol. 22, No. 7, (March 1983), pp. 1521-1525, ISSN 0006-2960
- Lee, J.; Wang, Y. Y. & Gibson, B. G. (1991). Electronic Excitation Transfer in the Complex of Lumazine Protein with Bacterial Bioluminescence Intermediates. *Biochemistry*, Vol. 30, No. 28, (July 1991), pp. 6825-6835 ISSN 0006-2960
- Moon, H.; Chang, IS.; Kang, KH.; Jang, JK. & Kim, BH. (2004). Improving the dynamic response of a mediator-less microbial fuel cell as a biochemical oxygen demand (BOD) sensor. *Biotechnology Letters*, Vol. 26, No. 22, (November 2004), pp. 1717-1721, ISSN 0141-5492
- Mounier, J.; Monnet, C.; Vallaey, T.; Arditi, R.; Sarthou, AS.; Helias, A. & Irlinger, F. (2008). Microbial interactions within a cheese microbial community. *Applied and Environmental Microbiology*, Vol. 74, No. 1, (November 2007), pp. 172-181, ISSN 0099-2240
- Pooley DT. (2011). Bacterial bioluminescence, bioelectromagnetics and function. *Photochemistry and Photobiology*, Vol. 87, No. 2, (March 2011), pp. 324-328, ISSN 0031-8655
- Raushel, F. M. & Baldwin, T. O. (1989). Proposed Mechanism for the Bacterial Bioluminescence Reaction Involving a Dioxirane Intermediate. *Biochemical and Biophysical Research Communications*, Vol. 164, No. 3, (November 1989), pp. 1137-1142 ISSN 0006-291X
- Rees, J. F., B. de Wergifosse, O. Noiset, M. Dubuisson, B. Janssens and E. M. Thompson; (1998). The Origins of Marine Bioluminescence: Turning Oxygen Defense Mechanisms into Deep-Sea Communication Tools, *The Journal of Experimental Biology*, Vol. 201, No. 8, (April 1998), pp. 1211-1221, ISSN 1010-061X

- Sasaki, S.; Okamoto, T. & Fujii, T., (2009). Bioluminescence intensity difference observed in luminous bacteria groups with different motility. *Letters in Applied Microbiology*, Vol. 48, No. 3, (March 2009), pp. 313-317, ISSN 0266-8254
- Sasaki, S.; Mori, Y.; Ogawa, M. & Funatsuka, S. (2010). Spatio-temporal control of bacterial suspension luminescence using a PDMS cell, *Journal of Chemical Engineering of Japan*, Vol. 43, No. 11, (August 2010), pp. 960-965, ISSN 0021-9592
- Sato, Y. & Sasaki, S. (2006). Control of the Bioluminescence Starting Time by Inoculated Cell Density. *Analytical Sciences*, Vol. 22, No. 9, (September 2006), pp. 1237-1239, ISSN 0910-6340
- Sato, Y. & Sasaki, S. (2008). Observation of Oscillation in Bacterial Luminescence. *Analytical Sciences*, Vol. 24, No. 3, (January 2008), pp. 423-425, ISSN 0910-6340
- Sato, Y.; Shimizu S; Ohtaki A; Noguchi K; Miyatake H; Dohmae N; Sasaki S; Odaka M & Yohda M. (2010). Crystal structures of the lumazine protein from *Photobacterium kishitanii* in complexes with the authentic chromophore, 6,7-dimethyl- 8-(1'-D-ribose) lumazine, and its analogues, riboflavin and flavin mononucleotide, at high resolution., *Journal of Bacteriology*, Vol. 192, No. 1, (January 2010), pp. 127-33, ISSN 0021-9193
- Septer, AN.; Bose, JL.; Dunn, AK. & Stabb, EV. (2010). FNR-mediated regulation of bioluminescence and anaerobic respiration in the light-organ symbiont *Vibrio fischeri*. *FEMS Microbiology Letters*, Vol. 306, No. 1, (February 2010), pp. 72-81, ISSN 0378-1097
- Shirazy, N. H.; Ranjbar, B.; Hosseinkhani, S.; Khalifeh, K.; Madvar; A. R. & Naderi-Manesh, H. (2007). Critical Role of Glu175 on Stability and Folding of Bacterial Luciferase: Stopped-Flow Fluorescence Study. *Journal of Biochemistry and Molecular Biology*, Vol. 40, No. 4, (July 2007), pp. 453-458, ISSN 1225-8687
- Szpilewska, H., A. Czyz and G. Wegrzyn; (2003). Experimental Evidence for the Physiological Role of Bacterial Luciferase in the Protection of Cells Against Oxidative Stress, *Current Microbiology*, Vol. 47, No. 5, (November 2003), pp. 379-382 ISSN 0343-8651
- Timmins, G. S., S. K. Jackson and H. M. Swartz; (2001). The Evolution of Bioluminescent Oxygen Consumption as an Ancient Oxygen Detoxification Mechanism, *Journal of Molecular Evolution*, Vol. 52, No. 4, (April 2001), pp. 321-332, ISSN 0022-2844
- Tsuchiya, HM.; Drake, JF.; Jost, JL. & Fredrickson, AG. (1972). Predator-prey interactions of *Dictyostelium discoideum* and *Escherichia coli* in continuous culture. *Journal of Bacteriology*, Vol. 110, No. 3, (June 1972), pp. 1147-1153, ISSN 0021-9193
- Tu, S. C.; Lei B.; Liu M.; Tang, C. K. & Jeffers C. (2000). Probing the Mechanisms of the Biological Intermolecular Transfer of Reduced Flavin. *The Journal of Nutrition*, Vol. 130, No. 2, (February 2000), pp. 331-332, ISSN 0022-3166
- Urbanczyk, H.; Ast, JC. & Dunlap, PV. (2011). Phylogeny, genomics, and symbiosis of *Photobacterium*. *FEMS Microbiology Reviews*, Vol. 35, No. 2, (September 2010), pp. 324-342, ISSN 0168-6445
- Vaiopoulou, E.; Melidis, P.; Kampragou, E. & Aivasidis, A. (2005). On-line load monitoring of wastewaters with a respirographic microbial sensor. *Biosensors and Bioelectronics*, Vol. 21, No. 2, (December 2004), pp. 365-371, ISSN 0956-5663

- Varon, M. & Zeigler, BP. (1978). Bacterial predator-prey interaction at low prey density. *Applied and Environmental Microbiology*, Vol. 36, No. 1, (July 1978), pp. 11-17, ISSN 0099-2240
- Wu, BM.; Subbarao, KV. & Qin, QM. (2008). Nonlinear colony extension of *Sclerotinia minor* and *S. sclerotiorum*. *Mycologia*, Vol. 100, No. 6, (November 2008), pp. 902-910, ISSN 0027-5514
- Yano, Y.; Numata, M.; Hachiya, H.; Ito, S.; Masadome, T.; Ohkubo, S.; Asano, Y. & Imato, T. (2001). Application of a microbial sensor to the quality control of meat freshness. *Talanta*, Vol. 54, No. 2, (April 2001), pp. 255-262, ISSN 0039-9140

IntechOpen



## **Bioluminescence - Recent Advances in Oceanic Measurements and Laboratory Applications**

Edited by Dr. David Lapota

ISBN 978-953-307-940-0

Hard cover, 190 pages

**Publisher** InTech

**Published online** 01, February, 2012

**Published in print edition** February, 2012

We now find ourselves utilizing luciferase - luciferin proteins, ATP, genes and the whole complex of these interactions to observe and follow the progress or inhibition of tumors in animal models by measuring bioluminescence intensity, spatially and temporally using highly sophisticated camera systems. This book describes applications in preclinical oncology research by bioluminescence imaging (BLI) with a variety of applications. Chapters describe current methodologies for rapid detection of contaminants using the Milliflex system, and the use of bioluminescence resonance energy transfer (BRET) technology for monitoring physical interactions between proteins in living cells. Others are using bioluminescent proteins for high sensitive optical reporters imaging in living animals, developing pH-tolerant luciferase for brighter in vivo imaging, and oscillation characteristics in bacterial bioluminescence. The book also contains descriptions of the long-term seasonal characteristics of oceanic bioluminescence and the responsible planktonic species producing bioluminescence. Such studies are few and rare.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Satoshi Sasaki (2012). Oscillation in Bacterial Bioluminescence, *Bioluminescence - Recent Advances in Oceanic Measurements and Laboratory Applications*, Dr. David Lapota (Ed.), ISBN: 978-953-307-940-0, InTech, Available from: <http://www.intechopen.com/books/bioluminescence-recent-advances-in-oceanic-measurements-and-laboratory-applications/oscillation-in-bacterial-bioluminescence>

**INTECH**  
open science | open minds

### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen