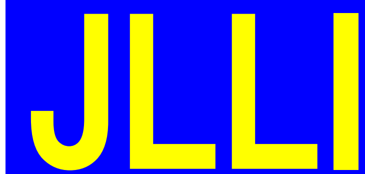

THE JOURNAL OF LIGHT EMITTING AND LIFE INNOVATION

VOL 2 March 2015

Published by the Society of Light Emitting and Life Innovation

The Journal of
Light Emitting and Life Innovation

An Official Journal of the Society of Life Emitting and Life Innovation.

This journal will consider for publication manuscripts of original investigations including clinical and experimental researches, case reports and high quality of review articles in medicine. These reports include full-length papers on original research, rapid communications of new discoveries, letters, commentaries, and invited reviews. Manuscripts are written in English.

Editor-in-Chief : Yohsuke Kinouchi

Publication Office :
Department of Preventive Enviroment and Nutrition
Institute of Health Biosciences
The University of Tokushima Graduate School
3 Kuramoto-cho, Tokushima 770-8503, Japan
Tel : +81-88-633-9428
Fax : +81-88-633-9072
e-mail : yobou@tokushima-u.ac.jp

THE JOURNAL OF
Light Emitting and Life Innovation

An Official Publication of the Society of Life Emitting and Life Innovation.

INFORMATION FOR AUTHORS

The Journal of Light Emitting and Life Innovation is published by the Society of Light Emitting and Life Innovation for the issue of original articles, case reports and reviews in the medicine. Four numbers that make a volume are published a year. Authors submitting a paper do so on the understanding that the work has not been published before, is not being considered for publication elsewhere and has been read and approved by all authors.

Send manuscripts to :
Department of Preventive Enviroment and Nutrition
Institute of Health Biosciences
The University of Tokushima Graduate School
3 Kuramoto-cho, Tokushima 770-8503, Japan
Tel : +81-88-633-9428.   Fax : +81-88-633-9072.
e-mail: yobou@tokushima-u.ac.jp

Manuscripts should be carefully revised before submission. As a general rule, papers will be evaluated by two independent reviewers. Revised manuscripts should be accompanied by a point-by-point reply to the recommendations of reviewers and editor, specifying the changes made in the revised version. When the manuscript is accepted for publication, the authors must agree to automatic transfer to the copyright to The Journal of Light Emitting and Life Innovation.

Manuscripts Submit manuscripts in triplicate (one original and two copies) and provide your manuscript on one newly fomatted 3.5-inch floppy disk, MO, CD or USB Memory filed in text style ; complete style printed manuscripts on white A 4 paper with 3 cm margin in each sides, double-spaced copies including figures and tables must accompany the disk. Mark a special character such as italic, bold, subscript, superscript, Greek letters, mathematical symbols et al. The printed manuscript and the file on the disk must be the same. Label the disk clearly with authors' names and file content.

The paper should be submitted in English. Manuscripts should be organized and numbered consecutively as follows : title, abstract, introduction, patients and methods / materials and methods, results, discussion, acknowledgements, reference list, tables, legends to figures and figures in that order. Drawings and charts for reproduction should be made in black and laser quality. Color illustraions are acceptable. Author (s) should insert the appropriate position of the text figures and tables on the manuscripts.

Abbreviations should conform to the style accepted in the Japanese Medical Sciences and used in the Quarterly Cumulative Index Medicus.

Manuscripts should be checked by native speaker.

All human investigation must be conducted according to the principles expressed in the Declaration of Helsinki.

All studies involving animals must state that guidelines for the use and care of laboratory animals of the authors' institution or any national law were followed.

Tittle The tittle page should contain : 1. a concise infomative tittle ; 2. author(s)'s names ; 3. name of department(s) / institution(s) to which the work is attributed ; 4. name, address, telephone and fax numbers of the author to whom correspondence about the manuscipt, and requests for offprints should be referred ; 5. a running head of no more than 40 characters.

Abstract and key words The abstract must not exceed 200 words. It should state the purpose of the study, basic procedures (study subject / patients / animals and methods), main findings, and principal conclusions. Below the abstract, provide 3-5 key words that will assist indexers in cross-indexing your article. Use terms from the Medical Subject Headings list from Index Medicus, whenever possible.

Acknowledgements Acknowledge persons and grant sources that have made substantive contributions to the study

References Number references consecutively in the order in which they are firstmentioned in the text. Identify references in text, tables and legends by arabic numerals(in parentheses). All references cited, and only these, must be listed at the end of the paper. All authors' names should be described. Abbreviations of journals must conform to those used in Index Medicus of the National Library of Medicine. The format should conform to the examples listed below :

References to a journal :

1. Yano S, Yanagawa H, Nishioka Y, Mukaida N, Matsushima K, Sone S : T kelper 2 cytokines differently regulate monocyte chemoattractant protein-1 production by human peripheral blood monocytes and alveolar macrophages. J Immunol 157 : 2660-2665, 1996

Reference to a book :

2. Kinney JM, Tucker HN : Energy Metabolism. Tissue determinants and cellular corollaries. Raven Press, New York, 1992

Reference to a chapter in a book :

3. Takaue Y, Kawano Y, Kuroda Y : Mobilization of peripheral blood stem cells for autografts. In : Levit DJ, Mertelsmann R, eds. Hematopoietic Stem Cells. Biology and Therapeutic Application. Marcel Dekker Inc, New York, 1995, pp.611-639

Effects of silver nanoparticles on bactericidal efficiency

Le Thi Tuyet Nhung[a,†], Hirofumi Nagata[b,†], Mutsumi Aihara[a], Akira Takahashi[a], Toshihiro Okamoto[b] Takaaki Shimohata[a], Kazuaki Mawatari[a], Masatake Akutagawa[c], Yohsuke Kinouchi[c], and Masanobu Haraguchi[b]
[a] Department of Preventive Environment and Nutrition, Institute of Health Biosciences, University of Tokushima Graduate School. [b]Department of Optical Science and Technology, Institute of Socio Techno Sciences, University of Tokushima Graduate School. [c]Department of Electrical and Electronic Engineering, Institute of Socio Techno Sciences, University of Tokushima Graduate School.
[†]These authors contributed equally to this work.

Abstruct
Photocatalytic is an effective disinfection method. In this study, TiO_2, TiO_2/Ag(nanoparticle), SiO_2, SiO_2/Ag(nanoparticle), TiO_2-SiO_2 and TiO_2-SiO_2/Ag(nanoparticle) films were prepared by sol-gel method at various sintering temperature and dip-coat speeds. The results indicated that the bactericidal efficiency of TiO_2 plus Ag(nanoparticle) is much better than that of pure TiO_2, pure SiO_2 or SiO_2 plus Ag nanoparticle. For TiO_2/Ag(nanoparticle) film, the inactivation of bacteria is highest at low sintering temperature and high dip-coat speed of film. These results contract with TiO_2 film, inactivation of bacteria is highest at high sintering temperature and high dip-coat speed of film. The photocatalytic activity increased at increasing concentration of Ag(nanoparticle) plus TiO_2 or SiO_2, increasing irradiation time and increasing irradiation dose. These indicated that the presence of UV light was a significant factor under conditions tested, presumably due to its role in generation of reactive oxygen radical species.

1.Introduction

TiO_2 is the most suitable chemical for photocatalytic disinfection of water (1). TiO_2 is widely used as a photocatalyst because it is highly efficient, nontoxic, chemically and biologically inert and photostable, inexpensive and has good mechanical hardness (2). Silver nanoparticle dispersed in TiO_2 film enhances electron-hole separation, the visible light excitation of TiO_2 and, by extension, enhancement of the photocatalytic inactivation of microorganisms (3). The aim of this work was to produce TiO_2 and/or TiO_2/Ag (nanoparticle)-coated glass substrates for surface sterilization by studying the effect of sintering temperature; ratios of TiO_2, SiO_2 and Ag(nanoparticle) in coating films; and dip-coating speed on the elimination of *Bacillus atrophaeus*.

2.Methods and Materials

Bacterial strains. *Bacillus atrophaeus* strain ATCC9372 was purchased from Shikoku Kakoki Co., Ltd (Japan), and used as a model microorganism for the disinfection experiments.

Preparation of Catalysts. TiO_2, TiO_2/Ag(nanoparticle), SiO_2, SiO_2/Ag(nanoparticle), and SiO_2-TiO_2/Ag(nanoparticle) films were prepared using *tetra-isopropoxytitanium* $Ti(i\text{-}OC_3H_7)_4$ [Kantokagaku], *silver paste* (Ag(nanoparticle)) [Nihon Paint]. The glass substrate were immersed in sol solution at rates between 0.75 and 2.25 mm/s and dried at room temperature for 10 min. The sol films were calcined at various temperatures between 250 - 600ºC for 60 min to achieve cohesion and adhesion of the film to the substrate.

Fifty microliters of bacterial suspension was added drop-wise onto the surface of each film and exposed to UVA-LED. After irradiation, the sample was placed in a petri dish with 5 ml of PBS, and shaken for 10 min. Following the appropriate dilutions, the sample was plated on LB agar plates and incubated at 37ºC for 24 h to obtain a colony count.

3.Results and discussion

Effect of calcination temperature. The survival of *B. atrophaeus* was investigated as a function of sintering temperature. Different dip-coating speeds, such as 0.75 mm/s, 1.50 mm/s, and 2.25 mm/s, were used to coat the glass substrates. The efficiency of the bactericidal activity of TiO_2 increased with increasing sintering temperature. However, the bactericidal efficiency of TiO_2/Ag(nanoparticle) increased with decreasing sintering temperature.

Effect of dip coating speed. The use of different dip-coating speeds showed that the bactericidal activities of both pure TiO_2 and TiO_2/Ag(nanoparticle) -coated glass sheets were maximal at the highest dip-coating speed. Therefore, the optimal conditions for the use of pure TiO_2 were high dip-coating speed and high sintering temperature, and the best conditions for the production of TiO_2/Ag(nanoparticle)-coated samples were low sintering temperature and high dip-coating speed. These optimized parameters were used for all subsequent experiments.

Effect of irradiation time. In the absence of UVA, neither TiO_2 nor TiO_2/Ag(nanoparticle) film exhibited bactericidal activity. Bactericidal efficiency was increased in a time-dependent manner by UVA irradiation of both TiO_2- and TiO_2/Ag(nanoparticle)-coated samples.

Effect of catalyst ratio and irradiation dose. TiO_2/Ag(nanoparticle) and SiO_2/Ag(nanoparticle), both present in atomic ratio of 6:0 and 6:1 were used to investigate the effect of a particular catalyst on photocatalytic disinfection. Because the biological activity of SiO_2 is very low, it served as the negative control for TiO_2. UVA irradiation of SiO_2 samples induced little killing; the bactericidal activity of SiO_2 was significantly lower than that of TiO_2. The addition of Ag(nanoparticle) enhanced UVA bactericidal activity in a dose-dependent manner. Likewise, the coating composed of SiO_2:TiO_2:Ag(nanoparticle) required UV exposure to activate photocatalytic bacterial inhibition. The level of SiO_2 affected antibacterial activity much less than the presence of TiO_2, as indicated by the rise in activity with catalyst loading of TiO_2. The rate of disinfection is remarkably enhanced in the presence of TiO_2 as a result of not only increasing TiO_2 content but also the presence and increasing Ag content. When Ag(nanoparticle) contacts TiO_2, electrons are transferred from TiO_2 to silver. Those electrons are loaded on the surface of silver and scavenged by an electron acceptor, thereby decreasing electron-hole recombination. Therefore, the presence of Ag(nanoparticle) in TiO_2/Ag or SiO_2/Ag coatings can help more holes transport to the surface and enhance photocatalytic efficiency.

4.Conclusion

Inactivation of bacteria is highest at high sintering temperature and high dip-coat speed of film. The photocatalytic activity increased at increasing concentration of Ag(nanoparticle) plus TiO_2 or SiO_2, increasing irradiation time and increasing irradiation dose.

References

(1) Wang, Q., S. Wang, W. Hang and Q. Gong. 2005. J. Phys. D: Appl Phys 38:389-391.
(2) Salih, F. M. 2002. J. Appl. Microbiol. 92:920-926.
(3) Ohtani, B. 2008. Chem. lett. 37:217-229.

Gallium Nitride Crystal Growth In Situ Monitoring Techniques

Vincent Robichaud[1], Yoshiyuki Majima[1], Yoshio Honda[2], Tomotaka Sano[2], Tomohiro Doi[2], Hiroshi Amano[2], Yves Lacroix[1,3]

[1] YSystems Ltd., [2] Nagoya University, [3] YSystems Kokusai Co., Ltd.

We present a novel *In Situ* monitoring technique for the growth of gallium nitride based materials and device structures. Using the bulk transmission characteristics of GaN, the temperature dependency of the band-edge is analyzed to extract key parameters which can be used for quality control in Light Emitting Diode (LED) production. Results obtained from a LED structure growth process demonstrate the potential of the technique for precise wafer temperature measurement compared to traditional pyrometry systems, which measure the temperature of the substrate below the infrared-transparent wafer. Using a time-correlated single wavelength band-edge transmission approach, a highly reproducible temperature measurement of the wafer surface was obtained. Further, precise determination of the surface residual roughness during the initial phase of the growth, or pit density, was precisely monitored in a quantitative fashion. Finally, effects of the Indium incorporation in multiple quantum well (MQW) structures used in optical emitting devices was measured to unprecedented detail. Further analysis of this information should lead to real-time monitoring of the Indium concentration, in turn bringing a new level of precision to the epitaxial deposition process of GaN based materials.

1. Introduction

In manufacturing, obtaining real time feedback of the process is important in order both to determine problems at early stages as well as to optimize the throughput of production. In the case of LEDs as well as most semiconductor based device fabrication, the initial process and the semiconductor wafer growth process can be considered most crucial. Maximizing the within-wafer (WIW) uniformity, minimize wafer defect density, and control the emission wavelength of the resulting LED, all depends on the crystal growth quality control. *In situ* monitoring tools providing real time measurements of the process are more and more being seen as basic necessity for this purpose. A particular important parameter to optimize is the temperature at the wafer surface before performing the active layer of the LED (MQW) in order to control the final emission wavelength. Traditionally, *In Situ* temperature measurements are based on an optical pyrometry system which measures the amount of black body emission. To further improve the accuracy of pyrometric measurements, such system usually have to be corrected for emissivity changes at the surface of the thin film.[1,2] However, a fundamental remaining problem is that such systems cannot distinguish between a lowering of the temperature and an unrelated decrease in the measured emission intensity such as due to a dirty window on the growth chamber. Furthermore, in the common case of GaN based LEDs grown on sapphire, where the material is transparent in the near-infrared (NIR), most of the black body emission seen by optical pyrometry comes from the heated susceptor behind the actual wafer.[6] In order for pyrometry to be effective in measuring the wafer temperature of GaN on Sapphire, the measurement would need to be made in the non-transparent region of the spectrum, typically at wavelengths shorter than 400nm.[9] This in turn has the disadvantage that black body emission decreases exponentially toward shorter wavelengths and nearly no photons are emitted at temperatures near the ones critical to the process. In this paper, we introduce a novel method allowing absolute measurement of the wafer surface temperature. This method is based on the time-correlation of a single transmitted wavelength near the band-edge of GaN, allows for rapid measurement (μsec sampling) even at low temperature, and is emissivity independent. Furthermore, due to the nature of this measurement not depending directly on emission intensity[5], traditional pyrometry may be calibrated by this

Figure 1: YGrowthMonitor (YGM) family product

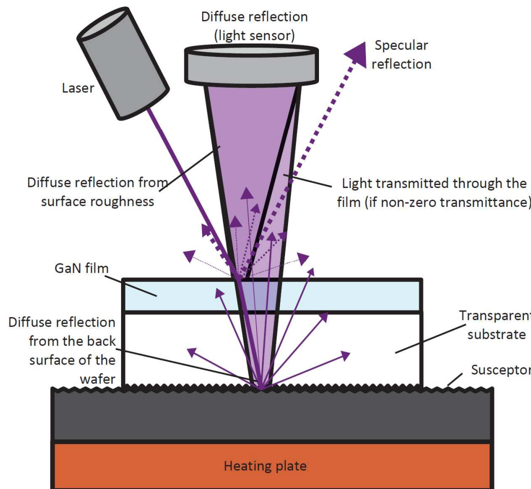

Figure 2: YGM-BandTime schematic diagram

method. Systems using only pyrometry for temperature determination require frequent calibrations to compensate for changes in the intervening optical components (such as window of the port chamber, or any optics required to access the chamber).[4,5,6] Exploiting the band-edge characteristic has the big advantage of being able to obtain a measurement of the temperature without any required calibration procedure. This new technology has been integrated in the latest generation of YGrowthMonitor(tm) tools, a family of *In Situ* monitoring products built by YSystems. Figure 1 shows an example research level system which includes the YGM-BandTime(tm)[7,8] module making use of the technology discussed in this paper.

2. Method

Figure 2 shows the principle behind YGM-BandTime. The incident light on the wafer is first transmitted through the thin film, and then reflected by the rough back surface of the substrate and/or the susceptor surface. The diffused reflection is collected by a sensor placed at a different angle compared to the specular reflection. When temperature of the GaN layer increases, its band-edge

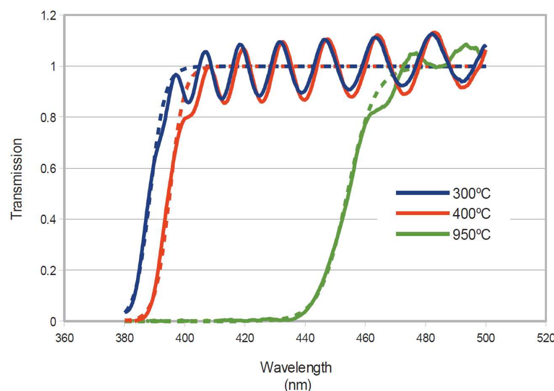

Figure 3: Temperature dependence of the GaN transmission spectrum

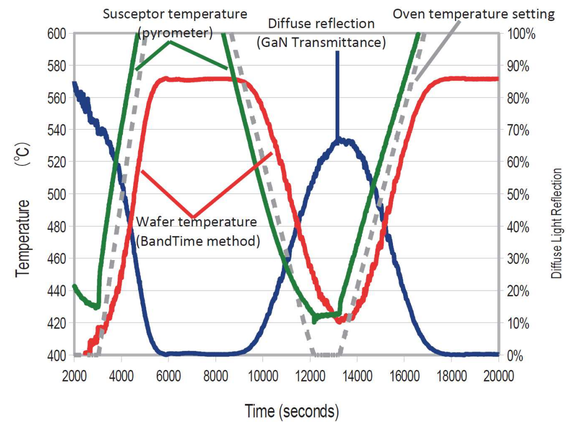

Figure 4: Pyrometer temperature versus wafer temperature for GaN on sapphire

transmission characteristic shifts to longer wavelength, as shown in Figure 3. The band-edge transmission is directly related to the bulk temperature of the GaN epitaxial layer, and therefore it is possible to extract an absolute temperature value of the wafer surface. An important point is that the diffuse reflectance used is independent from the emissivity changes of the substrate surface.[3] Furthermore, using single monochromatic laser light (single wavelengths) allows for rapid monitoring, even at low temperature, compared to full spectral acquisition, or compared to long light integration times that would be required to acquire black body emission signals at short wavelengths.[6] As mentioned above, in order to detect pyrometric information at the wafer surface, measurements made in the near ultra-violet portion of the spectrum have to be performed, since the GaN becomes transparent at longer wavelength. In spectral acquisition, white light or broad band emission light sources are usually used. Due to the large amount of data required to obtain a spectrum, and due to the relatively weak signal as compared to laser light, it is technically difficult to make such a measurement in microsecond time scales. The method presented here doesn't suffer from these drawbacks.

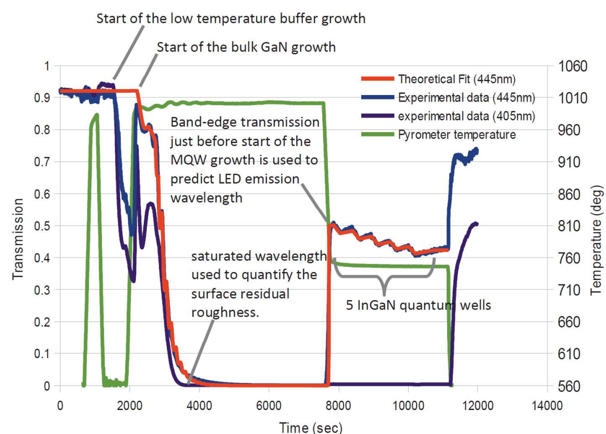

Figure 5: YGM-BandTime growth monitoring

High power blue lasers are used, making the reflected signal easy to distinguish from the background radiation of the heater. As each individual wavelength is specific to a certain temperature range, multiple wavelengths (lasers) are used to cover a wide range of temperatures.

3. Results

Measurements made of a GaN/Sapphire wafer in a controlled radiative oven are shown in Figure 4. For this experiment, the wafer was placed on a quartz susceptor. Wafer surface temperature has been extracted using the band-edge transmission of GaN to demonstrate how important can be the difference between the susceptor temperature measured by a pyrometer and the wafer temperature. Those measurements have also been made at low temperature, illustrating the capacity of this method to monitor low temperature processes. With the collaboration of the university of Nagoya, *In Situ* monitoring of a LED structure grown by metal-organic chemical vapor epitaxy (MOCVD) had been measured using YGM-BandTime. Results obtained are illustrated in Error: Reference source not found. The GaN transmission for two different wavelengths are shown (405nm and 445nm). In this process, the shortest wavelength is used to quantify the surface roughness, as it will be explained in the next section. The 445nm band-edge transmission is used to obtained the absolute temperature at the wafer surface. Another interesting aspect is the result obtained while performing the MQWs. Variations observed in the transmission signal are directly correlated with the deposition process of indium, and will also be discussed further below.

4. Discussion

For GaN layers above a certain thickness, wavelengths below the transmission band-edge (called here "saturated wavelength") will result in technically 0% of the source being reflected from the back of the wafer. The only remaining signal is therefore a diffused reflection from the wafer surface, as shown in Figure 2. By monitoring the diffuse portion of this reflection, information about the surface roughness can be obtained. In the initial phase of the growth, a high density of pits are present at the wafer surface, resulting in a strong diffused reflection. As the growth progresses, pit density reduces, and consequently the surface roughness induced signal gradually disappears, as it can be seen in Error: Reference source not found. The time at which the saturated wavelength becomes flat is used to precisely quantify the surface residual roughness, or the complete overgrowth of pits. Knowing this precisely can be used to greatly improve the efficiency of LED growth as most of the process time is currently being used to guarantee a complete GaN buffer layer. The thickness of this layer can be optimized

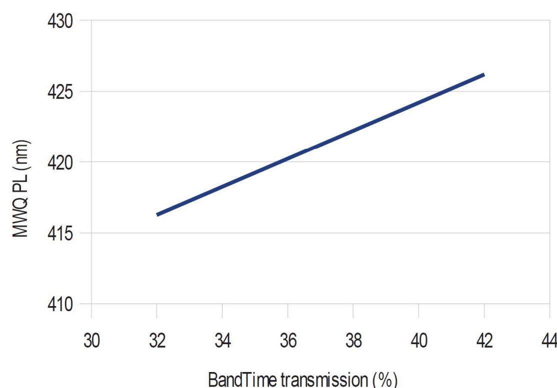

Figure 6: MQW PL trend vs GaN transmission band-edge @ 445nm

In Situ if the time required to form the complete epitaxial layer is known.

Another important parameter for LED production is the absolute temperature at the wafer surface when growing the Indium containing active layer of the LED. The temperature during this process is very important since it will directly affect the indium incorporation during the deposition process. The final indium composition of a LED in turn determines its emission wavelength. Using traditional pyrometry system, it is impossible to get an absolute temperature, since pyrometer measures the susceptor temperature. As shown in Figure 4, there is a considerable difference between the susceptor temperature and the temperature at the wafer surface. Pyrometer measurements are also affected by the transmission changes of optical components in its field of view, and therefore increase the difficulty of obtaining accurate and reproducible information. With the BandTime technology, there is no such problem since the band-edge characteristic is independent of the light intensity. Highly reproducible temperature measurement from one process to another can be achieved. As the band-edge transmission of the GaN is directly related to temperature, the

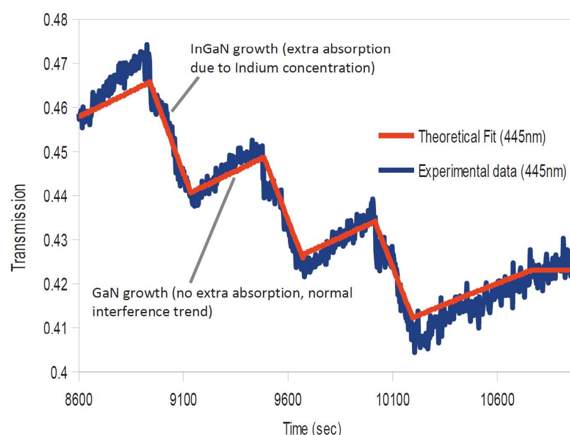

Figure 7: InGaN quantum wells growth

LED emission wavelength can be said to be dictated by the bulk band-edge transmission. The dependence of the LED emission wavelength as function of bulk transmission, or BandTime temperature, has been observed and the trend obtained is shown in Figure 6. From this trend, a direct reading of the band-edge transmission just before the start of the MQW growth (as illustrated in Error: Reference source not found) enables the prediction of the final LED emission wavelength.

Finally, details of the growth monitoring during the MQW structure warrant further discussion. Figure 7 shows a blow-up of this section taken from Error: Reference source not found, where the impact of the indium deposition on the transmitted signal is clearly seen. Each phase of the process can be observed, wherein for each step of InGaN deposition the absorption is seen to increase, while in each step of the GaN barrier the absorption remains constant, except for the optical interference change due to the layer thickness changes. The degree of absorption caused by the indium deposition gives unprecedented information related to each quantum well. Theoretical fitting has been performed and results obtained so far show great potential for exploiting this information

5. Conclusions

In the present paper, we have introduced YGM-BandTime, a new generation *In Situ* monitoring technology based on time-correlated single wavelength band-edge transmission. The temperature dependence of the band-edge is used to infer the wafer temperature, promising enhanced quality control for LED production. This technology enables rapid measurement of the absolute temperature of the wafer, even at low temperature. As the band-edge characteristic is independent of the light intensity, no particular calibration process is required, as opposed to traditional pyrometric system, which are affected by the transmission changes of intervening optical components. Highly reproducible temperature measurement of the wafer surface was achieved. Furthermore, use of saturated wavelengths was shown to provide information on the surface residual roughness during the initial phase of the growth. Finally, results obtained during the MQW process showed unprecedented detail, in which we could distinguish each phase of the indium deposition process. Those results demonstrate a great potential in a new avenue of monitoring epitaxial deposition process of GaN based materials.

Acknowledgment

Some of the results presented in this article were obtained from research supported by the New Energy and Industrial Technology Development Organization (NEDO).

References

1. J. Bodycomb, P. Helm, M. Kane, D. Nicol, I. Ferguson, Advanced Thermal Processing of Semiconductors, 2003. RTP 2003. 11th IEEE International Conference on, pp. 117- 123, 23-26 Sept. 2003
2. J.C. Ramer, B. Patel, V. Boguslavskiy, A. Patel, M. Schurman, A. Gurary, Electronic-Enhanced Optics, Optical Sensing in Semiconductor Manufacturing, Electro-Optics in Space, Broadband Optical Networks, 2000. Digest of the LEOS Summer Topical Meetings, pp. II49-II50, 2000
3. T. P. Pearsall, R. Saban Stevan, J. Booth, T. Beard Barrett, S. R. Johnson, Review of Scientific Instruments, vol.66, no.10, pp.4977-4980, Oct 1995
4. N. Daniel, NASA Technical Memorandum, n 208808, Nov 1998
5. Z. Wang, L. Kwan Siu, T.P. Pearsall, J.L. Booth, B.T Beard, S.R. Johnson, Journal of Vacuum Science and Technology B: Microelectronics and Nanometer Structures, v 15, n 1, p 116-121, Jan-Feb 1997
6. M.K. Weilmeier, K.M. Colbow, T. Teidje, T Van Buuren, Canadian Journal of Physics, v 69, n 3-4, p 422-6, March-April 1991
7. Y. Lacroix, “Method and device for measuring temperature during deposition of semiconductor”, Patent application No. JP2008/073702 (2008)
8. Y. Lacroix, “Method and apparatus for measuring temperature of semiconductor layer”, Patent application No. PCT/JP2011/067678 (2011)
9. A. Majid, A. Ali, Z. Jianjun, Journal of Materials Science: Materials in Electronics, v 18, n 12, p 1229-33, Dec. 2007

Comparison of Gene expression differences by UV irradiation on *Vibrio parahaemolyticus*

Mutsumi Nakahashi[1], Takashi Uebanso[1], Takaaki Shimohata[1], Kazuaki Mawatari[1], Akari Tsunedomi[1], Airi Honjo[1], Risa Nishisaka[1], Tomoko Yamashita[1], Natsumi Iwamoto[1], Teppei Hoshiyama[2], Masatake Akutagawa[2], Takahiro Emoto[2], Yohsuke Kinouchi[2] and Akira Takahashi[1]

[1]Department of Preventive Environment and Nutrition, Institute of Health Biosciences, The University of Tokushima Graduate School

[2]Department of Electrical and Electronic Engineering, Institute of Socio Techno Sciences, The University of Tokushima Graduate School

Abstract

Vibrio parahaemolyticus is a marine bacteria and causative food-poisoning associated with seafood consumption. *V. parahaemolyticus* is irradiated by sun light including ultraviolet (UV) light constantly therefore expected to have ultraviolet sensitivity but little is known about this. UV is classified by wavelength into UVC (100-280 nm), UVB (280-320 nm), and UVA (320-400 nm). We irradiated 255, 290, 310, 340, 365 and 385 nm of UV light on *V. parahaemolyticus* to compare gene expression differences of UV effects by microarray for the purpose of food hygiene technology improvement.

Introduction

V. parahaemolyticus is a gram negative, rod shape, marine and halophilic bacteria. It is causative food-poisoning associated with seafood consumption in many parts of the world (1,2). Range of *V. parahaemolyticus* is in the sea and it has been irradiated by sun light including ultraviolet (UV) light constantly. Therefore, *V. parahaemolyticus* is expected to have ultraviolet resistant but little is known about this. Previous analyses have demonstrated that SOS response was induced by UVC irradiation but not by UVA irradiation in *V. parahaemolyticus* (3). SOS response is induced when bacteria receive severe DNA damage and repairing DNA damage. We have reported disinfection system using 365nm of UVA-LED (4, 5, 6). In this study, variety of UV wavelengths were irradiated on *V. parahaemolyticus* which has totally 4832 genes, to compare gene expression level by microarray analysis for the purpose of food hygiene technology improvement.

Materials and Methods

Bacterial strain and culture condition

V. parahaemolyticus RIMD2210633 (GenBank ID: BA000031.2 and BA000032.2) was used in this study. *V. parahaemolyticus* was pre cultured 18 h at 37°C in Luria-Bertani (LB) broth (3% NaCl, 1%Tryptone, 0.5% East Extract) and it was inoculated on to fresh LB broth and cultured 6 h at 37°C to the stationary phase. Then cells were collected by centrifugation at 12000g, 3min and washed three times by phosphate buffered saline (PBS, 140 mM NaCl, 2.5 mM KCl, 1.6 mM KH_2PO_4, 15 mM Na_2HPO_4). *V. parahaemolyticus* solution were adjusted in OD 600nm = 0.5 (1.3×10^8 cfu/ml) by PBS and 5 ml in 3 cm dish were irradiated by UV light. After UV irradiation bacterial cells were collected by centrifugation and pellets were resuspended by LB broth then 30 min cultured for UV damage repair. Then mRNA were extracted by RNeasy (QIAGEN) according to the manufacture's instruction. RNA Integrity Number (RIN) were 8.2 to 10 by 2100 Bioanalyzer (Agilent Technologies).

UV irradiation

255, 290, 310 and 340 nm light were irradiated by Xenon UV light device (Asahi Spectra, Japan). 365 and 385 nm light were irradiated by UV-LED (365nm; NCSU033B, 385nm; NC4U134A, Nichia corp. Japan). Distance from light source to bacterial solution was

fixed at 5cm by the dish stand which originally made. UV intensities were adjusted respectively to achieve same UV damage level, the survival ratios (log_{10}) were -0.5.

Microarray

DNA microarray was customized by Agilent technology. 100ng of total RNA were put into cyanine 3 labele treatment and labeled cRNA samples were hybridized for 17 hours at 65 °C according to the manufacture's instruction (Agillent Technologies). Array slides were scanned and image data were extracted by Agilent Feature Extraction Software.

Results and Discussion

V.parahaemolyticus gene expression differences of UV irradiation effects were shown (Figure1).

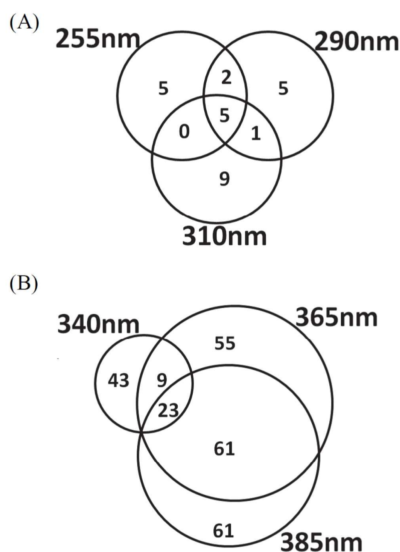

Figure.1 Venn diagram of up or down regulated genes in response to UV irradiation against control (no UV irradiated). (A) 255nm of UVC irradiation, 290nm and 310nm of UVB irradiation. (B) UVA irradiation. *V.parahaemolyticus* gene expression differences were analyzed by GeneSpring (Agillent technology), listed more than ± 1.5 of fold change.

All of wavelength effected on gene expression, the numbers were as follows, 255nm; 12, 290nm; 13, 310nm; 15, 340nm; 75, 365nm; 148 and 385nm; 145. UVA irradiation effected more gene expression numbers than UVB or UVC. As common denominator among UVC and UVB, 5 genes were up regulated (*VP0648*, *VPA0789*, *VP2342*, *VP2035*, *VP2034*). In UVA irradiation, 23 genes were changed. 17 genes were up regulated, (*VP0326*, *VP1119*, *VP1120*, *VP1121*, *VP1122*, *VP1123*, *VP1124*,*VP1125*,*VP1126*, *VP2086*, *VPA1159*, *VPA1470*, *VPA1471*, *VPA1472*, *VPA1473*, *fliH*, *fliR*) and 6 genes were down regulated (*VP1888*, *VPA0242*, *VPA0243*, *VPA0318*, *VPA0319*, *VPA1527*).

VP0648, one of up regulated genes by UVC or UVB irradiation, encodes recN protein which has a significant role in early phase of SOS response, recognizing DNA damage and coordinating DNA Double Stranded Breaks (7, 8). It was suggested that SOS response was induced by not only by UVC but UVB also on *V. parahaemolyticus.*

fliH (VP2247) and *fliR* (VP2237) were up regulated by UVA irradiation and encode polar flagellar assembly protein FliH and FliR. 520nm of green light also up regulated flagellar related genes and flagellar motility was increased on *V.parahaemolyticus* (data not shown). These flagellar proteins are related to the type III secretion system which injects virulence factor directly into the host cells (9). It is not well understand relationship between light and bacterial virulence yet. Further analysis will be needed for effective disinfection system development and food hygiene improvement.

Acknowledgements

This study was supported in part by a Grant-in-Aid for Scientific Research to M.N. (no. 26870411), T.S. (no. 90609687), K.M. (no. 24500857), M.A. (no. 90294727) and A.T. (no. 90304047) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

(1) Velazquez-Roman et al. (2013) *Front Cell Infect Microbiol* 3,110

(2) Chowdhury G. et al. (2013) *Foodborne Pathog Dis* 10(4), 338-342.

(3) Hamamoto A. et al. (2010) *Environmental Microbiology* Reports 2(5), 660-666.

(4) Hamamoto A. et al. (2007) *Journal of Applied Microbiology* 103(6), 2291-2298

(5) Mori M. et al. (2007)*Medical & Biological Engineering & Computing* 45(12), 1237-1241.

(6) Lian X. et al. (2010) *Biocontrol Sci* 15(1), 33-37.

(7) Odsbu, I. and Skarstad, K. (2014) *Microbiology* 160(Pt5), 872-882.

(8) Alonso, J.C. et al. (2013) *DNA Repair* 12(3), 162-176.

(9) Morimoto, Y.V.et al. (2014) *Mol Microbiol* 91(6), 1214-1226

Development of the disinfection system for hydroponic nutrient solution with UVA-LED

Akari Tsunedomi[1], Akinori Masamura[2], Mutsumi Nakahashi[1], Risa Nishisaka[1], Kazuaki Mawatari[1], Takaaki Shimohata[1], Takashi Uebanso[1], Katsuyuki Miyawaki[3], Masatake Akutagawa[4], Yohsuke Kinouchi[4] and Akira Takahashi[1]

[1]Department of Preventive Environment and Nutrition, Institute of Health Biosciences, The University of Tokushima Graduate School

[2] CKD Corporation

[3]Center for Collaboration among Agriculture, Industry and Commerce, The University of Tokushima

[4] Department of Electrical and Electronic Engineering, Institute of Socio Techno Sciences, The University of Tokushima Graduate School

ABSTRACT

The plant factory has been increasing every year in Japan. It can keep a constant quality to cultivate a lot of crops in artificial environment. In general, hydroponics which circulates the nutrient solution is carried out in the plant factory. When the pathogens contaminate hydroponic nutrient solution, it might propagate and spread immediately. Therefore, it is important to disinfect hydroponic nutrient solution. We developed the disinfection device for hydroponic nutrient solution with UVA-LED. The system did not use chemical and had low damage crops or nutrient solution. We suggest that it is possible for disinfectant system with UVA-LED to disinfect pathogen in plant factory.

INTRODUCTION

Japan relies 60% of foods consumed on the food that is imported from other countries, and can no longer support its people's diet without imported food. The problem that the maintenance of farmland and radioactive contamination happened by the Great East Japan Earthquake that happened in 2011. Thus, it has been required for the stable food supply systems which guarantee the quality of the food. For these reason, the plant factory is promoted establishment. This factory can keep a constant quality to cultivate a lot of crops in artificial environment. However, hydroponics, which circulate through nutrient solution in general, are carried out in the plant factory. When hydroponic nutrient solution is contaminated by the pathogen, the pathogens will propagate and spread. Therefore, it is important to disinfect hydroponic nutrient solution.

The consciousness of the society about safety and security of food has increased, and it demanded the methods of disinfection, which do not use chemical. In order to supply food that safety, security, and high quality, it is necessary for (A) do not use chemical such as disinfectant, (B) kind to environment and the human body, (C) the disinfection system is simple build and easy for use. Disinfection of light is excellent in not using a chemical substance [1～3]. In addition, UVA irradiation has low damage to crops and nutrient solution [4]. Therefore, we develop the disinfection device with UVA-LED, and we tried to apply the system for disinfection of nutrient solution in plant factory.

MATERIALS AND METHODS

We developed a circulating disinfection device including a cylindrical disinfection tank of 1L volume (Fig1). *Escherichia coli* strain ATCC25922 was used as model microorganism for the disinfection experiments. Inactivation level was determined by a colony-forming assay. After UVA irradiation, bacterial suspensions were diluted appropriately, plated on LB agar plates, and incubated at 37℃ for 18h. After incubation, the number of colonies was counted, and log survival ratio or inactivation percentage was calculated using the following equation;

$$\text{Log survival ratio} = \log\left(\frac{N_t}{N_0}\right)$$

N_t; the colony count of UVA irradiated sample

N_0; the colony count of the sample before UVA irradiation

When we downsized a device, we used 96-well plate. Aliquots (volume, 200μL) were placed into the wells of a sterile UVA-penetrable 96-well plate and irradiated with UVA-LED.

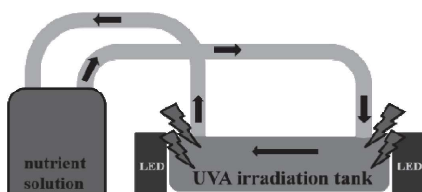

Fig1. Test model for disinfection device. 560 millimeter long and 1200 millimeter wide. The cylindrical tank is 67millimeter inside diameter and 300 millimeter in total length. This device has eight LED.

RESULTS AND DISCUSSION

The effect of disinfection was not changed when the volume of UVA irradiation tank was changed from 1L to 3L in the test model for disinfection. Furthermore, when the volume of circulating nutrient solution was changed from 5L to 20L in the test model, 10L of circulating nutrient solution has best disinfection efficiency. Next, we examined the circulation speed dependency of the bactericidal effect. 4L/min of circulation speed of the nutrient solution indicated the highest bactericidal effect.

For more detail examination about the kinetics of bactericidal effect, we used the small scale model for the disinfection model (data not shown). The small scale model indicated good similarity about the kinetics of bactericidal effect (data not shown). We found that the Log survival ratio significantly related to fluence (Fig2).

As the experimental results in the small scale model, we estimate kinetics equation which indicated the relation between volume of the nutrient solution and log survival ratio, as followed;

$$T(V) = 2.22 \times 10^{-6} \times V^2 - 2.28 \times 10^{-3} \times V + 0.975$$

T (V); log survival ratio

V; volume of the nutrient solution

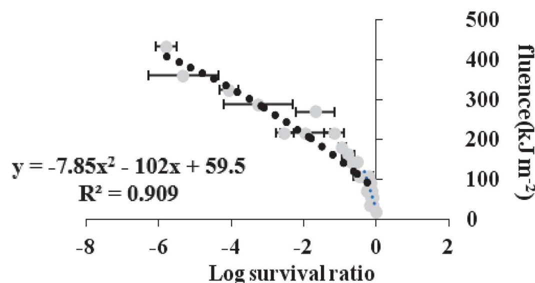

Fig2. Correlation of Log survival ratio and fluence

Vertical axis indicated fluence to the bacterial solution and the horizontal axle indicated Log survival ratio of the bacteria in the hydroponic nutrient solution. Regression analysis was perform for estimation the relation between fluence and Log survival ratio. *F*-test was applied and $p < 0.05$ was onsidered siginificant.

Next, we modified the above equation followed by the experimental results.

$$E(V) = -7.85 \times \frac{S^2}{T(V)^2} - 102 \times \frac{S}{T(V)} + 59.5$$

S; log survival ration for the goal

E (V); the energy of fluence

If Log survival ratio of target is decided, it will be possible to estimate the required fluence used by above equation.

CONCLUSION

In conclusion, it is possible for UVA irradiation to disinfect hydroponic nutrient solution. If Log survival ratio of goal is decided, it will be possible to estimate the required fluence. In this study, we obtain the necessary condition that develop disinfection device of hydroponic nutrient solution. We suggest that it is possible to manufacture disinfection device supposed plant factory.

REFERENCES

1. Hamamoto A.et al (2007) J Appl Microbiol, 103, 2291-8.
2. Mori M.et al (2007) Med Biol Eng Comput, 45, 1237-41.
3. Pi Xu.et al (2002) WATER RESEARCH, 36, 1043–1055.
4. Aihara M. et al (2014) J Med Invest. 61, 285-290.