RGC Ref. No.:
UGC/FDS25/E07/17
(please insert ref. above)

**RESEARCH GRANTS COUNCIL**
**COMPETITIVE RESEARCH FUNDING SCHEMES FOR THE LOCAL SELF-FINANCING DEGREE SECTOR**

**FACULTY DEVELOPMENT SCHEME (FDS)**

**Completion Report**
*(for completed projects only)*

| | |
|---|---|
| ***Submission Deadlines:*** | 1. *Auditor's report with unspent balance, if any: within* ***six*** *months of the approved project completion date.*<br>2. *Completion report: within* ***12*** *months of the approved project completion date.* |

## Part A: The Project and Investigator(s)

### 1. Project Title

Development of a high-efficiency and low-cost UV-LED/chlorine process for removal of antibiotic resistant bacteria and their resistance genes in municipal wastewater disinfection

### 2. Investigator(s) and Academic Department(s) / Unit(s) Involved

| **Research Team** | **Name / Post** | **Unit / Department / Institution** |
|---|---|---|
| Principal Investigator | NGAI KING WAH/ Teaching Fellow I | Faculty of Science and Technology/ Technological and Higher Education Institute of Hong Kong |
| Co-Investigator(s) | WANG Chao/ Assistant Professor | School of Environmental Science and Engineering/ South University of Science & Technology of China |
| Co-Investigator(s) | SHANG Chii/ Professor | Department of Civil and Environmental Engineering/ HKUST |
| Co-Investigator(s) | GUO Meiting/ Associate Professor | College of Environmental Science and Engineering/ Tongji University |
| Others | NA | |

### 3. Project Duration

**3. Project Duration**

| | **Original** | **Revised** | **Date of RGC / Institution Approval** *(must be quoted)* |
|---|---|---|---|
| Project Start Date | 1/1/2018 | NA | NA |
| Project Completion Date | 31/12/2020 | 30/6/2021 | Approved by THEi on 9/4/2020 |
| | | 31/12/2021 | Approved by the RGC on 20/11/2020 |
| Duration *(in month)* | 36 | 42 | Approved by THEi on 9/4/2020 |
| | 42 | 48 | Approved by the RGC on 20/11/2020 |
| Deadline for Submission of Completion Report | 31/12/2021 | 30/6/2022 | Approved by THEi on 9/4/2020 |
| | 30/6/2022 | 31/12/2022 | Approved by the RGC on 20/11/2020 |

**<u>Part B: The Final Report</u>**

**5. Project Objectives**

5.1 Objectives as per original application

1. To optimize the UV-LED/chlorine process in terms of way of chlorine addition (co-exposure or sequential exposure), disinfectant dosages and UV-LED wavelengths for removal of selected ARB and ARGs and alleviation of ARGs conjugative transfer.
2. To study the effects of multiple wavelengths and pulsed illumination by the UV-LED/chlorine process on removal of selected ARB and ARGs and alleviation of ARGs conjugative transfer.
3. To examine wastewater matrix effects including EfOM, suspended solids, ammonium, pH and alkalinity on the removal of selected ARB and ARGs, and reduction of ARGs conjugative transfer by the optimized UV-LED/chlorine process in simulated wastewater.
4. To extend the fundamental understanding of the UV-LED/chlorine process to a real-case application of wastewater disinfection either for direct discharge or for wastewater reuse.

5.2 Revised objectives

Date of approval from the RGC: NA

Reasons for the change: NA

*1.*

*2.*

*3. ....*

5.3 Realisation of the objectives
*(Maximum 1 page; please state how and to what extent the project objectives have been achieved; give reasons for under-achievements and outline attempts to overcome problems, if any)*

**All objectives achieved 100% completion.**
**For objective 1**, two ESBL-producing *E. coli* strains (i.e., *E. coli* HK01 & *E. coli* HK02), with different DNA characteristics, were isolated from the effluent (before UV disinfection) of the Sha Tin WWTP and selected for disinfection in this project. The observed general trend of UV-LEDs inactivation efficacy followed bacterial spectral sensitivity: 265 nm > 285 nm >> 300 nm, using the example of *E. coli* HK02. However, if comparing different wavelengths based on the required electrical energy (kWh/$cm^2$) necessary to achieve the same log inactivation, the trend was 285 nm ≈ 265 nm >> 300 nm. At 285 nm, it is suspected that protein denaturation plays the main role in bacteria inactivation (Horikawa-Miura et al., 2007; Santos et al., 2013). At a chlorine dosage of 0.8 mgCl2/L, *E. coli* HK01 achieved a 4.8 log reduction in 30 seconds. The log inactivation value of *E. coli* HK01 demonstrated first-order inactivation over time, fitting into Chick-Watson's Law. The inactivation rate constant is 0.203 $(mgCl2/L·s)^{-1}$, and the required CT value (disinfectant concentration × contact time; mgCl2/L·min) for achieving 1 log inactivation of *E. coli* is 0.072 mgCl2/L·min. At a dose of 26.7 mJ/$cm^2$, *E. coli* HK01 achieved a 4.11 log reduction under continuous photolysis by 285 nm UV-LED. The log inactivation value of E. coli HK01 demonstrated first-order inactivation over time, fitting into Chick-Watson's Law. Moreover, the inactivation rate constant is 0.160 $cm^2$/mJ, in line with previously published rate constants of *E. coli* inactivated by UV-LED ranging from 0.15 to 0.81 $cm^2$/mJ (Sholtes & Linden, 2019). In the continuous UV-LED/Cl2 process, the inactivation of *E. coli* HK01 was significantly greater than that by continuous UV-LED alone or chlorine alone. At a UV dose of 13.3 mJ/$cm^2$ and chlorine dose of 8 mgCl2/L·s, the inactivation of *E. coli* HK01 achieved a 4.63-log inactivation by continuous UV-LED/Cl2 process, compared with 1.63-log inactivation by continuous UV-LED alone and 1.74-log inactivation by chlorine alone processes. The synergistic effect of the combined process is illustrated by comparing the log inactivation value of continuous UV-LED/Cl2 with the sum of the log inactivation values achieved by UV-LED alone and chlorine alone processes. The additional increase resulting from the continuous UV-LED/Cl2 synergy was 1.26 log. The inactivation rate constant of the continuous UV-LED/Cl2 process is 0.411 $cm^2$/mJ, which is 2.57 times and 2.02 times as those of continuous UV-LEDs alone and chlorine alone, respectively. The synergistic effect of the combined process may be attributed to the generation of reactive oxygen species, such as ·OH and Cl·, during the process (Liu & Hu, 2020; Phattarapattamawong et al., 2021; Wang et al., 2021). However, compared to the inactivation of ARB, the degradation of ARGs is more difficult and critical to control the dissemination of antibiotic resistance in water treatment. Thus, the reduction of intracellular blaCTX-M-15, a plasmid-encoded CTX resistance gene of *E. coli* HK01, by the UV-LED/chlorine process in terms of chlorine addition (co-exposure or sequential exposure) were compared. Under the experiment conditions, the inactivation of blaCTX-M-15 by the continuous UV-LED/Cl2 process was greater than that by chlorine alone, UV-LED alone, UV-LED followed by chlorine, and chlorine followed by UV-LED processes. At a UV dose of 480 mJ/$cm^2$ and chlorine dose of 720 mgCl2/L·s, the inactivation of blaCTX-M-15 by continuous UV-LED/Cl2 process achieved a 2.10-log inactivation, compared with 1.43-log inactivation by UV-LED alone and 0.34-log inactivation by chlorine alone. The additional increase as a result of the continuous UV-LED/Cl2 synergy was 0.33 log. The contribution of UV-LED, chlorine, and ·OH radical were 67.4%, 15.2% and 17.4%, respectively, while Cl· radical did not contribute in the inactivation of blaCTX-M-15 by the continuous UV-LED/Cl2 process.
**For Objective 2**, at a UV dose of 13.0 mJ/$cm^2$ and chlorine dose of 8 mgCl2/L·s, the inactivation of *E. coli* HK01 by pulsed UV-LED/Cl2 process achieved a 4.86-log inactivation, compared with 1.63-log inactivation by pulsed UV-LED alone and 1.74-log inactivation by chlorine alone. The additional increase as a result of the pulsed UV- LED/Cl2 synergy was 1.49 log. The inactivation rate constant

of the pulsed UV-LED/Cl2 process is 0.512 $cm^2$/mJ, comparable to that of the continuous UV-LED/Cl2 process. Similarly, the inactivation rate constant of the pulsed UV-LED process (0.160 $cm^2$/mJ) was comparable to that of the continuous UV-LED process (0.141 $cm^2$/mJ). The inactivation values of blaCTX-M-15 by the continuous and pulsed UV-LED processes were the same at UV doses ranged from 0 to 480 mJ/$cm^2$.

**For Objective 3**, at a chlorine dose of 720 mgCl2/L·s and UV dose of 480 mJ/$cm^2$, the inactivation of blaCTX-M- 15 by the continuous UV-LED/Cl2 process decreased by 55.5% and 27.5%, respectively, with the increasing pHs from pH 6.5 to 8.5 and with the increasing ammonia concentrations from 0.5 mgN/L to 2 mgN/L. The inactivation of blaCTX-M-15 slightly increased by 8.2% with the increasing turbidity from 5 NTU to 20 NTU. The effects of dissolved organic matter ranging from 1 mgC/L to 5 mgC/L and bicarbonate ranging from 50 mg/L to 200 mg/L were insignificant in the inactivation of blaCTX-M-15 by the continuous UV-LED/Cl2 process. The results suggested that decreasing the pHs and ammonia levels and increasing turbidity assisted ARGs in inactivation. However, the concentrations of dissolved organic matter and bicarbonate did not affect its inactivation under the experimental conditions.

**For Objective 4**, at a chlorine dose of 24 mgCl2/L·s and UV dose of 10.7 mJ/$cm^2$, *E. coli* HK01 achieved a 2.42 log reduction in tertiary sewage effluent by continuous UV-LED/Cl2 process. At a chlorine dose of 1080 mgCl2/L·s and UV dose of 480 mJ/$cm^2$, blaCTX-M-15 achieved a 1.31 log reduction in tertiary sewage effluents by continuous UV-LED/Cl2 process.

References:

Horikawa-Miura, M., Matsuda, N., Yoshida, M., Okumura, Y., Mori, T., & Watanabe, M. (2007). The greater lethality of UVB radiation to cultured human cells is associated with the specific activation of a DNA damage-independent signaling pathway. *Radiation research*, *167*(6), 655-662.

Liu, X., & Hu, J. Y. (2020). Effect of DNA sizes and reactive oxygen species on degradation of sulphonamide resistance sul1 genes by combined UV/free chlorine processes. *Journal of Hazardous Materials*, *392*, 122283.

Phattarapattamawong, S., Chareewan, N., & Polprasert, C. (2021). Comparative removal of two antibiotic resistant bacteria and genes by the simultaneous use of chlorine and UV irradiation (UV/chlorine): Influence of free radicals on gene degradation. *Science of the Total Environment*, *755*, 142696.

Santos, A.L., Moreirinha, C., Lopes, D., Esteves, A.C., Henriques, I., Almeida, A., ... & Cunha, A. (2013). Effects of UV radiation on the lipids and proteins of bacteria studied by mid-infrared spectroscopy. *Environmental science & technology*, *47*(12), 6306-6315.

Sholtes, K., & Linden, K. G. (2019). Pulsed and continuous light UV LED: microbial inactivation, electrical, and time efficiency. *Water Research*, *165*, 114965.

Wang, L., Ye, C., Guo, L., Chen, C., Kong, X., Chen, Y., ... & Fang, J. (2021). Assessment of the UV/Chlorine Process in the Disinfection of Pseudomonas aeruginosa: Efficiency and Mechanism. *Environmental Science & Technology*, *55*(13), 9221-9230.

5.4 Summary of objectives addressed to date

| **Objectives** *(as per 5.1/5.2 above)* | **Addressed** *(please tick)* | **Percentage Achieved** *(please estimate)* |
|---|---|---|
| 1. To optimize the UV- LED/chlorine process in terms of way of chlorine addition (co-exposure or sequential exposure), disinfectant dosages and UV-LED wavelengths for removal of selected ARB and ARGs and alleviation of ARGs conjugative transfer. | ✓ | 100% |
| 2. To study the effects of multiple wavelengths and pulsed illumination by the UV-LED/chlorine process on removal of selected ARB and ARGs and alleviation of ARGs conjugative transfer. | ✓ | 100% |
| 3. To examine wastewater matrix effects including EfOM, suspended solids, ammonium, pH and alkalinity on the removal of selected ARB and ARGs, and reduction of ARGs conjugative transfer by the optimized UV-LED/chlorine process in simulated wastewater. | ✓ | 100% |
| 4. To extend the fundamental understanding of the UV- LED/chlorine process to a real-case application of wastewater disinfection either for direct discharge or for wastewater reuse. | ✓ | 100% |

## 6. Research Outcome

### 6.1 Major findings and research outcome
*(Maximum 1 page; please make reference to Part C where necessary)*

The major findings obtained from this study have formulated 1 prestigious journal paper and 4 conference presentations in outstanding international conferences so far. (please see Part C, items 8 & 9) We are also working on another 1-2 journal papers under preparation. This study provided training of 1 PhD student. This study also involved close collaboration between THEi and HKUST. The specific major findings are:

The inactivation of ARB by UV-LEDs at different wavelengths was evaluated. Synergistic effects of the combined UV-LED/$Cl_2$ process were observed compared to the UV-LED alone and chlorine alone processes in the inactivation of ARB and ARGs. ·OH radical was the dominant contributor to the synergistic effect of the inactivation of ARGs by continuous UV-LED/$Cl_2$ process. In terms of reducing ARB, the continuous and pulsed UV-LED/$Cl_2$ process showed comparable performance. Besides, the removal of ARGs were the same under continuous and pulsed UV-LED irradiation. Decreasing pHs and ammonia concentrations and increasing turbidity increased the inactivation of ARGs. However, the concentrations of dissolved organic matter and bicarbonate did not significantly affect the inactivation of ARGs under the experimental conditions. In tertiary sewage effluents, continuous UV-LED/$Cl_2$ process successfully inactivated ARGs.

### *6.2* Potential for further development of the research and the proposed course of action
*(Maximum half a page)*

Standard microbiological testing of the impacts of different processes to the bacterial systems is often done in the nutrient-rich environment. This is optimal for studying particular pathways, while minimizing strain on bacteria caused by environmental stressors such as starvation. However, in real-world engineering systems, the nutrient-rich environment is rare. Thus, microbiological knowledge obtained from a cell-optimized environment does not directly translate to real-world scenarios. This underlines the importance of mimicking and studying actual conditions in engineering systems. In this research, we have observed that co-exposure of UV-LED and chlorine (UV-LED/$Cl_2$ process) is promising to inactivate ARB and ARGs under realistic conditions. During the inactivation of ARGs in real-world operation, decreasing pHs and ammonia levels are suggested. In addition, the pulsed UV-LED/$Cl_2$ process is as efficient as the continuous UV-LED/$Cl_2$ process in the inactivation of ARB on a fluence basis. However, the research demonstrates the necessity to examine the inactivation mechanisms of commonly employed disinfection processes. Therefore, we can further identify different cell states during the disinfection processes, using a flow cytometry- based method to accurately detect membrane permeability, metabolic activity, and intracellular production of reactive oxygen species within a single bacterial cell.

## 7. Layman's Summary

*(Describe <u>in layman's language</u> the nature, significance and value of the research project, in no more than 200 words)*

Antibiotic resistance has become an emerging global health concern. Municipal wastewater treatment plants (WWTPs) are resources of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) spread to the environment owing to fluid mixtures of bacteria, abundant nutrients, and antimicrobial agents. The primary and biological treatment processes in WWTPs have limited contribution on the treatment of ARB or ARGs, and commonly applied disinfection processes such as UV or chlorine could probably partially reduce ARB or ARGs. The combined UV-LED/$Cl_2$ process may be a promising alternative in the removal of ARB or ARGs.

In this study, we provided a breakthrough in filling the knowledge gap of the inactivation of ARB or ARGs by different disinfection processes, including single UV-LED processes at different wavelengths, single chlorine process, and continuous and pulsed UV-LED/$Cl_2$ processes. The effects of water matrices, including pH, ammonia, turbidity, dissolved organic matter, and bicarbonate, on the inactivation of ARGs by the continuous UV-LED/$Cl_2$ process were unveiled. In addition, we also inactivate ARGs successfully by the continuous UV-LED/$Cl_2$ process in tertiary sewage effluents, suggesting that the process can be adopted in municipal wastewater disinfection.

## Part C: Research Output

### 8. Peer-Reviewed Journal Publication(s) Arising Directly From This Research Project

*(Please attach a copy of the publication and/or the letter of acceptance if not yet submitted in the previous progress report(s). All listed publications must acknowledge RGC's funding support by quoting the specific grant reference.)*

| The Latest Status of Publications | | | | Author(s) *(denote the correspond-ing author with an asterisk*)* | Title and Journal / Book *(with the volume, pages and other necessary publishing details specified)* | Submitted to RGC *(indicate the year ending of the relevant progress report)* | Attached to this Report *(Yes or No)* | Acknowledged the Support of RGC *(Yes or No)* | Accessible from the Institutional Repository *(Yes or No)* |
|---|---|---|---|---|---|---|---|---|---|
| Year of Publication | Year of Acceptance *(For paper accepted but not yet published)* | Under Review | Under Preparation *(optional)* | | | | | | |
| **2021** | **2021** | | | **Oriana Jovanovic, Carlos F. Amábile-Cuevas, Chii Shang, Chao Wang, King Wah Ngai** | **"What Water Professionals Should Know about Antibiotics and Antibiotic Resistance: An Overview", ACS EST Water 2021, 1, 1334-1351** | NA | **Yes** | **Yes** | **No** |

### 9. Recognized International Conference(s) In Which Paper(s) Related To This Research Project Was / Were Delivered

*(Please attach a copy of each conference abstract)*

| Month / Year / Place | Title | Conference Name | Submitted to RGC *(indicate the year ending of the relevant progress report)* | Attached to this Report *(Yes or No)* | Acknowledged the Support of RGC *(Yes or No)* | Accessible from the Institutional Repository *(Yes or No)* |
|---|---|---|---|---|---|---|
| **05/2018/ Nanjing, China** | **Modelling the UV-LED based chlorine photodecay and radical formation at different wavelengths and pHs** | **The 15th IWA Leading Edge Conference on Water and Waste Water Technologies** | **2019** | **No** | **Yes** | **Yes** |
| **05/2018/ Beijing, China** | **Inactivation of Escherichia coli Using Ultraviolet Light Emitting Diodes (UV-LEDs), Monochloramine ($NH_2Cl$) and the $NH_2Cl$/UV-LED Process** | **The 2nd Disinfection and Disinfection By-Products Conference** | **2019** | **No** | **Yes** | **Yes** |

| | | | | | | |
|---|---|---|---|---|---|---|
| **09/2019/ Vienna, Austria** | **Diversity of plasmids, antibiotic-resistance phenotypes and UV resistance among ESBL-producing E. coli isolates from WWTP effluent in Hong Kong** | **20th International Symposium on Health Related Water Microbiology** | **2020** | **No** | **Yes** | **Yes** |
| **10/2019/ Seoul, South Korea** | **Are ESBL-producing *E. coli* isolated from a WWTP effluent more resistant to UV light at different wavelengths?** | **11th Micropol & Ecohazard Conference 2019** | **2020** | **No** | **Yes** | **Yes** |

**10. Whether Research Experience And New Knowledge Has Been Transferred / Has Contributed To Teaching And Learning**
*(Please elaborate)*

The new knowledge and experimental methods have been delivered to the students in THEi and HKUST during the related courses including "Introduction to Environmental Engineering" , "Water and Wastewater Engineering", "Industrial Wastewater Treatment", etc.

**11. Student(s) Trained**
*(Please attach a copy of the title page of the thesis)*

| **Name** | **Degree Registered for** | **Date of Registration** | **Date of Thesis Submission / Graduation** |
|---|---|---|---|
| [redacted] | [redacted] | [redacted] | [redacted] |

**12. Other Impact**
*(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, teaching enhancement, etc.)*

To complete this project, THEi built close collaboration with HKUST in terms of co-supervising the PhD student, sharing the lab facilities, and co-publishing journal papers. All the knowledge obtained from this project were delivered in the courses of both THEi and HKUST including "Introduction to Environmental Engineering", "Water and Wastewater Engineering", "Industrial Wastewater Treatment", etc.

## 13. Statistics on Research Outputs

| | Peer-reviewed Journal Publications | Conference Papers | Scholarly Books, Monographs and Chapters | Patents Awarded | Other Research Outputs (please specify) | |
|---|---|---|---|---|---|---|
| **No. of outputs arising directly from this research project** | 1 | 4 | 0 | 0 | Type | No. |
| | | | | | NA | 0 |

## 14. Public Access Of Completion Report

*(Please specify the information, if any, that cannot be provided for public access and give the reasons.)*

| Information that Cannot Be Provided for Public Access | Reasons |
|---|---|
| NA | NA |