

UV BASED PROCESS FOR *E. COLI* AND COLIPHAGE IN SECONDARY EFFLUENT FOR WASTEWATER RECLAMATION AND REUSE

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ABSTRACT

The persistency and survival of pathogenic contamination related to biological risk has been a public concern when implementing water reclamation and reuse. To secure the public health risk when implementing water reuse, adequate treatment is needed. In this study Ultraviolet (UV), based treatment was studied for the applicability to remove microorganisms from secondary effluent. *E. coli*, Q β , T4 and Lambda phages were used as targeted microorganisms during the UV treatment process. The objectives of this study were to investigate the inactivation characteristics of *E. coli*, Q β , T4 and Lambda phage to UV treatment process and investigate the effect of combine UV/H₂O₂ for *E. coli* and Q β phage inactivation. As a result, T4 and Lambda phage from DNA group phage were found more resistant to UV treatment process compared to Q β phage from FRNA phage group and *E. coli* from coliform group. Combined process UV/H₂O₂ in secondary effluent could increase the inactivation efficiency of *E. coli* and Q β . DOC and UV 254 were found strongly affected the disinfection processes. Secondary effluent treated with UV processes was found suitable for wastewater reclamations.

Keywords: *E. coli*; Lambda phage; Q β ; T4; UV; Wastewater

1.0 INTRODUCTION

The concept of converting waste to resource is not a new concept. Waste from wastewater industry has been converted into useful resources [1]. At the source, grey water can be reused for toilet flushing as it is uneconomical to use treated water just to flush toilets [2]. Grey water reuse will reduce the demand for treated water. The reuse of effluent for irrigation purposes has occurred for centuries, and with increasing pressure on potable water supplies, the extent of reuse is increasing [3]. Secondary effluent can also be reused for toilet flushing purposes. Secondary effluent have been used for landscaping and general washing purposes as researchers have found the levels of faecal coliforms are acceptable [4-5]. The utilisation of secondary effluent for reclaimed wastewater also contributes in reducing potential negative impacts of wastewater discharges to receiving waters.

Over the past few years, interest in the existence of pathogens in reclaimed wastewater has rapidly increased with the increased in public awareness on human health and safety. The pathogenic microorganisms in treated wastewater for reclamation purposes pose potential health risks. It was found that apart from faecal coliforms, other pathogens have also been found in effluent, such as *Klebsella spp.*, *Shigella spp.*, *Enterobacter spp.*, etc. [6]. Therefore, human health risk should be managed related to pathogenic microorganisms contained in the wastewater. In order to reduce biological risk from reclaimed wastewater, a few guidelines were developed.

- California State regulation [7] proposed less than 2 Total Coliform/100ml involved specified multi-steps treatment. Proposed regulation would add log₁₀ microbes reduction levels for bacteria (6-logs) viruses (coliphages) (5-logs) and bacterial spores (*C. perfringens*) as protozoan parasite indicator (4-logs).
- US EPA regulation [8]: Proposed ≤ 200 FC/100ml for less stringent reclaimed water uses and 4-logs reduction of *Cryptosporidium* (22 mJ/cm²), *Giardia* (22 mJ/cm²) and virus (186 mJ/cm²).
- WHO guidelines [9]: Proposed about 6-7 logs pathogen reduction for unrestricted agricultural uses, 3-4 logs for restricted irrigation and 2-4 logs for drip irrigation purposes.
- Australian Guideline for Water Reuse [10]: This guideline proposed reduction of bacteria (5-logs), virus (6.5-logs), protozoa (5-logs), helminth (5-logs) recommended for Class A+ (dual- reticulation system). Further, reduction of bacteria (8-logs), virus (9.5-logs), protozoa (8-logs), helminth (8-logs) for augmentation of drinking water supplies.
- Malaysian Sewerage Industry Guidelines [11] stipulated that continuous disinfection is required for sewage treatment plants serving more than 20,000 population equivalent (PE) discharging into bathing or recreation waters. For continuous disinfection, Chlorination, UV or Ozone is recommended.

Other sewage treatment plants are to be equipped with dosing tank structure capable of intermittent disinfection facilities and only chlorination is recommended.

The guidelines were developed to ensure the reclaimed water is safe enough and reliable to be used by consumers. In order to attain adequate water quality standard, appropriate treatment is needed. Disinfection process is considered as the last barrier to control pathogenic microorganisms in reclaimed wastewater. However, secondary treatment and chlorination are insufficient to inactivate persistent microorganisms like viruses and protozoa. Therefore, an advanced technology is necessary to consider for achieving high reclaimed water quality.

There are many countries that have even implemented the effluent reuse up to potable water quality. Singapore implemented the production of NEWater (potable quality effluent reuse), initiated in 1998 to supplement Singapore's water resources [12]. United States had implemented the effluent reuse up to potable water quality since 1976 at Water Factory 21, Orange County Water District, Southern California, where high quality water reclaimed from treated used water has been injected into ground water. Similarly, at Upper Occoquan Sewage Authority (UOSA), North Virginia, high quality reclaimed water is discharged into Occoquan Reservoir since 1978 [12]. For both countries, ultrafiltration and reverse osmosis membrane systems were used to treat the secondary effluent and UV was used as the disinfection tool.

UV provides a fast-acting, residual free method of coliform inactivation. UV has been shown to be a potential option for coliform inactivation in stringent wastewater reclamation applications [13]. Previous study by Darby *et al.* [14] had proven that UV disinfection satisfied the requirement for reclaimed wastewater in the State of California at reasonable doses. The advantages of UV treatment include providing microbial inactivation equivalent to chlorine while reducing the formation of known carcinogenic disinfection by-products and the formation of chronic effluent toxicity [15]. The hypothesis was that the addition of H₂O₂ to UV process will increase OH radical formation and could increase the inactivation rate of targeted microorganisms. It was also expected that the addition of H₂O₂ will reduce the UV dose and thus saving the energy consumption for actual application. UV became an attractive option because of UV effectiveness for inactivation of waterborne pathogens including cysts, oocysts of protozoan and intestinal parasites which are highly resistant to chlorination [16-17].

There are various types of bacteria and viruses in wastewater and it is impossible to analyse all types of these microorganisms. Total coliform, faecal coliform and *E. coli* which have been used as conventional indicators, are insufficient to evaluate the efficacy of the disinfection processes [18]. Studies to find other indicators that may provide a better indication of disinfection performance is important. In some other studies, coliphages were proposed to be used as surrogates to evaluate the efficiency of various water and wastewater treatment processes [20-21]. Evidence suggested that coliphages were more persistent in the environment and had been shown to be good indicators of human enteric viruses in polluted waters [21-22]. Coliphages are often proposed as indicators to identify the origin of faecal pollution [23]. Coliphages are easy to culture compared to actual human viruses and non-pathogenic because they are viruses of *E. coli*. They possess similar structure, morphology, origin, release and tolerance to environmental conditions compared to actual human

viruses [19, 24].

In this study, *E. coli* and Q β , T4 and Lambda phage were used as targeted microorganisms. The objective of this study was to evaluate the inactivation characteristics during UV based disinfection processes on secondary effluent for reclaimed wastewater. The effect of secondary effluent constituents have also been investigated by measuring TOC and UV 254 during the inactivation processes.

2.0 MATERIAL AND METHODS

2.1 Selected Microorganisms

Four microorganisms were selected in this study namely *Escherichia coli* (*E. coli*), Q β phage, Lambda phage and T4 phage. Most of *E. coli* in water environment originates from faeces of human and animals, and their occurrence is, thus, very important in determining the origin of faecal pollution. Typically, *E. coli* account for approximately 11% of the coliforms in human faeces [25].

Q β phage has an icosahedral capsid structure and genome consisting of positive-sense linear single-stranded RNA (ss-RNA). Q β phage poses similar size and morphology with enteric viruses and has been used as indicator for water quality assessment and routine monitoring [19, 23, 24]. In this study, Q β phage was selected to represent RNA group viruses in water.

Lambda phage, a virus that infects other bacteria with the ability to transfer genes among them, infects cells of the bacterium *E. coli*, where it can either exist as a quiescent prophage or undergo replication leading to lyses of the host cell and release of new phage particles. T4 phage has a non-segmented genome that contains a single molecule of linear double-stranded DNA (ds-DNA) [26,36]. T4 phage is from the family of *Caudovirales* and sub-family of *Myoviridae*. In nature, T4 phage is non-enveloped double-stranded DNA (ds-DNA). Lambda phage and T4 phage were selected to represent DNA-containing viruses [35].

2.2 Preparation of Selected Microorganisms

Q β phage (NBRC20012), Lambda phage (NBRC) and *E. coli* K12F⁺ (A/λ) (NBRC13965) were obtained from National Institute of Technology and Evaluation Biological Resource Centre of Japan (NBRC). The Department of Urban and Environmental Engineering, Tokyo University provided T4 phage. Q β , T4 and lambda phage cultures were produced by adding the phages stock solution into exponentially growing *E. coli* K12F⁺ (A/λ) and pure culture growing in the Difco™ LB Broth Lennox at 37°C.

Suspended phages were collected by centrifugation (10000rpm, 20 min, 4°C) and filtration through 0.45μm membrane filter (Milipore) to remove the cell lysate and to remove any other bacteria that may present. The stock phages suspensions were diluted 10-fold with phosphate-buffer solution (PBS, pH 7.2) to prepare the working phage suspension, essentially to avoid UV absorption by any proteins carried over from cell culture growth medium. The phage stock solutions were preserved at 4°C for further experiment.

E. coli K12F⁺ (A/λ) (NBRC13965) stock solutions were cultured in Difco™ LB Broth Lennox at 37°C shaking for 4 hours. The stock *E. coli* K12F⁺ (A/λ) suspensions were diluted 10-fold with phosphate-buffer solution (PBS, pH 7.2) to prepare the working *E. coli* K12F⁺ (A/λ) suspension. The stock solutions were also used as a host cell for plaque assay of all the phages.

2.3 Inactivation Experiment

In these experiments, the temperature of tested water was maintained at 20°C by circulating water into a water jacket outside the reactor using a water circulator. The low-pressure mercury UV lamp was manufactured by Iwasaki Electric Co., Ltd, Japan. Applied UV lamp was stabilised for 5 min before each experiment. In order to simulate the actual condition, a laboratory scale reactor (as shown in Figure 1) was used. Experimental conditions are shown in Table 1.

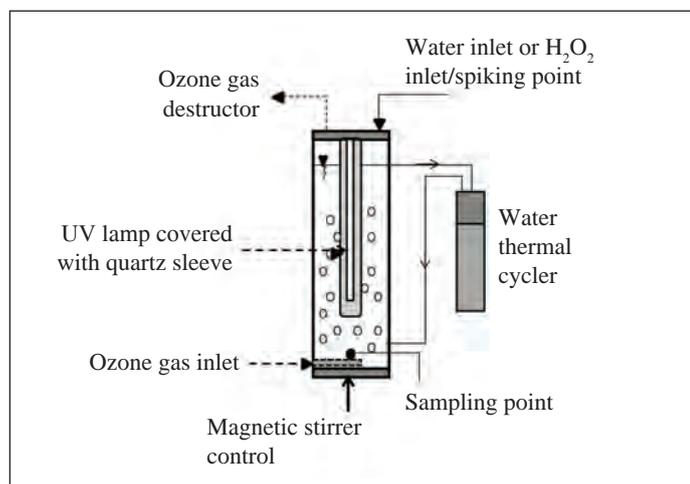


Figure 1: Schematic Diagram of Lab-Scale UV Reactor

Table 1: Experimental Condition

Microorganism	<i>E.coli</i> , Lambda, Q β , T4			
Tested water	Secondary effluent			
Effective volume (L)	1.7			
UV wavelength (nm)	254			
UV intensity(mW/cm ²)				
Time contact (min)	0-10			
UV dose (mJ/cm ²)	0, 38.4, 76.8, 153.6, 230.4, 384			
Process	UV	UV/ H ₂ O ₂	UV/ H ₂ O ₂	UV/ H ₂ O ₂
H ₂ O ₂ concentration (mg/L) for <i>E.coli</i> and Q β phage	0	2	4	6

2.4 UV Dose Measurements

UV is a physical disinfectant and the measurement of UV system performance occurred within the reactor. UV dose (mJ/cm² or mW-sec/cm²) is expressed by multiplying the UV intensity (typically expressed in mW/cm²) and contact time with the UV source (sec or min). UV intensity was determined from biosimetry test.

2.5 The Inactivation Quantification by UV Based Experiment

The inactivation of targeted microorganisms were calculated by the basic model relationship $\log(N/N_0) = -Kt$, where N_0 is the coliphages or *E.coli* count at the initial time (time = zero), N is the coliphage count at contact time t , and K is the inactivation rate (slope function). The logarithm of the average inactivation coliphage or *E.coli* ratios were plotted against UV dose (UV intensity x contact time) and was computed as a linear or pseudo-first order kinetic reaction.

2.6 Detection Method

The indicator microorganisms were treated with UV and UV/H₂O₂, and then 30 ml of tested water was collected from 0 to 10 minutes contact time. Contact time of 10 min was chosen based on a previous study [27]. It was shown that the most significant microbial reductions typically occur during the first 10-15 min of contact time. The samples were concentrated and purified with membrane filtration procedure [28]. In brief, 1M MgCl₂·H₂O was added to final concentration of 0.05M and 1M HCl was added to final pH of 3.5. Membrane filtration method using cellulose acetate membrane filter with 0.45 μ m of pore size was adapted to recover the phages. After membrane filtration, phages recovered on the filter were eluted by 3% beef extract with pH 9.0.

The concentrated viruses were diluted 10-fold with Difco™ LB Broth Lennox and assayed in double agar layer with Difco™ Agar and plated out with *E.coli* K12F⁺ (A/ λ) (NBRC13965) as the host cells. All samples were prepared in duplicated petri dishes and incubated invertedly at 37°C for 18 hours. The clear spot appeared in *E.coli* lawn represented dead cell of *E.coli* and known as plaque. The plaques were counted as Plaque Forming Unit per ml (PFU/ml).

E.coli was recovered using membrane filtration with 0.45 μ m of pore size after 10-fold dilution series [29]. The membrane filter containing filtered *E.coli* was laid on XM Nissui Agar to perform a blue colony after incubation at 37°C when the *E.coli* enzyme reacted with the agar. The colonies were counted as Colony Forming Unit per ml (CFU/ml). All samples were prepared in duplicated dishes.

Each selected microorganism was spiked separately into the secondary effluent before treatment experiments to the final concentration of 10⁶ PFU/mL.

2.7 TOC and UV₂₅₄ Analysis

UV absorbance (UV₂₅₄) was measured by spectrometer (UV-16000, Shimadzu). The absorbance at 254nm (UV₂₅₄) was measured and indicates amount of unsaturated bonds in compounds in tested water. TOC concentration measured with Total organic carbon analyzer (TOC-5000A, Shimadzu). TOC is a measurement of organic compound in tested water.

3.0 RESULTS AND DISCUSSIONS

3.1 Inactivation Characteristics of Q β , T4, Lambda phage and *E.coli* with UV Alone Process

The observed inactivation rates are shown in Figure 2. In comparison with coliphage Q β under the same condition, coliphage T4 and Lambda demonstrated slower inactivation kinetics. *E.coli* was very sensitive to UV and displayed the fastest inactivation rates compared to the others.

The UV doses needed for 1-log to 4-logs inactivation for *E.coli*, coliphage Q β , T4 and Lambda were calculated for comparison. The doses required for the 1-log and 4-logs inactivation, were calculated based on the regression analysis of each experiment. The doses of 46.9, 44.6, 32.1 and 27.8 mJ/cm² were needed to inactivate Lambda, T4, Q β and *E.coli* respectively, by 1-log. The inactivation increased with UV doses. In order to achieve more than 4-logs inactivation, 128.2 mJ/cm² was needed for Q β , whereas 178-188 mJ/cm² were required for T4 and Lambda.

Lambda phage was found to be the most resistant to UV treatment process in secondary effluent, followed by T4, Q β and *E. coli*. Different UV doses were observed to achieve the same log inactivation of *E. coli*, Q β , T4 and Lambda. Among all coliphage, Q β was more susceptible to UV inactivation compared to T4 and Lambda.

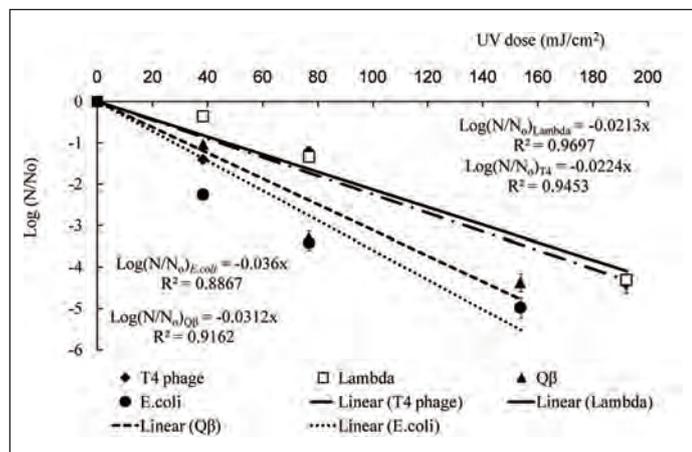


Figure 2: UV Inactivation of Targeted Microorganism (The error bars show maximum and minimum value for each duplicate dishes)

Previous study found that MS2, rotavirus, poliovirus and hepatitis A virus needed 64-93 mJ/cm², 50 mJ/cm², 23-29 mJ/cm² and 6-15 mJ/cm² respectively with UV process for natural waters to achieve 4-logs inactivation [30]. Based on the condition in this study, high dosage is required to achieve the same inactivation indicated the effect of the quality of tested water.

The significant differences in viral susceptibility to UV irradiation that of the cytosine content, or the complexity of the viral capsid and organisation of amino acids, carbohydrate and lipid composition of the protein capsid [17, 31]. Based on the characteristics of targeted microorganisms it could be suggested that protein coat (capsid) of T4 and Lambda may act as a partial shield against UV penetration to the genetic material. Capsid structure, as well as nucleic acid size, render ds-DNA virus less susceptible to UV inactivation [32].

UV disinfection could cause damage to the nucleic acid is well described in previous study [33]. But the knowledge of the ability to repair UV-induced effect or photo-reactivation to different microorganisms is still unclear. This ability could reduce the UV disinfection efficiency.

In order to ensure minimal biological risks, UV has the capability to establish as a primary disinfection for water reclamation facility. The susceptibility of selected microorganisms differed with the microorganism's characteristic. In this study, dsDNA bacteriophages T4 and Lambda demonstrated greater resistant compared with Q β and *E. coli*. This finding is consistent with previous studies [29, 37].

3.2 Effect of H₂O₂ Addition on the Inactivation of Q β phage and *E. coli* during UV Treatment

In order to reduce the energy consumption during treatment of high resistance virus and to control the photoreactivation of microorganisms, combination of UV with H₂O₂ is necessary and should be considered for actual wastewater reclamation facility.

The effects of H₂O₂ addition on the inactivation of Q β and *E. coli* during UV treatment are illustrated in Figure 3 and Figure

4. These experiments were performed under three different initial H₂O₂ concentrations in order to compare the effects of H₂O₂ addition during UV treatment.

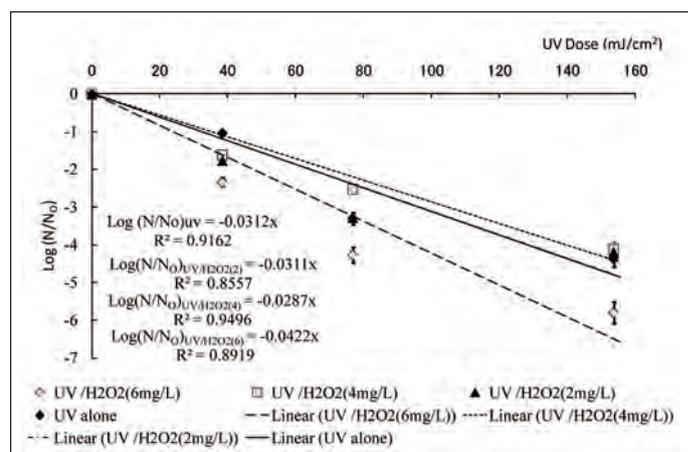


Figure 3: The Effect of UV/H₂O₂ Treatment Processes to Q β Inactivation Rate (Error bars represent maximum and minimum value for duplicate experiments)

Figure 3 shows 3-logs inactivation occurs at 96 mJ/cm² for UV alone process. On the other hand, additional of 6 mg/L H₂O₂ reduced UV dosage to 71mJ/cm². By increasing the UV dosage to 160 mJ/cm², 5-logs inactivation could be achieved by UV alone process. UV combined with 2 mg/L H₂O₂ and 4 mg/L H₂O₂ did not significantly increased the inactivation rate. However, the combination with 6 mg/L of H₂O₂ yielded higher inactivation rate with lower UV dose at 118mJ/cm². It was calculated that combination of UV/H₂O₂ enable approximately 26% reduction in UV dosage to achieve the same log inactivation compared to UV process alone.

For the combined process, during coliphage Q β inactivation in secondary effluent, the combination of UV and H₂O₂ promoted the production of hydroxyl radical and OH^o radical formation increased the inactivation rate.

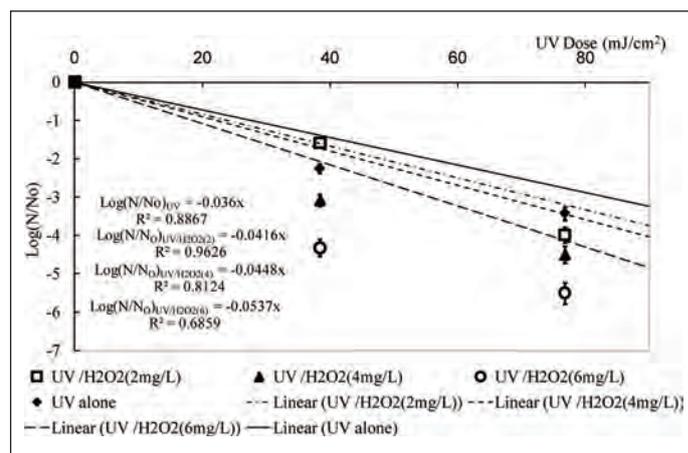


Figure 4: The Effect of UV/H₂O₂ Treatment Processes to *E. coli* Inactivation Rate (Error bars represent maximum and minimum value for duplicate experiments)

The combined processes of UV/H₂O₂ were tested for *E. coli*. The results showed that the addition of H₂O₂ significantly increased the inactivation of *E. coli* (Figure 4). The inactivation of *E. coli* gradually increased with additional of H₂O₂. For *E. coli*, 3-logs inactivation were achieved with 72, 67 and 55 mJ/cm²

UV doses with addition of 2, 4 and 6 mg/L H_2O_2 respectively. The rates of inactivations of 5-logs were reached with 120, 112 and 93 mJ/cm² with 2, 4 and 6 mg/L H_2O_2 , respectively. The combined processes were able to achieve approximately 32% reduction of UV dosage with 6 mg/L H_2O_2 compared to UV process alone.

The differences in disinfection efficiencies of UV and H_2O_2 could be explained by differences in mechanisms or susceptibility of indicator microorganisms to disinfectants. The main target of UV treatment are the nucleic acids, meanwhile the chemical H_2O_2 probably attacked the membrane walls, capsid or protein function for infectivity. The combination of UV/ H_2O_2 produced OH° radical during the reaction to increase the inactivation rate of *E.coli* and Q β . OH° radical is a strong oxidant and produced during advance oxidation processes such as UV/ H_2O_2 and UV/ O_3 treatment processes. Based on the results, the efficacy of combined UV/ H_2O_2 depended on the ratio of UV dosage to H_2O_2 concentration. OH° radical is non-selective oxidant and react very fast in water. The combination of UV/ H_2O_2 has been proposed as a measure to control photo-reactivation of microorganisms [38].

This study had shown that the secondary effluent treated with UV based processes had high potential to be used as reclaimed wastewater. Reduction of 1-log,3-logs,4-logs to 5-logs during UV based processes for *E.coli* and coliphages suggested that secondary effluent treated with UV based processes were suitable for restricted and non-restricted usage. Examples of potential usage include irrigation purposes and for receiving water being used for recreational activities with body contact [8, 9]. However, for more resistant viruses or for augmentation of drinking water supplies and dual reticulation in the building [10], higher quality is needed. The approach in producing NEWater by Singapore can then be considered, where microfiltration and reverse osmosis with UV disinfection were used to produce reclaimed wastewater for potable reuse [12]. This study was also found that the doses required for *E.coli* and coliphages were reasonable compared to USEPA requirement [39].

3.3 The Effect of Secondary Effluent Constituents

The significant effect of secondary constituents on coliphage Q β and *E.coli* inactivation were examined. The required doses for 3 to 5-logs inactivation as determined from the experiment were correlated to the measured value of TOC and UV absorbance (UV254). The effect of dissolved organic compound (DOC) and UV absorbance are shown in Figures 5 – 8.

Most organic compounds absorbed UV energy, thus it can be expected that a correlation between DOC and inactivation would exist. DOC demonstrated strong correlations as seen in Figures 5 and 6 during Q β phage and *E.coli* inactivation. The combined processes by addition of H_2O_2 during UV exposure produced OH° radical. OH° radical is highly reactive and can oxidize almost all contaminant in wastewater. However, organic compound in wastewater will undergo oxidative degradation and increased the competition of OH° radical consumption during the inactivation of microorganism.

UV absorbance reflects the quantity of UV energy absorbed by the medium (secondary effluent). Increased absorbencies mean that less UV energy is available for disinfection. In secondary effluent might contain many water matrixes showed strong correlation between UV_{254} and inactivation for indicator microorganisms as shown in Figures 7 and 8.

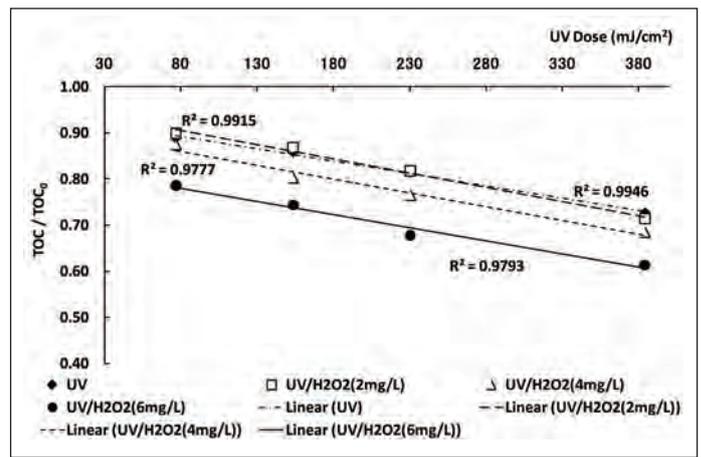


Figure 5: Correlation between UV Dose during Q β Inactivation and DOC Reduction

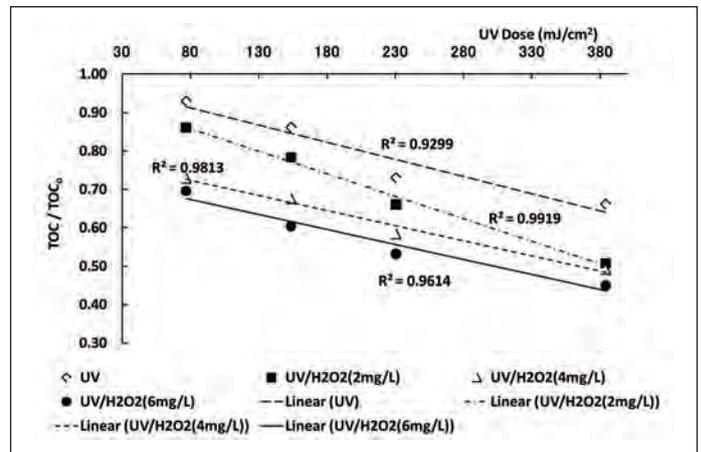


Figure 6: Correlation between UV Dose during *E.coli* Inactivation and DOC Reduction

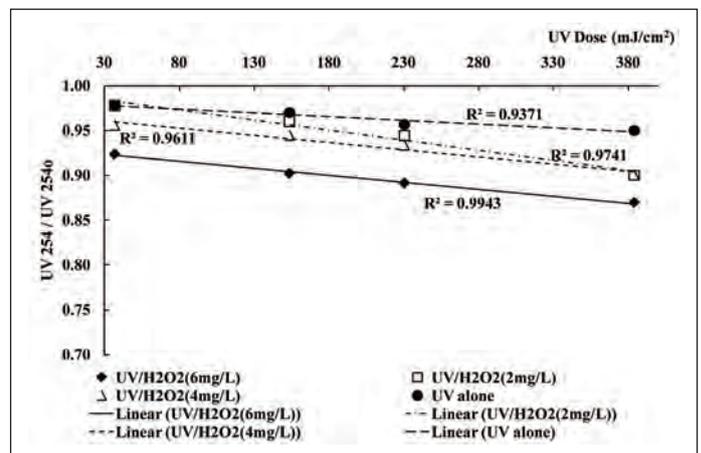


Figure 7: Correlation between UV dose during Q β Inactivation and UV Absorbance

Other study has also found that constituents of secondary effluent will have significant impacts on reclaimed water quality [40]. Microfiltration and reversed osmosis membrane systems were used to treat secondary effluent from sewage treatment plant in Malaysia. The reclaimed water quality complied with all parameters set by WHO Guidelines for Drinking Water [41], except for Ammoniacal Nitrogen and total plate count [40]. The non-compliance of Ammoniacal Nitrogen was because the sewage treatment plant was not designed with nutrient removal

facilities. Thus, secondary effluent contained high Ammoniacal Nitrogen, which affects the reclaimed water quality. The total plate count did not comply because no disinfection facilities were provided. Therefore, the secondary effluent quality is expected to affect the reclaimed water quality in terms of chemical properties.

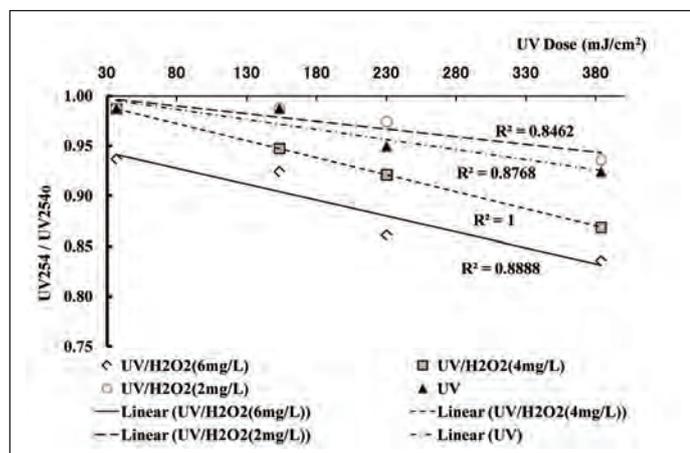


Figure 8: Correlation between UV Dose during *E. coli* Inactivation and UV Absorbance

4.0 CONCLUSION

This study emphasised on UV based processes for disinfection of selected microorganisms from secondary effluent. Secondary effluent was recognised as having a potential to be an alternative water resources as reclaimed wastewater. Nevertheless, the biological risks to end users should be considered. In order to reduce the biological risks of secondary effluent as reclaimed wastewater, proper disinfection is needed.

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