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Sensitive low-cost CCD-based detector for determination of UV-LED water microbial disinfection

Reuven Rasooly*, Ziv Magoz, Ji Luo, Paula Do, Bradley J. Hernlem

Western Regional Research Center, Foodborne Toxin Detection & Prevention Research Unit, Agricultural Research Service, United States Department of Agriculture, Albany, CA 94710, USA, Tel. +1-510-559-6478; email: reuven.rasooly@ars.usda.gov (R. Rasooly)

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ABSTRACT

Ultraviolet (UV) is widely used for bacterial disinfection of water, mainly derived from low-pressure mercury UV (LP-UV) lamps. The newly developed UV light-emitting diodes (UV-LEDs) have been of great interest as an alternative to LP-UV. Because of the lack of uniformity in research materials and methods and because no standard methods are available for UV-LEDs, it becomes difficult to make comparisons of alternative microbial disinfection. To overcome some of these limitations we present here a simple low cost new charge coupled device (CCD)-based sensitive method for determination of UV-LED microbial disinfection of water. The system was tested for UV-LED disinfection using a novel internal reflection UV-LED flow-through reactor. Samples of 200 mL water were spiked with 1,000,000 CFU (colony forming unit) pGlo fluorescent *Escherichia coli* and treated for 4 min with different UV-LED fluence (UV dose). To improve detection at low cell number we used filtration of a relatively large sample volume, the membrane filters were placed on agar plates containing arabinose that regulates the expression of the green fluorescent protein in the live bacterium and their viability was quantified by measuring their fluorescence with a CCD camera enabling detection of very low number of cells (0.62 cells/mL). The number of viable cells decreased with the increased level of UV illumination. At level of 100% illumination the disinfection was ~99.99% and the CCD-based detection was in agreement with a commercial detector system. These results demonstrate the potential of the CCD-based method combined with fluorescence *E. coli* to standardize UV-LED water microbial disinfection. Also, it compares the effectiveness of technologies for flow rate and UV radiation level for water disinfection.

Keywords: UV-LED; Disinfection; CCD; GFP; CFU

1. Introduction

Availability of safe clean drinking water is a problem for nearly 2 billion people according to the WHO [1] with 842,000 deaths, mostly children, from diarrheal disease in 2012 [2]. There are several methods available for the treatment of water, one recently expanding technique utilizes ultraviolet (UV) radiation because it effectively inactivates various micro-organisms in water [3]. UV technology is relatively simple to install, is chemical-free, and emits no harmful by-products [4]. However, the effectiveness of UV water disinfection depends upon many factors including: the type of micro-organism, the wavelength used, power of the source, the exposure time, the turbidity of the process fluid which limits its penetration depth and effectively shades and protects the micro-organisms from the UV light. Because of limitations to the depth of penetration, when organisms are embedded in a biofilm UV light may only be effective in surface sterilization and may not be effective in treating organisms protected by the biofilm. Micro-organisms are most vulnerable to cellular damage by light in the UV-C range of wavelengths (200–290 nm) [4]. During water disinfection UV radiation passes through the cell wall barrier of microbial pathogens and is absorbed by

* Corresponding author.

most organic materials including the DNA. The main UV damage is to DNA and the effectiveness of UV absorption by DNA peaks at wavelengths about 265 nm and at 185 nm. The effect is to damage and break the backbone of the DNA leading to improper fusing of DNA bases, altered nucleotide bases, and creation of new linkages between adjacent nucleotides such as thymine dimers. Although UV-A (320–400 nm) radiation is poorly absorbed by DNA and is less efficient in inducing damage on DNA, it still has the ability to inactivate micro-organisms. The main mechanism of UV-A inactivation involves an indirect effect by damaging proteins and producing hydroxyl and oxygen radicals that can destroy cell membranes and other cellular components [5] which may explain the lower level of disinfection at 365 nm [6]. In general, UV inactivation of micro-organisms probably follow the Bunsen–Roscoe reciprocity law which states that the photochemical effect is directly proportional to the total energy dose which is the product of the fluence rate and exposure time [7]. However, the combination of longer exposure time and lower fluence rate resulted in a higher degree of inactivation (up to 7 log) than the combination of a shorter exposure and higher fluence rate despite the total UV fluence (UV dose) being the same and this observation was attributed to biological processes. UV light-emitting diodes (UV-LEDs), like other LEDs, are not broad band sources but rather emit quasi-monochromatic light over a band typically 10–50 nm wide at half maximum and, further, radiometric power varies according to the chemistry of the semiconductor employed and thus it's emission spectrum [8]. Because of such factors and irradiation configuration, UV inactivation kinetics can be more completely predicted using a first-order model between dose and log inactivation [9]. For many food pathogens (e.g., *Salmonella*, *Escherichia coli*, and *Shigella*), to achieve >99.9% inactivation requires a UV radiation dose of 15.2 mW s/cm^2 at 253.7 nm [10]. Conventional germicidal UV radiation for water disinfection is obtained from low-pressure mercury UV lamps (LP-UV) emitting at 254 nm with typical 40 W output or high-output medium-pressure mercury lamps (up to 30 kW) with large surface area, however, the latter require significant amounts of energy with relatively low efficiency of around 15%–35% and they have a relatively short lifetime of about 10,000 h [11–13], are fragile and present the danger of mercury leakage, which is hazardous to the environment and requires proper disposal [6]. The United Nations Environmental Programme on Mercury has set the goal to phase out the production of the heavy metal mercury because of its associated hazards. In the last few years, alternative UV sources have been developed including light-emitting diodes (UV-LEDs). Current production UV-LEDs are typically limited to only several milliwatts output per single chip device, which is much lower than LP-UV. Combining multiple chips in one device yields higher power UV-LEDs that are typically heat sinked [14]. To maximize their effectiveness, UV-LEDs have to be very close to a water sample for deliverable UV energy. Despite their power limitations LEDs offer several advantages: compactness, long life of about 50,000 h, with broad wavelength diversity, low electrical power consumption, capable of pulsed illumination, favorable electrical to UV power conversion efficiency, lower voltage operation (which make them practical to area without electric infrastructure), and more robust, durable and do not contain mercury or other toxic materials. Another advantage and unique feature of UV-LEDs is their ability to be rapidly switched on and off with a high frequency, enabling adjustable UV-pulsed illumination. Such a feature makes UV-LEDs desirable for potentially enhancing the inactivation effectiveness by pulsed irradiation [15,16]. However, because of the wide array of micro-organisms analyzed, the lack of uniformity in research materials and methods reported [7] and because no standard methods are available for UV-LEDs [17] this makes the comparisons between disinfection technologies and the condition of disinfection (e.g., flow rate and UV radiation level for water disinfection) difficult. The radiometric characterization of LEDs is not trivial [14]. Techniques using silicon photodiodes and chemical actinometry have been applied to a range of commercial LEDs with emissions from the UV to IR [8]. Moreover, a system for analyzing water disinfection must be able to detect very few numbers of microbial cells in a large volume. While there are several technologies for such analysis including flow cytometry [18–20], such technologies are complex and expensive. To overcome some of these limitations, we present here a simple low-cost new CCD (charge coupled device)-based method for determination of UV-LED water microbial disinfection to high levels of efficiency which can be used for the comparison of the effectiveness of technologies for flow rate and UV radiation level for water disinfection.

2. Material and methods

2.1. UV-LED flow-through photoreactor

We designed, built, and characterized a flow-through photoreactor. The basic configuration of the UV-LED internal reflection illuminator is shown schematically in Fig. 1(a). The flow-through system is comprised of (1) a round aluminum tube with inner walls polished to increase reflection of UV light. Aluminum tubing was 6061-T6 Seamless Round. The reflective inner wall was polished with Mothers 05100 Mag & Aluminum Polish. Process liquid flows through the tube and is exposed to UV light emission through (2) quartz glass seals enabling illumination via the UV-LED light sources (3) positioned so that their emission is at an angle of 45° to the axis of the device to maximize internal reflection, (4) liquid inlet and (5) liquid outlet.

UV-LED wavelength of 275 nm was chosen because micro-organisms are most vulnerable to cellular damage by wavelengths of 200–290 nm [4] and the absorption spectrum of protein has a peak around 280 nm, which might help damage repair enzymes and prevent DNA repair [21]. The flow cell employed four UV-LEDs with emission at a wavelength of 275 nm and 10 mW optical power for each LED at 100 mA 8.5 V driving condition (Shenzhen Hanhua Opto Co. Ltd brand, Guangdong, China). The UV-LEDs were situated at the bottom of the reactor (Fig. 1(a)). More than 80% of the UV light is internally reflected by the polished aluminum reflective inner walls, the reflection is at the same angle as the incident angle, increasing the light path and increasing the exposure of the pathogen in the water to the UV irradiation. A low-cost Arduino controller board (Amazon) operated remotely (Fig. 1(b)) was used to control the level of UV

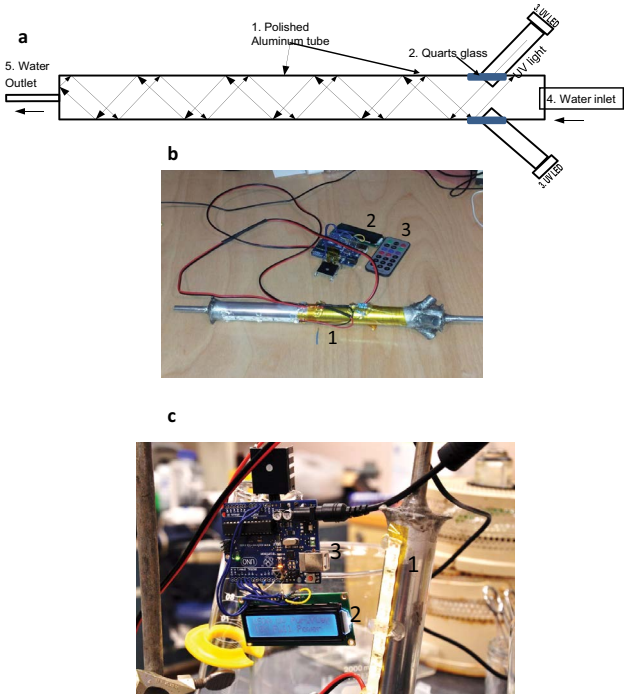

Fig. 1. **UV-LED system**. (a) A schematic of the flow-through UV reactor which is comprised of (1) a tube with polished reflective aluminum inner walls through which the process liquid flows and is exposed to UV light emission (2) quartz glass seals enabling illumination via the (3) UV-LED light sources positioned at angles to maximize internal reflection, (4) liquid inlet and (5) liquid outlet. (b) A photo of the system including (1) the flow-through UV reactor, (2) the Arduino controller remote control for controlling the UV light intensity of the system, and (3) the remote control. (c) The configuration of the complete system including (1) the UV reactor, (2) the controller, and (3) the LCD display indicating the current status (UV light intensity) of the system.

illumination. The water flow rate was adjusted by digital peristaltic pump with Easy Load II Head MasterFlex model 7524-40. The configuration of the complete system including the flow cells and the controller is shown in Fig. 1(c) including the LCD display presenting the status of operation (UV-on/off and output level).

2.2. Measurement of UV-LED disinfection efficiency

We used *E. coli* DH10B competent cells (Invitrogen, Carlsbad, CA, USA) which were transformed with Bio-Rad's pGLO plasmid containing the green fluorescent protein (GFP) gene under control of the arabinose-inducible *araBAD* promoter (Bio-Rad's, Hercules, CA, USA). For cell counting, 200 mL of the treated *E. coli* suspensions were filtered through a vacuum filtration system (Millipore, Hayward, CA, USA) that contained a 0.45 μm pore size, 47 mm nitrocellulose sterile membrane filter (Millipore Cat no. HAWP04700) to retain the bacteria present in the water. The membrane filters were placed on LB (Luria–Bertani, DIFCO, Franklin Lakes, NJ, USA) agar plates containing 100 μg/mL ampicillin and 6 mg/mL arabinose that regulates the expression of the GFP protein in the live bacterium. The inverted agar petri dishes were incubated at 37°C for up to 24 h. Only the live bacteria contain the GFP protein which can be excited by 406.0 nm photons (major excitation peak at a wavelength of 395 nm, a minor one at 475 nm) and then emits lower-energy green light photons with an emission peak at 509 nm wavelength as the molecule returns to its ground state. The bacterial viability was quantified by viewing the membrane filters under blue light of flashlight at 406.0 nm.

2.3. Colony counter system

The main components of the low-cost automatic colony counter system were: (1) a Point Grey Research Chameleon camera, Pentax 12 mm f/1.2 C-mount CCTV lens and a 50 nm passband 535 nm (HQ535/50M filters, from Chroma Technology Corp., Rockingham, VT, USA). For comparison, fluorescence measurements were also made using a commercial AlphaImager (Alpha Innotech-HP, San Diego, CA, USA). The colony forming units (CFUs) were counted manually and image counting using ImageJ [22].

3. Results and discussion

It was reported that in the field of UV-based microbial disinfection there are issues of uniformity in research materials and methods [7] and lack of standard methods for UV-LEDs [17]. To overcome these issues, we present here a rapid low-cost new CCD-based method for determination of UV-LED microbial disinfection of water which simplifies the quantification of UV microbial disinfection which can be used for the comparison of the effectiveness of technologies for flow rate and UV radiation level for water disinfection. It should be noted that this is a general method which can be applied to measure the efficacy of any disinfectant and is not limited to UV-LED disinfection. We used *E. coli* bacteria commonly found in the gut and feces of humans and warm-blooded animals. The presence of *E. coli* in drinking water indicates that water is contaminated with feces or sewage, and it has the potential to cause disease. We genetically engineered the *E. coli* bacteria cells to express GFP. We employ the engineered bacteria cells and a CCD camera to simplify cell counting. The CCD-based system was tested using a novel internal reflection UV-LED illuminator operated by an Arduino controller. Although *E. coli* are used here, this method could be calibrated to other micro-organisms to establish a relative coefficient of disinfection to apply the *E. coli* measurements to other micro-organisms including UV-resistant micro-organisms or spore.

3.1. Imaging-based quantitation of microbial disinfection effect by UV-LED

Our approach to measure microbial disinfection is based on fluorescence imaging of GFP transformed *E. coli* subsequent to UV treatment. For imaging of the plates, we used a simple configuration (Fig. 2(a)). The simple detection system includes a Point Grey Research Chameleon CCD camera (Fig. 2(a-1)) to image GFP fluorescence, a CCTV f1.2 lens mounted on the camera (Fig. 2(a-2)) with a 535/50 nm band pass (BP) emission filter mounted on the end of the lens Fig. 2(a-3)), flashlight 406.0 nm was used for excitation source (Fig. 2(a-4))

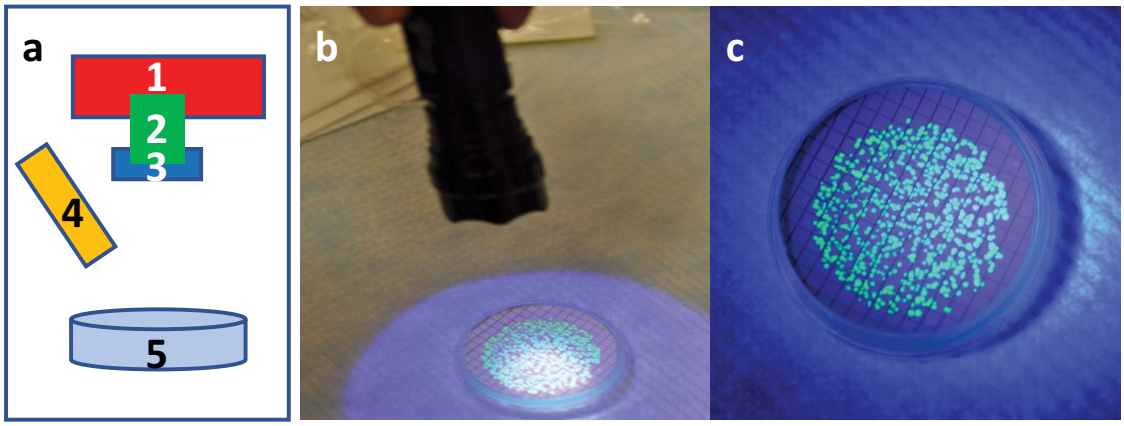

Fig. 2. **Florescence imaging system for quantitation of microbial disinfection**. (a) The configuration of the detector: (1) CCD camera, (2) CCTV f1.2 lens, (3) 535 nm emission filter, (4) flashlight 406.0 nm, and (5) the filter on a petri dish. (b) The 406.0 nm flashlight illuminating the plate and (c) the plate showing high GFP signal.

illuminating the filter on a petri dish (Fig. 2(b-5)). While the 535/50 nm BP filter is not centered on the GFP emission peak at 509 nm, as shown in Fig. 2(c), with the f1.2 lens and the high GFP signal this selection of emission filter was adequate in this application.

3.2. UV-LED internal reflection disinfection of water

To determine the efficiency of the UV-LED system, 200 mL water was spiked with engineered *E. coli* to create suspensions containing 1,000,000 CFU and this was treated for 4 min with different input power levels (0%–100% power of the total 40 mW UV-LED illumination). After UV treatment the water was filtered through a sterile nitrocellulose membrane filter with a 0.45 μm pore size to capture the bacteria present in the water. Sensitive detection of low concentrations of cells requires analysis of relatively large volumes of water because a low volume (e.g., 1 mL) is insufficient when the effectiveness of UV-LED disinfection or the level of contamination is so low as to reduce the probability of a single bacterium in 1 mL below 1. In our system designed to analyze unknown water sources, we chose to use a 200 mL sample. The membrane filters were placed on agar plates containing arabinose that regulates the expression of the GFP protein in the live bacterium. The inverted agar petri dishes were incubated at 37°C for up to 24 h and analyzed as described earlier and imaged with the simple CCD configuration (Fig. 2(a)). To estimate the number of viable bacteria that are able to multiply, we count the number of CFUs, each colony resulting from a single bacterium. As shown in Fig. 3 it was relatively easy to count the number of CFUs when the plate contained only a low number of CFUs as the colonies are large and well spaced. However, it is a difficult, tedious, and time-consuming task to count CFUs in an overcrowded plate containing many colonies above an upper countable limit. Additional errors occur when we try to estimate the number of CFUs in the entire overcrowded plate by dividing the plates into equal sectors, counting only subsections

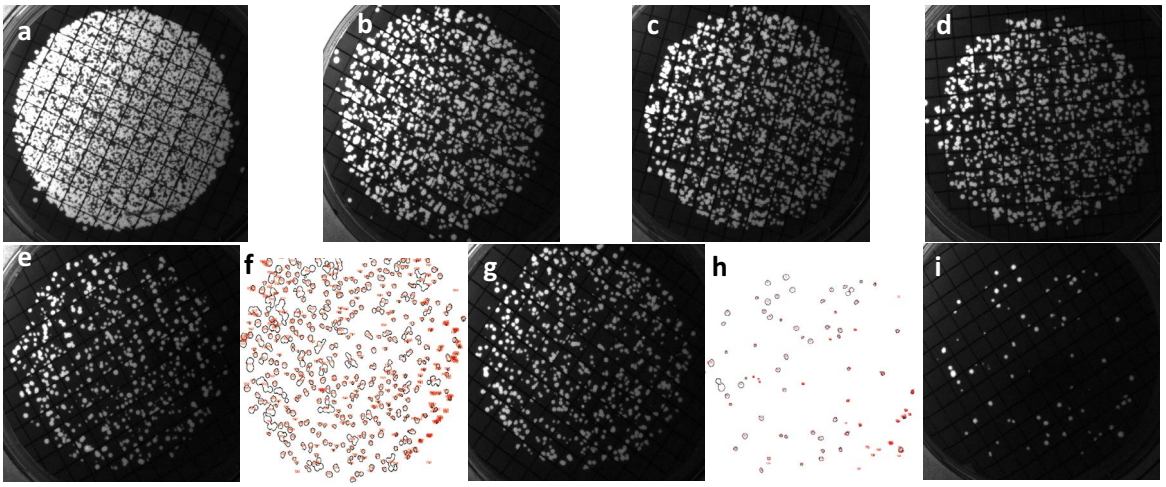

Fig. 3. **UV-LED internal reflection disinfection of water**; samples of 200 mL water were spiked with GFP expressing *E. coli* to create suspensions with 1,000,000 CFU and were treated for 4 min with different power levels: (a) 0%, (b) 30%, (c) 60%, (d) 70%, (e) 80%, (f) 90% ImageJ count, (g) 80%, (h) 100% ImageJ count, and (i) 100%.

of a plate to estimate the whole plate count after extrapolation. In order to turn colony counting into a fast procedure that reduces counting errors and simplifies the cell counting method, the bacterial filters were imaged using a CCD camera that captures the entire plate area in a single image. We applied open-source software ImageJ to automatically count the entire number of round white colonies of a particular size on the filter. As shown in Fig. 3, the number of cells decreased with increased level of UV fluence (UV dose). With full power of illumination (Fig. 3(i)) ImageJ cell counting estimated 126 cells/200 mL (Fig. 3(h)) which is 0.62 cell/mL. Using standard cell counting methods such as Most Probable Number Test will require at least 15 tubes in three dilutions to detect 0.62 cell/mL. However, because the possible number of cells is not known a priori, the volume needed for testing probably must be larger while using our approach does not require a priori knowledge and serial dilution procedure. Similarly, the ImageJ count of colonies at 90% illumination was determined as 602 and at 80% the count was 697. At adjustment power level of 100% illumination the number of bacteria (CFU) was reduced by 4-log, from 1,000,000 to 126 CFU for a disinfection efficiency of 99.99%. However, the water that was exposed to low UV fluence (UV dose) resulted in confluent lawns (Fig. 3(a)). Single colonies merged together and ImageJ was unable to count them accurately. For those levels, we used ImageJ to measure the fluorescence of the whole plate, rather than manual counting colonies. The bioluminescent imaging method described here measures the disinfection of genetically engineered luminescent *E. coli* rather than UV-resistant micro-organisms or spores employed in more stringent assays. Nonetheless, one could calibrate the method to other micro-organisms and establish a relative coefficient of disinfection to apply the results with the *E. coli* strain used here to other micro-organisms including UV-resistant micro-organisms or spore.

3.3. Imaging-based fluorescence analysis of microbial disinfection effect by UV-LED

To calculate the average signal fluorescence intensity value of each CCD pixel from the bacteria colony, the average brightness of each pixel was quantified for a series of frames using the free open-source imaging software ImageJ. The average optical brightness intensity of the bacterial colony is reported in analog-digital units (ADUs) and was plotted for different power levels of UV-LED fluence (UV dose) (Fig. 4(a)) revealing a dose–response relation between UV-LED fluence level and the intensity of fluorescence emission. These results were compared with a commercial AlphaImager (Fig. 4(b)). The average luminous intensity brightness values were similarly reported in ADUs. The results from both instruments are in agreement with high

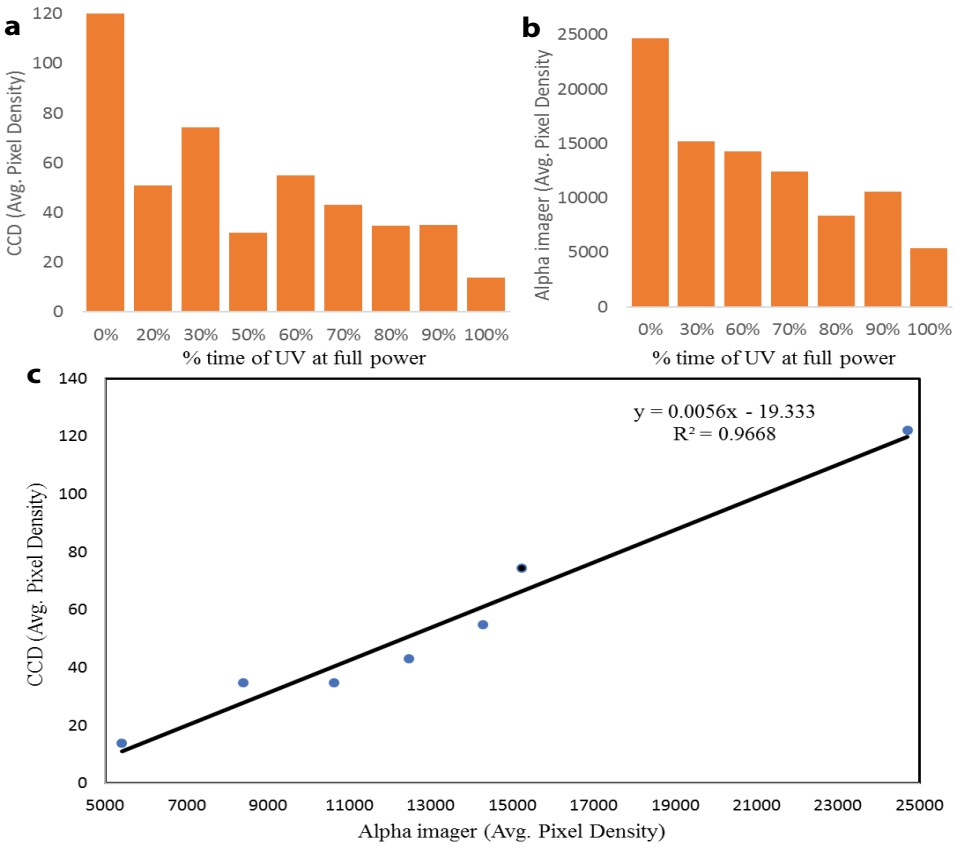

Fig. 4. **Imaging-based fluorescence analysis of microbial disinfection effect by UV-LED**. (a) Fluorescence imaging of the filters measured by CCD camera, (b) fluorescence imaging measured by AlphaImager, and (c) CCD measurements versus AlphaImager measurements.

correlation between the measurements (Fig. 4(c)) showing that there is a negative relationship between UV-LED fluence level and the GFP fluorescence intensity emitted by live bacteria. These results demonstrate proof of principle that the low-cost CCD camera and AlphaImager systems can be used for automatic live bacterial colony counting and demonstrate that the low-cost CCD camera is an effective technology as a fast automatic colony counter system at comparable levels to the AlphaImager.

4. Conclusion

The data presented here suggest that the detection system utilizing a fluorescence *E. coli* cells expressing GFP fluorescently detected by CCD camera enabled detection of very low number of cells (0.62 cells/mL) in water sample treated with a flow through UV-LED reactor which enabled 4 logs disinfection. The new method can be used for the comparison of the effectiveness of technologies for flow rate and UV radiation level for water disinfection.

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