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Enhancement of ultraviolet water disinfection process

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The aim of this study was to examine the efficiency of phages in combination with physical water treatment process to ensure optimal disinfection without subsequence water recontamination. The use of phages' cocktail was used instead of chemical reagents to overcome the non remanent effects of ultraviolet (UV) disinfection procedure and to enhance the worth of this clean and safe technology. This combination proved to be advantageous. Indeed, the lysis activity of phage combined with germicidal effects of ultraviolet (UVC) irradiation provides a decrease of viable bacterial density. Furthermore, the use of virulent virions can reduce the subsequent reactivation of UVC treated bacteria without addition of chemical reagents that can have a potential risk to public health.

Key words: UV radiation, water treatment, reactivation, remanent effects.

INTRODUCTION

Chlorination has been used for most water disinfection operation for many years. However, it is no longer the disinfection method automatically chosen for either water or wastewater treatment because of potential problems with disinfection by products and the associated toxicity in treated water (Gallard and Von Gunten, 2002). In addition, a large variety of pathogenic bacteria has been isolated from biofilms both in chlorinated and non-disinfected water distribution systems, including *Legionella pneumophila*, *Salmonella typhimurium* and Campylobacter spp. (Momba and Binda, 2002).

Among the alternatives to conventional chlorination, ultraviolet (UVC) irradiation is chosen most frequently. The effectiveness of UV light in biological inactivation

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arises primarily from the fact that DNA molecules absorb UV photons between 200 and 300 nm, with peak absorption at 260 nm (Bolton and Linden, 2003). This absorption creates damage in the DNA by altering nucleotide base pairing; thereby creating new linkages between adjacent nucleotides on the same DNA strand. If the damage goes unrepaired, the accumulation of DNA photoproducts can be lethal to cells through the blockage of DNA replication and RNA transcription, which ultimately result in reproductive cell death (Zimmer and Slawson, 2002).

Disinfection by ultraviolet light (UV) is considered as a cost effective and easily implementable system for water disinfection (Clancy et al., 2000). Unlike chemical biocides, UV does not create any appearance disinfection by-product DBPs. Indeed, according to Reckhow et al. (2010) on the study of the effect of UV treatment on the subsequent formation of disinfection by-products (DBPs) using two sets of water samples submitted to low-pressure (LP) and medium-pressure (MP) UV disinfection, it was found that germicidal doses of UV light LP did not substantially affect the tendency of the test waters to form trihalomethanes, haloacetic acids, or total organic carbon under conditions typical for drinking water treatment. Although, some minor DBPs were enhanced by pretreatment with MP UV, the final concentrations remained small.

In addition, disinfection by germicidal UV light provides no residual effect in the water to protect against post treatment contamination therefore; some post-irradiated microorganisms can develop mechanisms to appropriately respond to UV stress. Most of them can repair UV-DNA lesions in two ways: light dependent photoreactivation catalyzed by a photolyase and light-independent nucleotide excision repair (NER).

The major goal of water disinfection step is the production of safe water. Accordingly, to overcome the non remanent effects of UV disinfection water and to reduce the recontamination of treated water by pathogenic bacteria, you must add a complementary step to ensure a residual disinfecting effect in irradiated water such as the addition of chlorine. However, due to the negative impact of secondary by-products generated by chemical reagents (Amisha et al., 2011), we searched for a novel and an alternative approach.

In this study, we aimed at enhancing the tertiary water treatment by the addition of a bio-remanent reagent (BRR) instead of chemical one. The BRR consist of a pool or cocktail of phages in inlet or/and outlet UV reactor.

Bacteriophage (phage) is a virus that infect and lyses bacteria. These viruses are small infectious particles, typically 20 to 200 nm consisting of a nucleic acid core (single or double stranded RNA or DNA) enclosed by a protein coat (capsid) and in some cases a lipid envelope (Mudgal et al., 2006). Bacteriophages (phages) are viruses that infect prokaryotes. Like all viruses, phages are obligate intracellular parasites, which have no intrinsic metabolism and require the metabolic machinery of the host cell to support their reproduction.

Two categories of bacteriophages are recognized: temperate and virulent. During lytic infection, virulent phages inject their nucleic acid into the host cell following attachment. Expression of the phage genome directs the cellular machinery of the host to synthesize new phage capsule material. The resulting phage progeny are released by fatal cell lysis enabling the lytic cycle to continue as new cells infected. The number of progeny released (burst size) varies from 50 to 200 new phage particles (Wommack and Colwell, 2000). In contrast, during lysogenic infection, temperate phage nucleic acid recombines with the host cell genome forming a dormant prophage. The prophage is reproduced in the host cell line and confers immunity from infection by the same type of phage. Stress conditions such as ultraviolet light or chemical mutagens can induce a switch to the lytic cycle.

We proposed in the present study, to enhance the effectiveness of water disinfection process by the use of phages to prevent an eventual recontamination of treated water without addition of chemical reagents.

MATERIALS AND METHODS

Bacterial strains

All bacterial strains were obtained from American Type Culture Collection: *Escherichia coli* ATCC25922, *Salmonella typhi* ATCC 14028, *Pseudomonas aeruginosa* ATCC 15442 and *Enterococcus faecalis* ATCC 19433.

Isolation of phages

A pool of phages was isolated from wastewater and were tested for their specificity and lytic activity against tested bacterial strains. Phages were isolated using plaque assay. Briefly, wastewater samples were filtered through 0.22 µm millipore filter. The supernatant was mixed with ~10^6 CFU/ml of each tested bacteria strains. The mixture was inoculated into 0.7 % Luria Bertani (LB) soft agar, mixed, incubated at room temperature for 2 min and poured onto the pre-poured LB agar plate. Plates were then incubated for 24 h at 37°C. Then, phages that have a large spectrum of infectivity against tested bacteria were picked up.

Host range determination

The host range of bacteriophage was determined against different species of bacterial strains obtained from the American Type Culture Collection (ATCC). The plaque-forming ability of isolated phages was tested by the spot-on the-lawn technique. Bacterial suspensions were infected with phage at a multiplicity of infection (MOI). The lytic activity was observed after overnight incubation.

Phage titration of stock solution

Bacteriophage titer was analyzed as described by Ben Said et al. (2009). Briefly, 100 µl of diluted phage solution, 100 µl of a bacterial

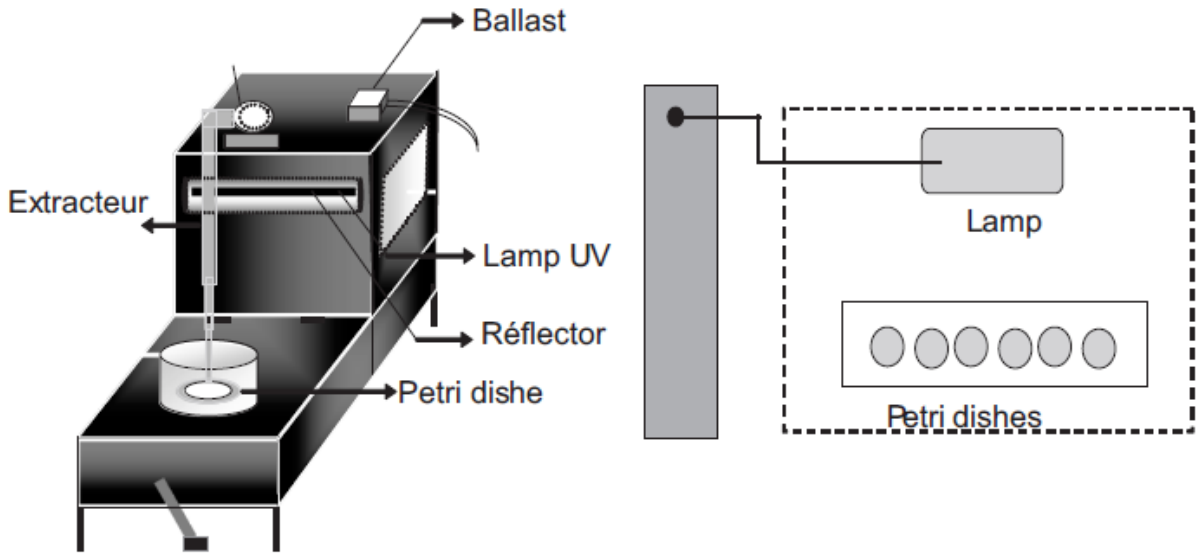

Figure 1. Experimental setup for UVC irradiation.

overnight culture and 3 ml of molten agar were mixed in a glass tube and poured into a Tryptone Soya Agar (TSA) containing Petri dish. Plates were incubated for 18 h after which plaque forming units (PFU) were counted.

The laboratory UV device

The laboratory UV-devise was built with the cooperation of the company, Guy Daric S.A (Aubervilliers, France). This prototype contained a sliding rack, with an irradiation board which could receive at the same time six Petri dishes (90 mm in diameter). A germicidal low-pressure mercury vapour discharge lamp (length = 900 mm, diameter = 13 mm, power of UV emission at 253.7 nm = 55 W) with reflector could be adjusted in height above the irradiation board (Figure 1). The lamp was supplied via electric ballast and the ozone produced in the irradiation room was removed by an extractor.

UVC irradiation

For the study of UVC dose/biofilm production relationship, the bacterial strains were cultured in Luria-Bertani broth (LB). Bacterial suspensions were diluted in saline phosphate buffer (PBS) and the resulting preparations were used for irradiation experiments. A volume of 20 ml of the suspended culture was transferred into a standard Petri dish for exposure to the continuous UV-light treatment. The samples were exposed to the UV-light for chosen increasing UV dose.

Viable cell counts

Viable cell counts were taken before and immediately after UVC exposure using a standard plate count method. A 100 µl portion of each UVC irradiated samples was removed in order to prepare serial dilutions in PBS buffer. A volume equal to 100 µl of the appropriate serial dilutions was spread in duplicate onto TSA agar. The number of colony-forming unit (CFU/ml) or a number of viable and cultivable bacteria was determined after 24 h of incubation at 37°C. The fraction of viable and cultivable bacteria was calculated by dividing the number of CFU in the UV-treated sample (N) by the number of CFU determined at time zero before UV irradiation (N_0).

Bacterial reactivation in darkness or visible light condition

UV-irradiated samples were divided and transferred into two separate sterile Petri dishes. One of the two Petri dishes was exposed to visible light to examine potential photo repair and one was covered with foil to allow for potential dark repair at room temperature.

The determination of a log increase methodology was employed to quantify the capacity of bacterial reactivation (photo repair and dark repair). In this method, the differences in the log bacteria survival after UV-C radiation, and after reactivation in darkness or in visible light condition were determined according to Lindauer and Darby (1994) with modifications.

As noted, all experiments were done three times, to verify the efficiency and the reproducibility of the BRR combination with UV disinfection process.

RESULTS AND DISCUSSION

Phage growth constants defining phage-host cell were directly related by the host cell quality (damaged cells by UV light, accumulation of photoproduct, enter in a VBNC state, etc) (Ben Said et al., 2010). Indeed, the infectivity rate of lytic phage was used as a bio-captor or bio-indicator of the physiological state of irradiated host cells mainly, the active but non cultivable bacteria and also to reduce the density of residual viable bacteria after disinfection process (Ben Said et al., 2011). Thus, we can use this propriety to control bacterial density and related virulence factors after irradiation.

In addition, this “bio-remanent reagent” can be used as a bio-indicator of water treatment effectiveness. For example, after UVC disinfection, we can titer the phage to

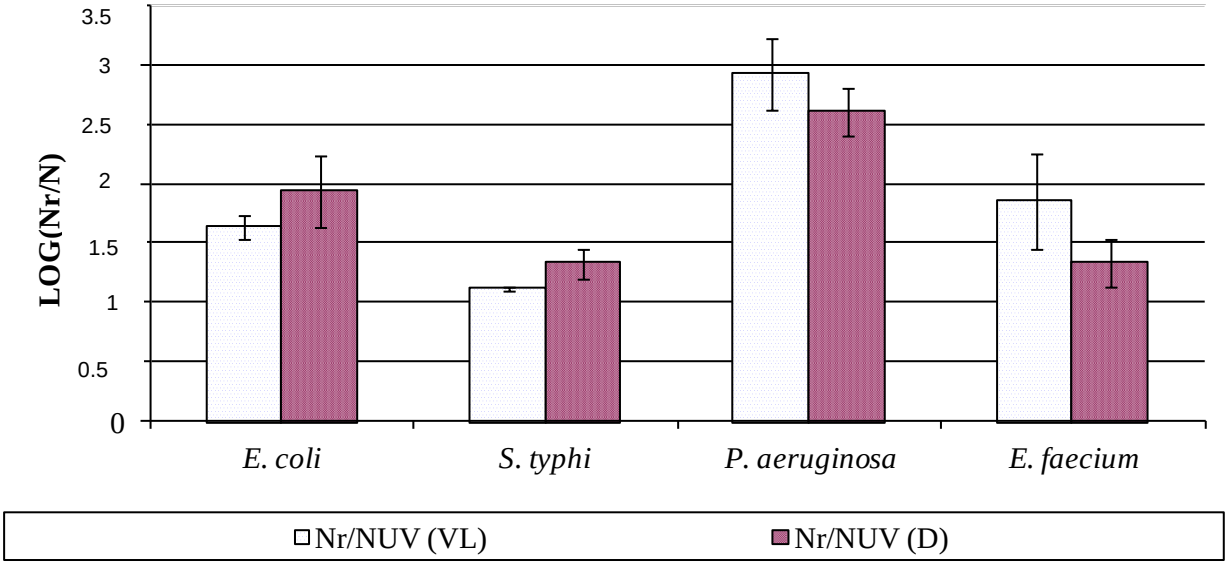

Figure 2. Coefficient of bacterial reactivation (C_R) or log increase in the presence of photoreactivation and dark repair conditions after UVC treatment without addition of biological remanent reagent (BRR). N_{UV} is the number of viable and cultivable bacteria after UVC irradiation; N_{VL} and N_D are the number of recovered bacteria after a rest time in the presence of visible light (VL) and in darkness (D).

show if the applied dose is adequate to inactivate microorganisms or not.

The control of pathogenic bacteria density (viable and cultivable bacteria and viable but non cultivable bacteria) under UVC irradiation and phage infectivity can be used as an effective approach to control and guarantee optimum water UVC disinfection step without any risk of water recontamination.

Bacterial reactivation after UV irradiation

Reactivation is of particular significance in water UV disinfection. Indeed, some post irradiated microorganisms can carry out replication and re-growth after a rest time in darkness or/and in the presence of visible light. Consequently, reactivation mechanisms can reduce directly the efficacy of UV-disinfection process and can represent a potential public health risk. In the experiments on reactivation, it was represented by an increase in the log counts of the tested bacteria.

The reactivation data is semi-quantified using the log increase method where reactivation is defined by the difference in log survival of the microorganism before and after reactivation (Ben Said et al., 2011) with modifications, as represented by the formula shown below:

$C_R = \log N_{VL}/N_0 - \log N_{UV}/N_0 = \log N_{VL}/N_{UV}$ (in the presence of visible light)

$C_R = \log N_D/N_0 - \log N_{UV}/N_0 = \log N_D/N_{UV}$ (in the darkness condition)

Where, C_R is the reactivation level or reactivation coefficient in the presence of visible light or in the darkness condition; N_0 is the number of viable and cultivable bacteria before UVC irradiation (at time zero); N_{UV} is the number of viable and cultivable bacteria after UVC irradiation; N_{VL} and N_D are the number of recovered bacteria after a rest time in the presence of visible light (VL) and in darkness (D).

The determination of a log increase methodology was employed to semi-quantify the capacity of UVC irradiated bacteria to reactivate and repair the damages caused by germicidal light (photo repair and dark repair).

The maximum photoreactivation levels of *E. coli* of up to 3.4 log have been reported (Harris et al., 1987). Such high levels of photoreactivation may cause bacteria concentrations in the treated water to exceed the regulatory limits. *E. faecalis* was found to perform pho-toreactivation to levels of about 2.3-log in the same study.

Figure 2 shows the reactivation level of tested bacteria, after irradiation with a conventional dose of UVC light; in the presence of visible light and in the darkness (C_R superior to 1 for the majority of tested bacteria). This result highlights the limit of UV water disinfection process (continuous UV irradiation and pulsed UV light). Indeed, UV disinfection does not provide a residual disinfectant that can help to suppress this current phenomenon (Bohrerova and Linden, 2006). Hence, it is very important that reactivation following UV disinfection be thoroughly investigated so that preventive measures can be designed and put in place. To overcome this inconvenience, we added a supplementary disinfection step: a biological

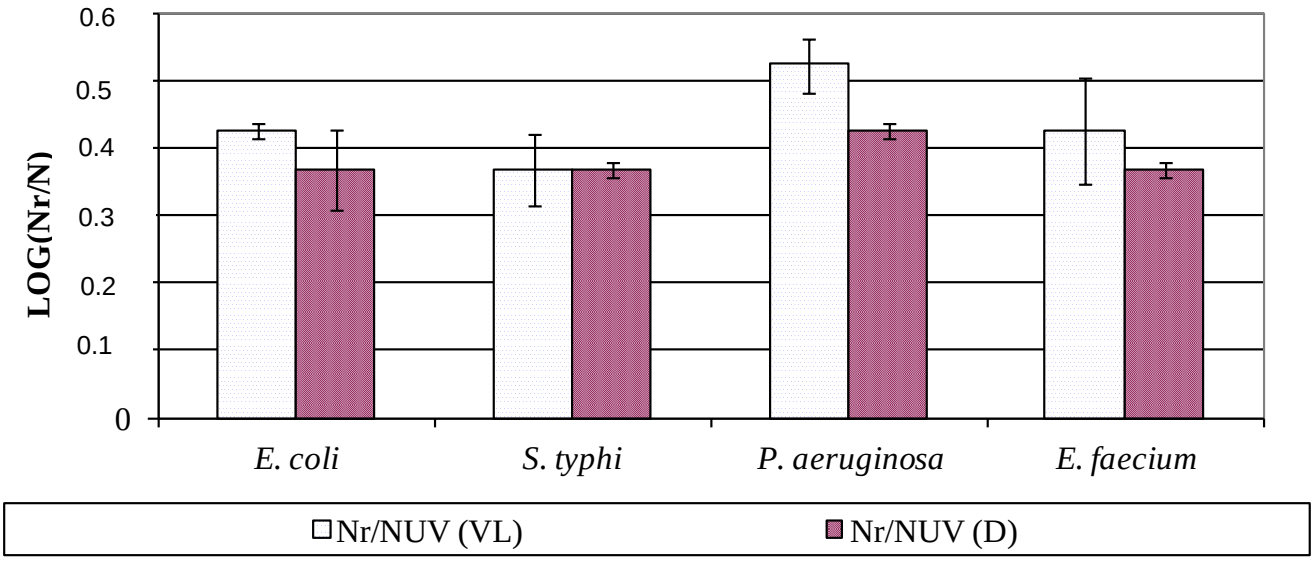

Figure 3. Bacterial reactivation (C_R) or log increase in the presence of photoreactivation and dark repair conditions after UVC irradiation with addition of biological remanent reagent (BRR). N_{UV} is the number of viable and cultivable bacteria after UVC irradiation; N_{VL} and N_D are the number of recovered bacteria after a rest time in the presence of visible light (VL) and in darkness (D).

treatment process by the use of phages or bacteriophages.

Figure 3 shows the decrease of the coefficient level of bacterial reactivation, after combination of physical and biological water treatment steps (C_R inferior to 1 for the majority of tested bacteria). This result confirms the importance and the efficiency of phage to prevent the bacterial reactivation and thus the recontamination of UVC treated water after a rest time. The application of biological treatment using infectious virions after water treatment can constitute an alternative to chemical reagents.

Phages are self-replicating in the presence of their host cells and self-limiting in their absence (Ben Said et al., 2009, 2010). Thus, phages are continually produced to infect new bacterial cells as long as the bacteria are able to support their replication and to overcome the non remanent effect of UVC water disinfection process by the addition of biological remanent reagent (BRR) without addition of chemical agents.

Before the addition or the injection of BRR, a prior study of treated water is very important. Then, physical and chemical proprieties of treated water and disinfection parameters (water flow, total suspended solid (TSS), pH, temperature, etc), were evaluated. Based on this prior study, the BRR injection can precede; inlet the disinfection and/or outlet the UV device (Figure 4).

The BRR is composed by a pool of phages that have a different genetic (DNA phages or/and RNA phages) and infectious proprieties (titer, life cycle of phages, the phages infectious spectrum). The composition of BRR change with the step of bio-injection: inlet or outlet of the disinfection system, and also with the indefinite use of treated water (agriculture, aquaculture and industrial use, recreational water, etc).

Inlet of the UV disinfection system used a high titer of phages with a large infectious spectrum. In contrast, outlet water disinfection system used inject BRR with lower titer of phages with specific or/and large infectious spectrum (it depends on water use and water storage conditions).

Conclusion

The enhancement of physical effectiveness of germicidal radiation, using a biological reagent instead of chemical reagent can ensure the remanent effect and protect against potential risk of water recontamination. The combination of physical and biological treatment of water can increase the bacteria inactivation without subsequent reactivation and without addition of chemical or toxic reagents.

The present study provides an alternative technology to prevent the post-UV reactivation of bacteria without addition of chemical reagents or change of disinfection parameters (dose, exposure time, etc). This approach consists of the addition of biological treatment of water by a cocktail of phages called BRR as a pretreatment and/or post-treatment step (according to the indefinite use of treated water) in combination with UV germicidal light.

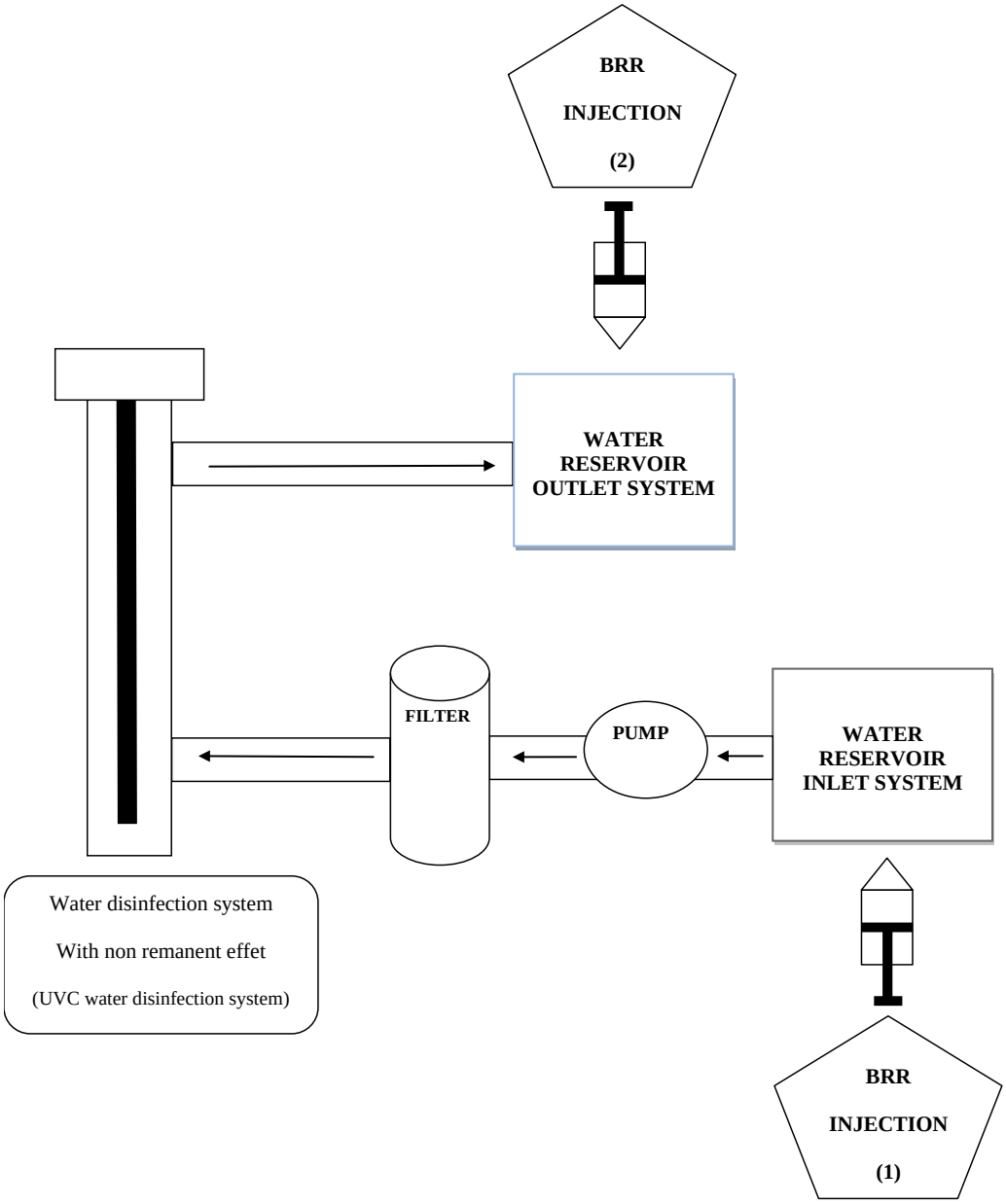

Figure 4. Application of bio-remanent reagent (BRR) injection process inlet (1) or/and outlet (2) disinfection system.

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