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ENHANCED INACTIVATION OF *E. COLI* IN DRINKING WATER USING SEQUENTIAL UV IRRADIATION AND CHLORINATION FOR IMPROVED DISINFECTION EFFICIENCY IN NORTHERN CYPRUS.

Sunday Onyebuchi Ukanwa[1*] and Joshua Tunde Olaifa[2].

[1]Department of Bioengineering, Faculty of Engineering, Cyprus International University, Nicosia, North Cyprus, Mersin 10, Turkey.

[2]Department of Crop Science, Faculty of Agriculture, University of IIesa Osun State, Nigeria.

[*]Corresponding Author's Email: sundayukanwa@gmail.com

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ABSTRACT: *Clean water is essential for human health, as it greatly reduces the risk of disease; however, water can also serve as a vehicle for pathogens. To safeguard public health, the Environmental Protection Agency (EPA) has established drinking water standards to control microbial contamination and disinfection byproducts. This study investigated the combined use of UV irradiation followed by chlorination to inactivate* Escherichia coli *under low UV doses and short contact times. The results showed that UV irradiation alone achieved a 2-log$_{10}$ reduction after 30 seconds, whereas chlorination alone was more effective, achieving a 2-log reduction at chlorine doses of 0.5, 1, 2, and 3 mg/L within 60, 5, 1, and 1 minute, respectively. The combined UV–chlorine treatment outperformed either method alone, demonstrating that UV disinfection is significantly enhanced when followed by low-dose chlorination.*

KEYWORDS: Chlorination, Disinfection, *E. coli*, Petri dish, UV irradiation.

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INTRODUCTION

The universe is covered by water, with the total volume being approximately 1,400 million km³. About 2.5% of this, or 35 million km³, is freshwater. The majority of this natural water (over 1.5% of all water and 60% of all freshwater) is frozen in the form of snow or perpetual ice in Antarctica and Greenland, making it inaccessible. Around 1% of all water, or 200,000 km³, of the freshwater is available in lakes, rivers, channels, and underground sources (WHO 2017). These sources are the primary means of water for human consumption. A significant issue, especially in developing countries, is the lack of access to safe drinking water (Mosler & Mosler 2008; Bosshard et al. 2010). Water security is crucial, and inadequate sanitation and hygiene lead to widespread microbial contamination of water sources. Out of the world's 6.8 billion people (U.S. Census Bureau 2010), 2.6 billion lack proper sanitation, and about 884 million, mostly in developing countries, do not have access to safe drinking water. Water is essential for various purposes, including drinking, food production, domestic use, recreation, and agriculture, all of which impact health (WHO 2017). The lack of potable and safe drinking water and sanitation in developing countries is largely due to poverty and the inability of governments to finance water projects and sanitation systems (WHO 2000).

Microbiological hazards can cause waterborne illness to humans which is as a result of water supply contamination. Just in 2004, South Bass Island, Ohio waterborne epidemic affected about 1,450 residence and visitors. (Fong et al., 2007). Runoff from microbiological contaminants of wastewater treatment plants is responsible for major contamination in lakes and groundwater area (Fong et al., 2007). In Cyprus, the drinking water supply is primarily derived from surface waters or groundwaters influenced by surface water. According to a new report, "The Provision and Quality of Drinking Water in Cyprus," the Environmental Protection Agency (EPA) found that 81.6% of the drinking water comes from surface water sources like rivers and lakes, with the remaining 18.4% derived from groundwater (10.3%) and springs (8%). These water sources are susceptible to surface contamination and contain microorganisms such as bacteria, viruses, and protozoan parasites (e.g., Cryptosporidium), which can pose health risks if not properly treated (Morris et al., 1996; Hoxie et al., 1997).

Drinking water contaminated with live fecal bacteria can cause gastrointestinal illnesses. One example is E. coli, a bacterium found in the gastrointestinal tracts of humans, pests, ruminants, non-ruminants, and wild animals, where it usually exists as a commensal organism (Feng et al. 2002; Frederick 2011). *E. coli* are gram-negative, facultative anaerobic bacteria (Feng et al. 2002) belonging to the *Enterobacteriaceae* family, and they ferment glucose or lactose (Feng et al. 2002). While most E. coli strains are harmless, pathogenic strains can cause hemolytic uremic syndrome, which can be fatal (Feng et al. 2002). In 2008, the EPA established that the most effective way to ensure the safety of drinking water is through a comprehensive risk assessment and management approach, covering all steps from the water source to the consumer. The EPA recommended that Water Service Authorities adopt the World Health Organization (WHO) Water Safety Plan for risk assessment and management. The main goal of water treatment is to remove or inactivate pathogenic microorganisms to prevent waterborne diseases. With safe water supplies from controlled catchments becoming increasingly rare, it is essential for water treatment facilities to have adequate disinfection systems. Typically, pathogenic organisms are removed through the addition of coagulant chemicals followed by sedimentation and filtration, as well as other filtration processes such as membrane filtration.

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E. coli is a water quality indicator, and therefore, when found in water systems, there are other possible pathogens, and viruses in the water that are connect with sewage waste, such as cholera and salmonella. In the United States of America, the EPA's (Environmental Protection Agency) standard of fecal coliform levels in recreation water is less than 200 colonies/100 ml using a thirty-day geometric mean (Hamzah et al. 2011). This standard is based on the World Health Organizations water quality guidelines for healthy drinking and recreation waters (van Rees & Reed 2015). In Cyprus, drinking water sources are at risk of contamination from both point sources, such as wastewater treatment plants, and non-point sources. Pathogenic microorganisms from these waste inputs can be present in drinking water, posing serious health risks to humans. Therefore, it is crucial to disinfect drinking water to prevent public health issues.

Chlorine is the primary disinfectant used for drinking water treatment in Cyprus because it is highly effective, affordable, and simple to apply. When added in appropriate amounts, chlorine can neutralize the majority of harmful microorganisms found in water and provides a lasting disinfectant effect that helps protect against further contamination within the water distribution network. However, its effectiveness can be reduced if microorganisms are shielded by attaching themselves to particles or other organisms, limiting chlorine contact. Moreover, certain pathogens such as *Giardia lamblia* and *Cryptosporidium parvum* show resistance to chlorine treatment. A significant drawback of using chlorine is the formation of disinfection by-products (DBPs), some of which are suspected to be carcinogenic. These DBPs, including trihalomethanes (THMs) and haloacetic acids (HAAs), are created when free chlorine interacts with natural organic materials in the water. The regulation and control of these disinfection by-products are overseen by the U.S. Environmental Protection Agency (EPA) through stringent rules.

There are several other important methods of disinfection, including UV irradiation, chloramination, and the use of multiple disinfectants. UV irradiation is a key physical method for disinfecting water and wastewater. It effectively eliminates enteric bacteria, viruses, bacterial spores, and parasite cysts without producing DBPs or other chemical residues (Oppenheimer et al., 1997; Lazarova et al., 1998). UV irradiation is particularly effective in inactivating Giardia lamblia and Cryptosporidium parvum, though it can be costly for large-scale plants. Chloramines, although weaker disinfectants than free chlorine, are often used as secondary disinfectants because they break down slowly, providing extended protection in distribution systems. This research aims to investigate the germicidal effects of UV irradiation and the combination of UV irradiation and chlorination on *E. coli*-contaminated drinking water, with the goal of generating data on the feasibility of applying a similar design at a larger scale in future treatment plants.

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MATERIALS AND METHOD

Sodium hypochlorite solution (NaOCl) was the reagent utilized for the chlorination process. UV lamp used for the irradiation water disinfection process was from Philip company (TUV16W-T54PSE). Ascorbic acid was gotten from Sigma-Aldrich company. Escherichia coli was obtained from the microbiology laboratory of Cyprus International University.

Preparative Work

Sterilization of glass wares

All glassware used in these experiments, including test tubes, petri dishes, dilution bottles, glass pipettes, forceps, and other heat-stable items, were sterilized in a hot-air oven (Memmert model 100-800) at 130°C or 140°C for 4 hours. Materials were either placed directly into the oven or wrapped in foil before exposure to heat, ensuring the oven lid was securely closed. Pipettes were sterilized in heat-resistant plastic bags or metal boxes, with each bag containing one size of pipette and the tips pointing in the same direction to protect them from contamination. All liquid solutions and diluents, including growth media and dilution water, were sterilized using an autoclave set to 121°C for 20 minutes.

E. coli Preparation

48 hours before the experiment, two sterile 250-mL Erlenmeyer culture flasks, each containing 50 mL of tryptic soy broth, were moved from the refrigerator to the incubator and maintained at 35°C overnight and 24 hours before the experiment, *E. coli* from the frozen stock culture was transferred to the culture flasks using a wire loop. The inoculated flasks were then placed on a rotating platform in the incubator at 35°C, shaking at a constant rate of 100 revolutions per minute (rpm). The *E. coli* cultures were allowed to grow in the incubator overnight for 16–18 hours. On the experimentation day, centrifugation was used to separate the *E. coli* from the broth. Sample was centrifuged at 3,650 rpm for 20 minutes at 4°C. After centrifugation, the broth in one tube was decanted, leaving an *E. coli* pellet at the bottom. This pellet was resuspended in a dilution bottle containing 25 mL of 0.01M CDF PBS. A slight volume of CDF PBS was added to the centrifuge tube and shaken until the pellet was fully dissolved. *E. coli* was added to the experimental water (CDF PBS) to achieve a starting concentration of 3 x 10^7 cfu/ml.

Experimental Design

The objective of this research was to analyse the effects of chlorination, UV irradiation and the combination of both disinfection process of UV irradiation followed by chlorination on artificially contaminated water samples. Figure 1. shows the UV irradiation chamber. The cylinder has two openings, an inlet and an outlet, for water circulation. It has a mechanical pump that controls the flow rate through the cylinder for desired contact time. Before the experiment proper, 650ml of tape water was pumped into the chamber through the right-hand inlet to rinse the chamber and let out through the left-hand outlet, and then 650ml distill was also pumped or introduced into the same process to rinse well to avoid contamination. Through the same process, 1000 ml *E. coli* contaminated water samples were pumped into the chamber for UV disinfection at 254nm and samples were collected at different contact times of 1,2,3,4,5,10,20,30,40,50 and 60sec.

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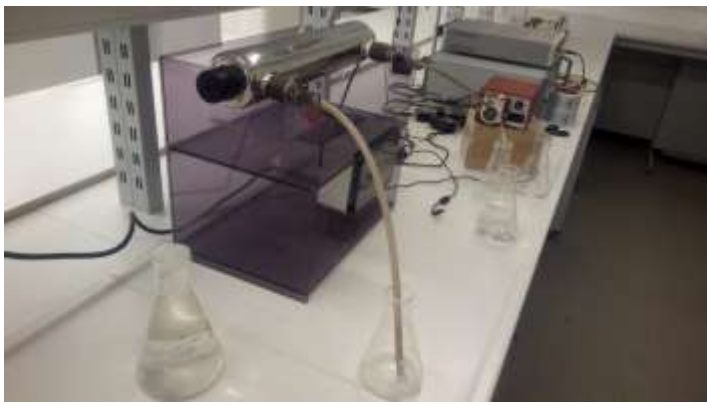
Figure 1. UV irradiation chamber.



Inactivation Experiment

Chlorination

NaOCl solution of varying concentration 0.5 to 3mg/l which is considered the standard used in communal treatment of water was utilized during the research in evaluating the deactivation of *E. coli.* Exposure time employed are; 1, 5, 10, 15, 30, 60, 120, 240, 480, 1440 min. Glass flask containing 100 ml water at 10^{o}C was utilized. 25 ml of Ascorbic acid was used the quench the amount of chlorine. There was an evaluation of free chlorine before and after the treatment and the pH of the water during the study was 7.0 all through.

UV Irradiation activity

To inactivate *E. coli* using UV irradiation, water samples were pumped into a 2-liter cylindrical chamber fitted with a UV lamp. The samples flowed through the chamber and were exposed to UV light before exiting through the outlet, with exposure time controlled by the flow rate. A power-driven pump regulated the water flow to achieve the desired UV exposure. Experiments were conducted at exposure times of 1, 2, 3, 4, 5, 10, 20, 30, 40, 50, and 60 seconds. The UV fluence ranged from 73.33 to 2239.5 mJ/cm^2, depending on the adjusted flow rate, and was calculated using the method described by Nourmoradi et al. (2012). Treated water samples were collected in sterile 50 mL test tubes from the chamber outlet for further analysis.

Joint Treatment

(UV irradiation and Chlorination)

To evaluate *E. coli* decontamination, experiments were conducted using combined UV irradiation and chlorination. UV treatment was applied first to prevent chlorine oxidation and the formation of harmful byproducts. UV exposure times were 1, 5, 10, 20, and 30 seconds. After UV treatment, samples were collected in glass flasks and treated with sodium hypochlorite. Chlorination was performed at chlorine concentrations of 0.5, 1, 2, and 3 mg/L with contact times of 1, 5, 10, 15, 30, and 60 minutes, as well as 24 hours. The chlorination reaction was stopped by adding 25 mg/L of ascorbic acid.

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Pour Plate Method

The pour plate method was conducted in accordance with Standard Methods 9215B (APHA et al., 2005). After preparing pre- and post-disinfection dilution series, 1 mL from each appropriate dilution was transferred into 100 mm Petri dishes. Duplicate plates were prepared for each dilution, along with one negative control. Approximately 10–12 mL of molten tryptic soy agar at 47°C was added to each plate and gently mixed for uniform distribution. Plates were allowed to solidify for 5 minutes, then incubated inverted at 35°C for 22–24 hours. Plates with 30–300 colonies were counted, and *E. coli* log reduction was calculated following disinfection.

Figure 2. Water sample before disinfection

RESULTS AND DISCUSSION

The findings present the individual and synergistic effects of chlorination, UV irradiation, and combined UV–chlorine treatment on *E. coli* deactivation.

Chlorination Effect

The effect of chlorination on *Escherichia coli* inactivation using sodium hypochlorite doses of 0.5, 1.0, 2.0, and 3.0 mg/L is presented in Figure 3. At 0.5 mg/l, a 3-log (99.9%) reduction was achieved after 120 minutes, reducing *E. coli* from 300 to 0 cfu/ml. At 1.0 mg/L, a 3-log reduction occurred after 60 minutes, with counts decreasing to 0.3 ± 0.6 CFU/mL and reaching 0 cfu/ml after 24 hours. A dose of 2.0 mg/l achieved a 3-log reduction within 10 minutes, with complete inactivation maintained throughout the 24-hour period. The highest dose, 3.0 mg/L, produced complete (*100%*) inactivation within 1 minute and remained effective for the duration of the experiment.

These results indicate that chlorine dosage has a greater influence on *E. coli* inactivation than contact time. Regression analysis showed no significant relationship between exposure time and inactivation at the 0.01 significance level, whereas chlorine dose had a statistically significant effect. Although 3.0 mg/L provided the most rapid inactivation, similar findings have been reported by Hamid Mohammad et al. (2013) using chlorine for the elimination of *Aspergillus flavus*.

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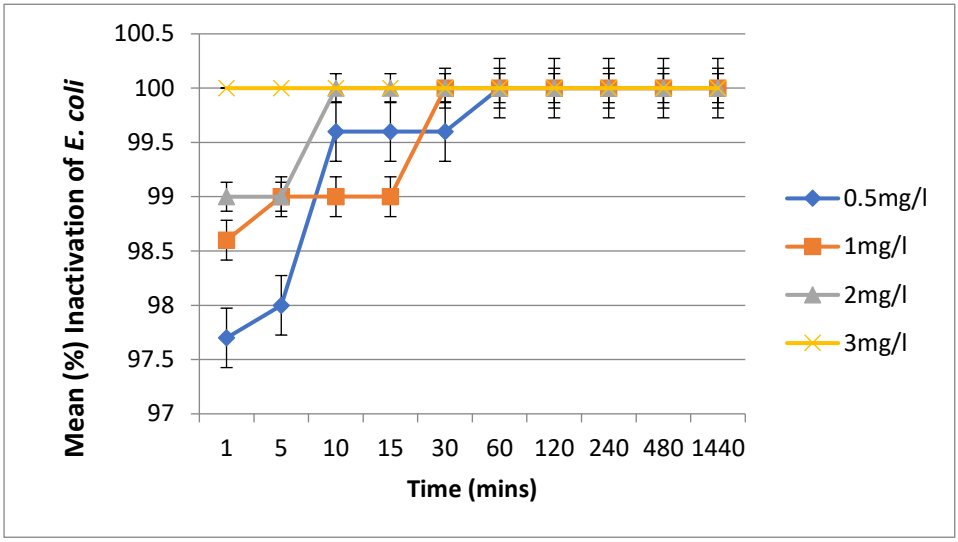
Figure 3. Effect of Chlorination in deactivation of *Escherichia coli.*



Evaluation of Contact Time Value

The CT value provides a clearer understanding of how chlorine concentration influences the inactivation of *Escherichia coli* in water. CT is determined by two key factors: chlorine concentration and the time required to achieve complete inactivation. Mathematically, the CT value is expressed as:

$$CT = Concentration \times Contact\ Time$$

Table 1: Contact time values for deactivation of *Escherichia coli* in drinking water using varying concentration NaOCl at 3 $\log^{10}$ (99.9%).

NaOCl conc. (mg/l)	Time (mins)	Ct rate
0.5	120	60
1	60	60
2	10	20
3	1	3

Statistical analysis showed a strong positive relationship between chlorine concentration and *E. coli* reduction ($R^2 = 0.947$). Chlorine doses of 2–3 mg/L were significantly more effective than 0.5–1 mg/L, with differences across all concentrations statistically significant at the 0.01 level. Microorganisms vary in their susceptibility to chlorination, with some being inactivated at lower chlorine concentrations than others (McDonnell & Russell, 1999). This variation is attributed to differences in sensitivity among microbial species, including fungi (Pereira et al., 2013).

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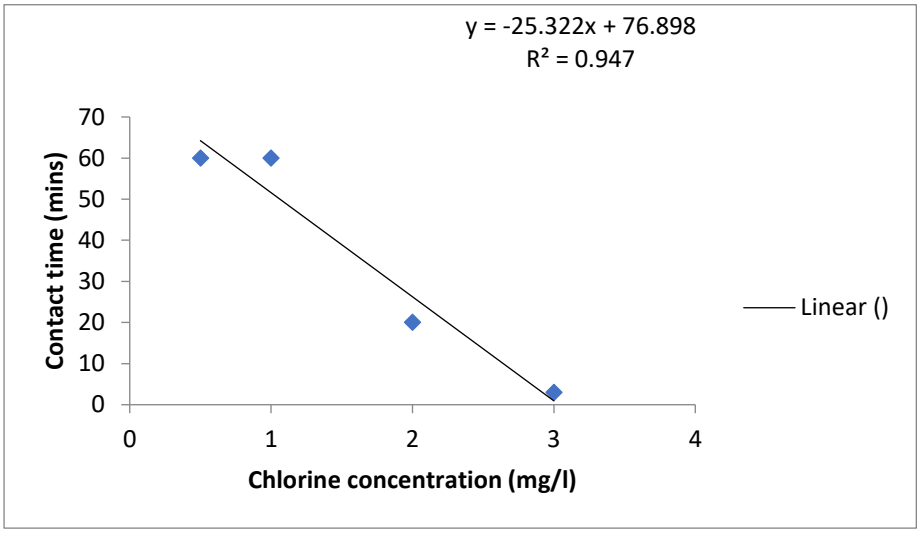
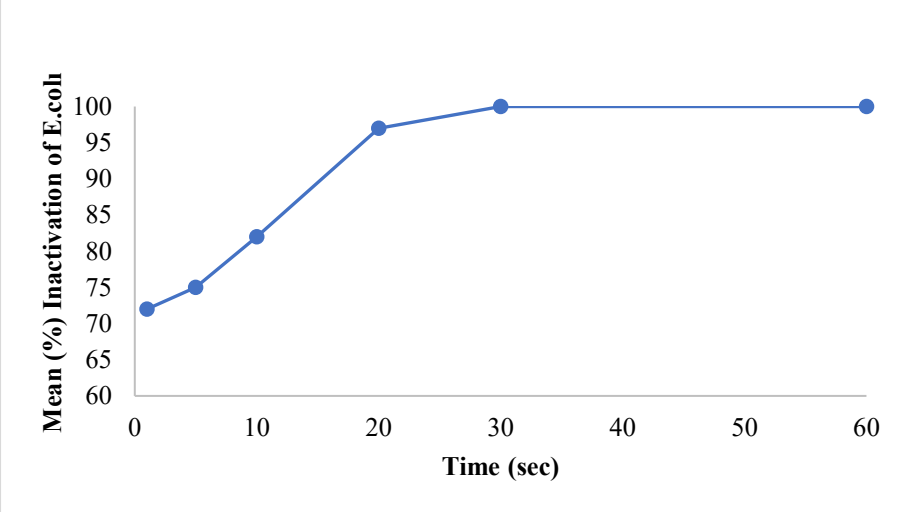
Figure 4. Correlation between chlorine concentration and inactivation of *E. coli.*



Effect of UV irradiation

A strong positive relationship was observed between UV irradiation time and the percentage of *E. coli* inactivation (Figure 5). *E. coli* counts decreased from 300 CFU/mL before treatment to 9 CFU/mL after 20 seconds of UV exposure, while complete (100%) inactivation was achieved at 30 seconds. These findings are consistent with Hamid Mohammad et al. (2013), who reported a 4-log reduction of *Aspergillus flavus* at a UV fluence of 20.75 mJ/cm² for an initial concentration of 1000 CFU/mL, despite using a higher microbial load than in the present study. Similarly, Labas et al. (2006) reported 99.99% inactivation of *Escherichia coli* using UV irradiation.

Figure 5. Inactivation of *E. coli* by UV irradiation at different exposure times



The results show relatively high *E. coli* counts after 1 and 5 seconds of UV exposure (71 and 75 CFU/mL, respectively), levels that are unacceptable for safe water quality. However, a 99% reduction was achieved after 20 seconds of irradiation, likely due to the higher resistance of *E. coli* to UV compared with *Aspergillus flavus* reported by Hamid Mohammad et al. (2013).

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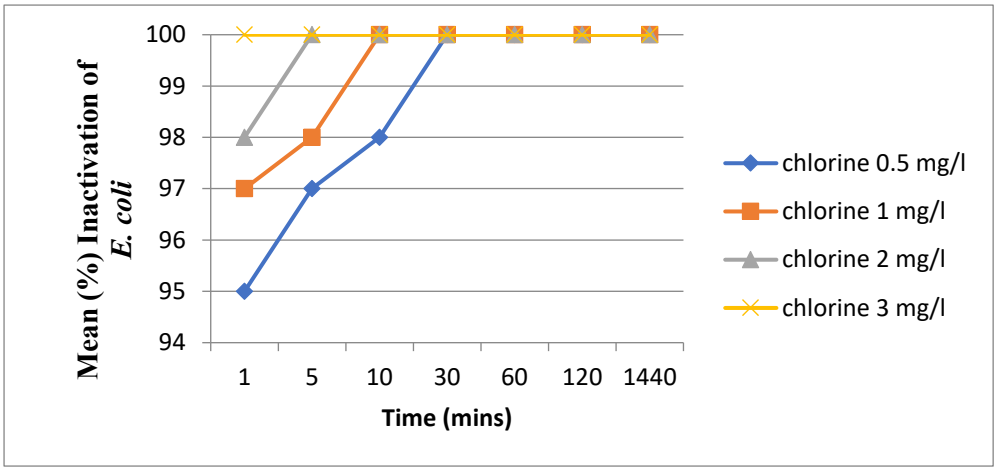
Residual *E. coli* detected before 30 seconds remains undesirable, as the organism can rapidly regrow as biofilm within water systems. *E. coli* is a known pathogen associated with serious human illnesses, including hemolytic uremic syndrome, particularly in children (Straub & Chandler, 2003).

Combined effect of UV irradiation and chlorination

Chlorination and UV irradiation when combined on the deactivation of *Escherichia coli* under room temperature as well as neutral pH demonstrated that UV irradiation of 5sec contact time followed with chlorination dosage of 0.5-3 mg/l showed a significant inactivation rate when compared with the treatment process of UV irradiation or chlorination alone (figure 6). It can be seen from the result that *E. coli* total (100%) inactivation was achieved after 30, 10, 5 and 1min on dosages of chlorine 0.5, 1, 2 and 3 mg/l, respectively; meaning as the concentration of chlorine increases, the time taken to achieve 100% inactivation of the bacteria reduces (contrary relationship amid treatment rate as well as deactivation bacteria).

Figure 6. *E. coli* reduction after 5-s UV irradiation and chlorination



The results revealed statistically significant differences ($P \leq 0.01$) in *E. coli* inactivation across various treatment stages involving UV irradiation (5, 10, 20, and 30 seconds) followed by chlorination at different doses (Figures 6–9). The most effective inactivation was achieved with 30 seconds of UV exposure, outperforming both 10- and 20-second treatments. Complete (100%) inactivation occurred after 10 seconds of UV irradiation followed by chlorination at 2 and 3 mg/L within 5 and 1 minute, respectively, while at 0.5 mg/L chlorine, full (4-log) inactivation required 30 minutes.

Overall, the findings indicate that longer UV exposure significantly reduces the chlorine contact time required for microbial inactivation, demonstrating the enhanced efficiency of the combined UV–chlorine treatment. This study confirms that UV irradiation increases chlorine effectiveness and shortens disinfection time compared with either treatment used alone. Similar trends have been reported for *Aspergillus flavus*, although fungal species exhibit greater resistance to chlorination than *E. coli* (Pereira et al., 2013). The results further suggest that UV irradiation followed by chlorination is a viable approach for complete *E. coli* removal from drinking water, with minimal risk of bacterial regrowth or biofilm formation in distribution systems. Comparable enhancements in disinfection efficiency have been reported for combined

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ozone–UV treatments (Sharrer & Summerfelt, 2007) and H_2O_2/UV processes in wastewater treatment (Koivunen & Heinonen-Tanski, 2005).

Figure 7. *E. coli* reduction after 10-s UV and chlorine treatment

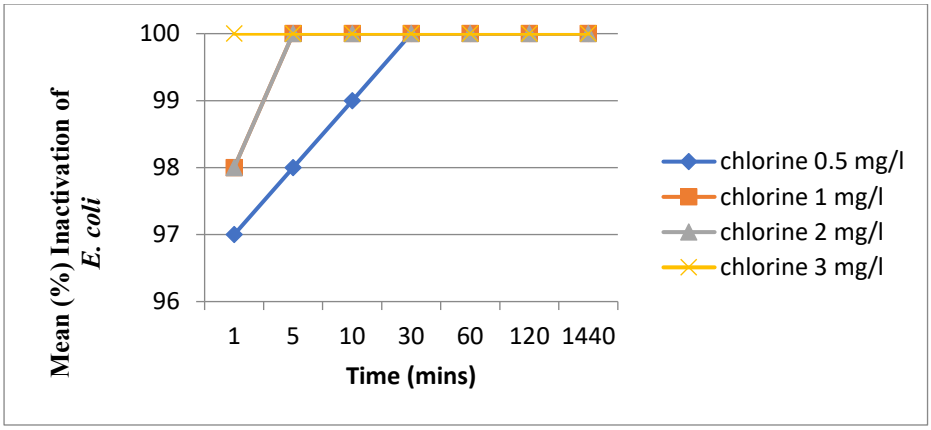


Figure 8. *E. coli* reduction after 20-s UV and chlorine treatment.

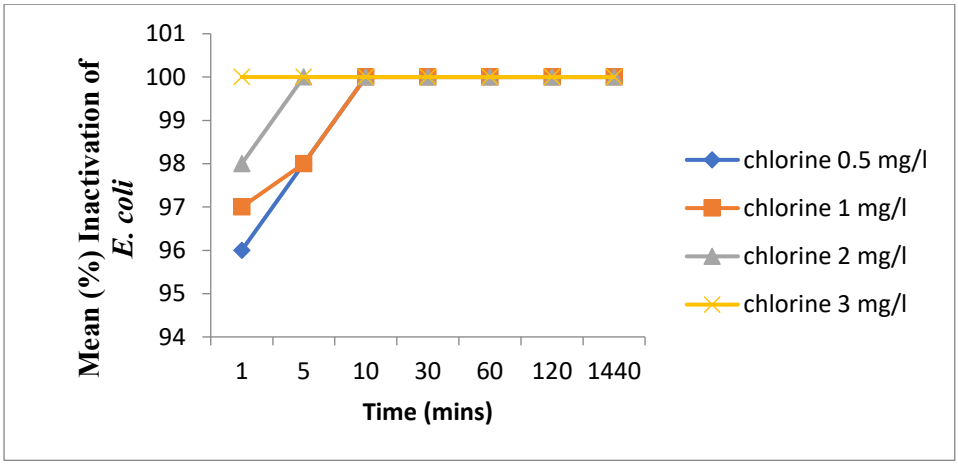
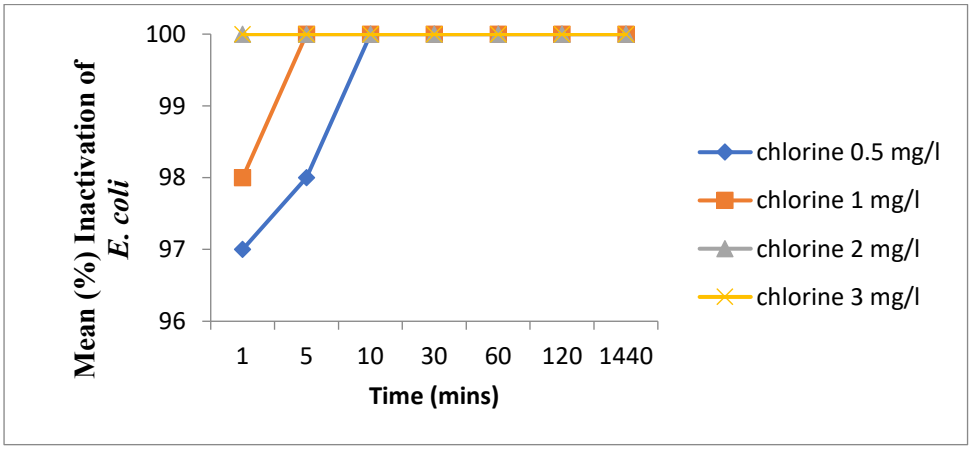


Figure 9. E. coli reduction after 30-s UV and chlorine treatment.



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CONCLUSIONS

This research was carried out to study the effects of various parameters on the disinfection abilities of chlorination, UV irradiation and the combined efficacy of both chlorination and UV irradiation. The parameters tested were, temperature, pH, volume and contact or exposure time. The disinfection procedure for E. coli in drinking water by utilizing UV irradiation gave great outcome as far as inactivating E. coli; in any case, the procedure stayed inadequate into the elimination of aggregate E. coli from the water, the few debris of E. coli could quickly grow back to form biofilms in the water supply system. Chlorine, as a disinfectant, seemed more viable than UV irradiation in lessening E. coli, additionally, demonstrating a positive connection between chlorine dosage and the log of E. coli decrease. Combination of UV irradiation followed by chlorination indicated significantly more compelling inactivation of E. coli than UV or chlorination alone; low dosage of chlorine and generally short contact time with UV could accomplish 4 log inactivation of E. coli. In this way, UV irradiation can enhance viability of chlorination (at low utilized rates) in decreasing E. coli, along these lines rendering more protected water. The combination of UV irradiation and chlorination in this manner, could decrease the availability of chlorine on the water supply system, so the chlorination DBPs won't be harmful to the consumer.

Finally, in analyzing the results from these experiments, the following conclusions were drawn:

- The Sodium hypochlorite, as a disinfectant shows more effectiveness than UV irradiation in inactivating *E. coli* and also it showed a positive correlation between sodium hyprochorite concentration and the log of *E. coli* reduction.
- The combined effect of UV irradiation followed by chlorination showed a more efficient inactivation of *E. coli* than UV irradiation alone; Sodium hypochlorite alone and the relative short contact time with UV could achieve a rather more efficiently 100% inactivation of *E. coli*.
- However, the UV irradiation shows here that it enhances the efficiency of chlorination in reducing *E. coli* for drinking water to be safe.
- The combined effect of both UV irradiation and chlorination reduces or decreases the dose of chlorine used in the disinfection process, thereby reducing or totally eliminating the production of DBP in the process which is beneficial to consumers.
- Temperature and pH do not have any significant effects on the inactivation of *E. coli* rather Concentration and contact time.

LIMITATIONS AND PROSPECT

Several more research work is needed to be worked on if the synergistic effect of chlorination and UV irradiation is to be seen as a better way of water treatment today and in the future. Additionally, the limited time and resource available for this study is not feasible for completion aside the data presented here. The following materials should be improved for subsequent research work:

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- A UV irradiation system should be designed that allows flexibility and better control of experimental parameters; that is, UV-reactor with significant worry to wavelength (adjustable wavelengths), dosage, contact time and power supplied and in addition checking power dispersion in the chamber keeping in mind the end goal to help its exploratory proficiency for drinking water.

- Removable components which contact experimental water. Specifically, any part of the apparatus which will contact experimental water should be chlorine free and autoclaved; that is, the UV lamp/bulb in the chamber and the chamber itself which comes in contact with the water sample should be made to be sterilized with chlorine before use or rather autoclaved, rather that rinsing with water and also PBS before use.

- *E. coli* is a typical test organism and inactivation comes about relate to the impacts of Chlorination and UV Irradiation disinfection on different organism, for example, pathogenic microscopic organisms. Nonetheless, it is prescribed that both UV irradiation and chlorination disinfection tests be directed on organisms other than bacteria alone. For instance, studies are lacking on the effects of UV irradiation disinfection on spore-forming organisms and protozoa.

- The power required to run the UV irradiation machine ought to be checked constantly with a specific end goal to completely assess the monetary attainability of utilizing an UV irradiation system to accomplish synergistic sterilization.

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