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Is Not It Time to Stop Using Chlorine for Treating Water?

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Abstract

Chlorine is largely used as a disinfectant in the water and wastewater treatment industries through the world despite the fact that is greatly poisonous for human beings. Its toxicity is more extended to generating disinfection by-products during its microorganisms' killing and action on organic matter present in water. More importantly, recent studies proved the potential impacts of disinfection on transmission of antibiotic resistance genes (ARGs), particularly for free-living ARGs in final disinfected effluent of urban wastewater treatment plants. Indeed, *Escherichia coli* concentration prior to chlorination depicted a powerful positive correlation with the extracellular ARGs plenty in the final effluents; however, lower temperature and higher ammonium concentration were suggested to relate with intracellular ARGs. Chlorination could elevate the plenty of ARGs, therefore, inducing danger of the diffusion of antibiotic resistance in nature. Consequently, chlorine toxicity is more and more proved, which appeals its urgent stopping from using it in the treatment of both water and wastewater. The same conclusion was also obtained at least for UV and UV/H_2O_2 disinfection. Chemical disinfection should be urgently avoided or at least deeply revised. For removing pathogens and treating water, safe multi-barrier methods, such as distillation and membrane processes, have to be adopted.

Subject Areas

Environmental Sciences, Hydrology

Keywords

Antibiotic-Resistant Bacteria (ARB), Antibiotic Resistance Genes (ARGs), Wastewater Treatment, Disinfection, Oxidation, *Escherichia coli*

1. Introduction

Antibiotic resistance has caused massive dangers to public health globally [1]. Every year, more than 700,000 persons die from antimicrobial-resistant infections worldwide; nevertheless, the envisaged disaster number risks to attain 10 million by 2050 if no measures are made immediately [2]. Even if the diffusion of antibiotic resistance in clinic environments has appealed considerable worries, the appearance and propagation of antibiotic resistance bacteria (ARB) or antibiotic resistance genes (ARGs) in natural environments have been disregarded yet [3].

Urban wastewater treatment plants (UWWTPs) remain at the interface joining the human population and the aquatic/soil mediums [4]-[10]. They can supply an exemplary environment for the procuration and dispersal of antibiotic resistance [11] [12] [13]. Due to an assorted blend of antibiotics and different contaminants, their metabolites and resistant bacteria attain UWWTPs via wastewater discharges from hospitals, households, industries, and animal farms [14]. The spread of ARB and ARGs in UWWTPs has caught more and more awareness lately [15] [16] [17] [18]. Especially, biological reactors [19] [20] [21] in activated sludge setups are considered as one of the serious boxes in nursing both ARB and ARGs [22] [23], via picking below subjection of antibiotic or resistance transfer between bacteria through horizontal gene transfer (HGT) [24] [25]. In particular, it has been mentioned that UWWTPs display restricted potential to eliminate or restrain ARGs and ARB [17] [26] [27]. More importantly, not only does the effluent constitute a huge tank comprising diverse ARGs, but also the plenties of several exemplary ARGs are surprisingly higher in effluent than that in influent through the treatment techniques, such as *blaCTX-M*, *bla-TEM* and *qnrS* [28] [29]. As a result, UWWTPs have been established to function a key part in the transmission of ARB and ARGs in nature, therefore causing hazards to public health [1].

To dominate the prevalence of microorganisms, different disinfection techniques have been largely employed in UWWTPs. Several investigations have studied the demobilization of ARGs via disinfection technologies like chlorination and ultraviolet (UV) irradiation [30] [31] [32] [33]. Nevertheless, not many new investigations mentioned that chlorination, as a largely utilized disinfection method, might co-select antibiotic resistance. As an illustration, Shi *et al.* [29] affirmed that the chlorination method augmented the plenties of *ampC* and *tetA* following metagenomic sequencing. Further, Xu *et al.* [34] published that chlorination elevated the comparative plenty of ARGs from 6.4- to 109.2-fold in tap water contrasted to the final water. On the other hand, Lin *et al.* [35] proposed that ARGs were more possibly eliminated rather than co-selected via chlorine, where they monitored 225 ARGs; however, only six were elevated following chlorination. Even if paradoxical deductions were gained concerning the effect of chlorination on ARGs, a small number of researches have been devoted to discerning among intracellular ARGs (iARGs) and extracellular ARG (eARGs).

Indeed, disinfection techniques (like chlorination) may destroy ARB; however, at the same time, DNA will be liberated into the water, where eARGs may remain existing in the free-living DNA. The presence of iARGs can assist ARB dispersal through conjugation and transduction; however, the competent non-resistant bacteria in the biofilm and sedimentation can take up eARGs stability in the aquatic medium for quite a time, that way conducting to the diffusion of antibiotic resistance through transformation [36] [37] [38]. Consequently, eARGs in the effluent from UWWPTs are also possible to raise ARGs and ARB transmission in environmental mediums. Nevertheless, the influence of chlorination on eARGs stays ambiguous because of the shortage of an efficacious procedure to extract extracellular DNA (eDNA), which is too small to collect in treated water. Lately, Wang *et al.* [38] presented a procedure to collect eDNA via employing a novel kind of nucleic acid adsorption particle (NAAP) with an elevated potential, which may attain an eARG recuperation average of more than 95% from 10 L of water samples. This advanced procedure may let us examine the effect of disinfection on eARGs [1].

2. Chlorination Augments Both iARGs and eARGs

Liu *et al.* [1] examined the impacts of chlorination on the presence and concentration of both eARGs and iARGs in a full-scale UWWTP during twelve months. They indicated that the concentrations of both eARGs and iARGs could be elevated via disinfecting with chlorine dioxide (ClO_2). More importantly, chlorination preferentially augmented the plenties of eARGs versus macrolide (*ermB*), tetracycline (*tetA*, *tetB* and *tetC*), sulfonamide (*sul*1, *sul*2 and *sul*3), *β*-lactam (*ampC*), aminoglycosides (*aph(*2'*)*-Id), rifampicin (*katG*) and vancomycin (*vanA*) up to 3.8 folds. In the same way, the plenties of iARGs were also elevated up to 7.8 folds following chlorination. In terms of correlation analyses, the plenty of *Escherichia coli* prior chlorination depicted a powerful positive correlation with the total eARG concentration; however, lower temperature and higher ammonium concentration were supposed to be linked with the concentration of iARGs. Liu *et al.* [1] concluded the chlorination could elevate the plenties of both iARGs and eARGs, just like that causing danger of the diffusion of antibiotic resistance in nature.

3. Microbial Selectivity of UV Treatment on Antibiotic-Resistant Bacteria

To comprehend the influence of UV disinfection on antibiotic-resistant bacteria, Guo *et al.* [39] investigated both total heterotrophic bacteria, and antibiotic-resistant bacteria (comprising cephalexin-, ciprofloxacin-, erythromycin-, gentamicin-, vancomycin-, sulfadiazine-, rifampicin-, tetracycline- and chloramphenicol-resistant bacteria) in secondary effluent samples from a domestic wastewater treatment plant (**Figure 1**). Bacteria resistant to both erythromycin and tetracycline were selected as the specimen of multiple-antibiotic-resistant

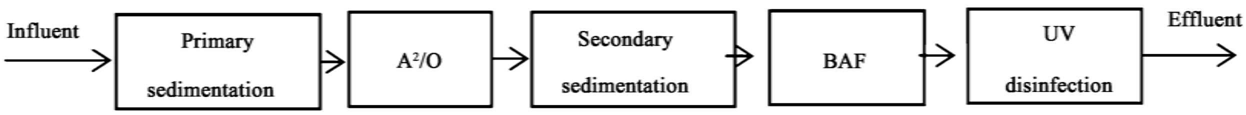

Figure 1. Treatment process of the municipal wastewater treatment plant (A^2/O: Anaerobic-Anoxic-Oxicprocess; BAF: Biological Aerated Filter) [39].

bacteria and their properties following UV application were also examined. They found that UV disinfection conducts to performant demobilization of total heterotrophic bacteria, as well as all antibiotic-resistant bacteria. Following UV application at a fluence of 5 mJ/cm^2, the log decreases of nine types of antibiotic-resistant bacteria changed from 1.0 ± 0.1 to 2.4 ± 0.1. Bacteria resistant to both erythromycin and tetracycline had an identical fluence reaction as did total heterotrophic bacteria. Their results propose that UV disinfection could remove antibiotic resistance in wastewater treatment effluents and therefore guarantee public health security. Guo *et al.* [39] mentioned that UV disinfection conducted to the enrichment of bacteria with resistance to sulfadiazine, vancomycin, rifampicin, tetracycline and chloramphenicol; however, the fractions of cephalexin-, erythromycin-, gentamicin- and ciprofloxacin-resistant bacteria in the wastewater diminished. This illustrates the microbial selectivity of UV disinfection for antibiotic-resistant bacteria.

4. Antibiotic Resistance Diffusion Capacity by UV/H_2O_2 Technique

Ferro *et al.* [40] assessed the influence of an advanced oxidation process (particularly UV/H_2O_2) [41]-[46] on antibiotic resistance diffusion capacity. They performed UV/H_2O_2 disinfection trials on real wastewater samples to estimate the: 1) demobilization of Total Coliforms, *E. coli*, and antibiotic-resistant *E. coli* as well as 2) probable elimination of objective ARGs (that is, bla_{TEM}, *qnr*S and *tet*W) (**Figure 2**). Especially, DNA was extracted from both antibiotic-resistant *E. coli* bacterial cells (intracellular DNA), grown on selective culture media, and the whole water suspension (total DNA) collected at various treatment periods. Polymerase chain reaction (PCR) check was realized to discover the absence/presence of the selected ARGs. Real Time quantitative Polymerase Chain Reaction (qPCR) was employed to measure the studied ARGs in terms of copies/mL. Regardless of the bacterial demobilization and a diminution of ARGs in intracellular DNA following 60 min treatment, UV/H_2O_2 method was not performant in eliminating ARGs from water suspension (total DNA). More importantly, an augmentation up to 3.7×10^3 copies/mL (p N 0.05) of bla_{TEM} gene was noted in total DNA following 240 min treatment; however, no variation ($p > 0.05$) was observed for *qnr*S gene among the initial (5.1×10^4 copies/mL) and the final sample (4.3×10^4 copies/mL) (**Figure 3**). Following their findings, Ferro *et al.* [40] concluded that the examined disinfection technique might not be able to diminish antibiotic resistance diffusion capacity.

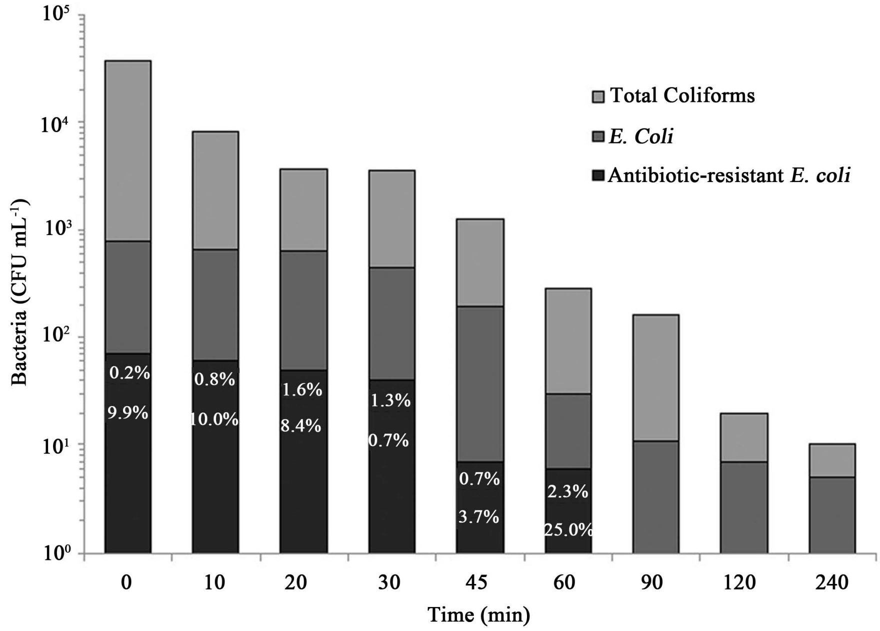

Figure 2. Relative abundance of bacterial population during UV/H_2O_2 treatment and AntibioticResistant *E. coli* percentages calculatedwith respect to total coliforms (upper placed values) and *E. coli* [40].

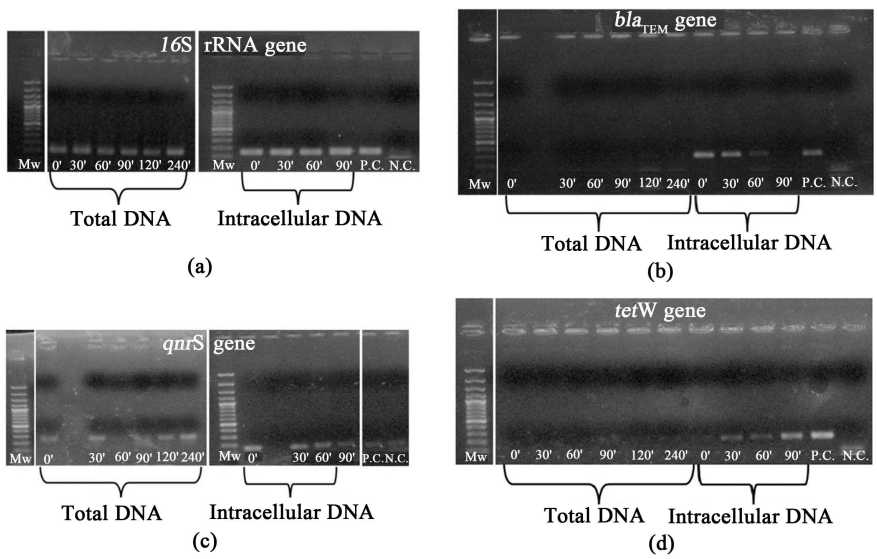

Figure 3. Results of electrophoretic run of PCR product on gel agarose: 16S rRNA gene (a); bla_{TEM} gene (b); *qnr*S gene (c); *tet* Wgene (d). P.C. = positive control; N.C. = negative control [40].

5. Electrodis Infection of Bacteria

Electrodisinfection has been illustrated to be an efficacious technique with low needed residence period for treating potable water supplies, industrial raw water supplies, liquid foodstuffs, and wastewater effluents [47]-[52]. Ghasemian *et al.* [53] explored the electrodis infection of saline water polluted with bacteria in chloride-containing solutions employing Sb-doped $Sn_{80\%}$-$W_{20\%}$-oxide anodes.

They focused on the impact of current density, bacterial load, initial chloride concentration, solution pH, and the kind of bacteria (*E. coli* D21, *E. coli* O157: H7, and *E. faecalis*) on disinfection performance. They tested the influence of the natural organic matter [54]-[60] and a radical scavenger on the disinfection efficiency. They found that their electrochemical setup was greatly efficient in demobilizing bacteria for a 0.1 M NaCl solution polluted with ~10^7 CFU/mL bacteria via implementing a current density ≥ 1 mA/cm^2 over the cell. They attained 100% demobilization of *E. coli* D21 during less than 60 s and power consumption of 48 Wh/m^3, via implementing a current density of 6 mA/cm^2 in a 0.1 M NaCl solution polluted with ~10^7 CFU/mL. Reactive chlorine species as well as reactive oxygen species (such as hydroxyl radicals) [61], formed *in situ* throughout the electrochemical technology, were discovered to be in charge of demobilizing bacteria.

6. Conclusions

The main points drawn from this work may be given as:

1) Chlorination augmented both eARGs and iARGs contamination in a full-scale UWWTP. *E. coli* concentration prior to chlorination depicted a powerful positive correlation with the eARGs plenty in the final effluents; however, lower temperature and higher ammonium concentration were suggested to relate with iARGs. Chlorination could elevate the plenty of ARGs, therefore inducing danger of the diffusion of antibiotic resistance in nature [1].

2) As the UV fluence used in actual WWTPs for disinfecting wastewater is usually less than the design parameters, regarding the life span and running circumstances of UV lamps, the potential hazard augmented by ARB following implementations of low fluences still needs awareness. For this reason, ARB removal researches of UV disinfection or different disinfection techniques in WWTPs are necessitated [39].

3) Consequently, chlorine toxicity is more and more established. This implicates its urgent stopping from using it in the treatment of both water and wastewater [62]. Chemical disinfection should be deeply avoided or at least revised [63]-[68]. For removing pathogens and treating water, safe multi-barrier processes, such as distillation and membrane processes [69]-[78], have to be adopted for the best future of humankind [79] [80] [81].

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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