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Comparison of different disinfection processes in the effective removal of antibiotic-resistant bacteria and genes

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ABSTRACT

This study compared three different disinfection processes (chlorination, E-beam, and ozone) and the efficacy of three oxidants (H_2O_2, $S_2O_8^-$, and peroxymonosulfate (MPS)) in removing antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in a synthetic wastewater. More than 30 mg/L of chlorine was needed to remove over 90% of ARB and ARG. For the E-beam method, only 1 dose (kGy) was needed to remove ARB and ARG, and ozone could reduce ARB and ARG by more than 90% even at 3 mg/L ozone concentration. In the ozone process, CT values (concentration × time) were compared for ozone alone and combined with different catalysts based on the 2-log removal of ARB and ARG. Ozone treatment yielded a value of 31 and 33 (mg·min)/L for ARB and ARGs respectively. On the other hand, ozone with persulfate yielded 15.9 and 18.5 (mg·min)/L while ozone with monopersulfate yielded a value of 12 and 14.5 (mg·min)/L. This implies that the addition of these catalysts significantly reduces the contact time to achieve a 2-log removal, thus enhancing the process in terms of its kinetics.

Introduction

Antibiotics are currently considered to be one of the emerging micro-pollutants that need attention in treatment. They are continuously being used in various applications such as in the livestock industry. Low dosages of antibiotics have been used in this industry to promote growth and to improve other properties to produce better livestock (Gustafson and Bowen, 1997). Antibiotics' ability to lower costs also increases their practicality. It is a common practice to incorporate the antibiotics in livestock feed. Due to these practices, it has been found that certain wastewater, especially that coming from a livestock operation, contains antibiotics at low mg/L levels (Kemper, 2008).

Treatment of these emerging pollutants has been the focus of much research due to its potential effect on the environment if left untreated. One effect is in the propagation of antibiotic resistance among organisms. Propagation of this resistance to pathogens would be an alarming issue, especially for public health and antibiotic research, making it harder to treat diseases and more difficult to formulate stronger pharmaceuticals (Baquero et al., 2008). The treatment of antibiotic resistance depends on the effective disinfection of antibiotic-resistant bacteria (ARB), and antibiotic resistance genes (ARGs) (Russel, 2003).

Chlorination is one of the most universal methods of disinfection (Bekink et al., 2013). It has been well known to avoid the spread of various waterborne diseases in treated water. Over the course of time, the manner of utilizing chlorine in disinfection treatment has changed due to certain factors such as safety and cost. This improvement involved the use of hypochlorite instead of gaseous chlorine. Although less potent, it showed more stability and safety than its gaseous form.

One of the typical treatment methods in bacterial disin-

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fection of wastewater is ozonation (Rice, 1996). Ozone is a strong oxidizing agent which has high effectivity in killing bacteria and removing other organic compounds. With the aim of improving efficacy, other studies have attempted to incorporate other methods. Some studies have looked into the increased disinfection effectivity of the combination of ozone and hydrogen peroxide (Sommer et al., 2004). Persulfate ($S_2O_8^-$) is a chemical that can produce a strong oxidizing agent, sulfate ion radical ($SO_4^{-\bullet}$). The ion can be thermally or chemically activated to produce this oxidant, which has a redox potential of 2.6 V and can degrade vast numbers of organic contaminants (Liang et al., 2003). On the other hand, peroxymonosulfate (MPS) ion is also an oxidant that is an analogue of H_2O_2. Previous works have shown that MPS can be more reactive than H_2O_2 both in oxidation potential and kinetics (Kotronarou and Hoffmann, 1991).

With the advent of emerging pollutants at low mg/L levels, it is a must to improve treatment processes. Very few studies have looked into the treatment of oxidants paired with ozone to disinfect ARBs and degrade ARGs. Recent studies have presented probable mechanisms of reaction with ozone, persulfate, peroxide, and peroxymonosulfate (Huang et Al., 2002):

$$S_2O_8^{2-} + 2e^- \rightarrow 2SO_4^{2-} \quad (1)$$

$$O_{3(g)} + 2H^+ + 2e^- = O_{2(g)} + H_2O \quad (2)$$

$$H_2O_2 + 2H^+ + 2e^- = 2H_2O \quad (3)$$

$$HSO_5^- \longrightarrow HO\bullet + SO_4^{-\bullet} \quad (4)$$

In the last decade, several studies have used electron beam (E-beam) as an alternative advanced oxidation process to degrade pollutants (Cho, 2010; Chung et al., 2008). E-beam irradiation is an established sterilization method to break the DNA chains in microorganisms, resulting in microbial death. Accordingly, this sterilization technique is applied in various industries such as food processing (Hong et al., 2008), medical devices (Matthews, 1994), or wastewater treatment (Farooq et al., 1993). However, little study has been conducted on the removal characteristics of ARB and ARGs using E-beam irradiation technology. In particular, the investigation of ARG transfer rate changes as a function of E-beam irradiation intensity has not been previously studied as far as the authors know.

The aim of this study therefore was to compare (1) three different disinfect processes (chlorination, ozone, and E-beam) and (2) the efficacy of three oxidants (H_2O_2, $S_2O_8^{2-}$, and MPS) with the ozonation process in removing ARB and ARGs in synthetic wastewater.

1 Materials and methods

1.1 Bacterial culture

In this study, *E. coli* DH5α, containing a multi-resistance gene (pB10), which enables the bacteria to be resistant to different antibiotics, was used. Cultures were grown in lysogeny broth (LB) and stored in an incubated shaker at 20°C and 150 r/min to an OD value of 1.3 before being used in the downstream process.

1.2 Chlorine disinfection

Sodium hypochlorite stock solution (NaOCL) was used for chlorination disinfection. Appropriate amounts of the stock solution were added to the *E. coli* DH5α cultures in phosphate buffer solution to obtain various final concentrations of chlorine (Cl_2) (0, 3, 6, 7.5, 10, 20, 30 mg/L). The chlorine contact time was fixed at 15 min.

1.3 Ozone process

Figure 1 shows the experimental setup. Ozone was generated from pure oxygen (99.9%) using an ozone generator (LAB 2B, Ozonia, Korea). The flow rate of pure oxygen to the ozone generator was maintained at 4–5 L/min. The ozone-oxygen mixture was introduced at a constant rate at the reactor bottom via a porous gas diffuser. Varying concentrations of ozone gas (0, 3, 5, 7, 10 mg/L) were continuously introduced and measured by an ozone analyzer (Orbisphere model 3600, Switzerland). When the ozone concentration in the reactor was saturated (after 60 min, 3 mg/L of ozone concentration), the sample (*E. coli*) and different concentrations (1, 5, 10, and 15 mg/L) of catalysts (hydrogen peroxide, potassium persulfate, monopersulfate) were then injected into the reactor. Samples were then taken at different intervals (1, 3, 5, 10, and 15 min) for analysis.

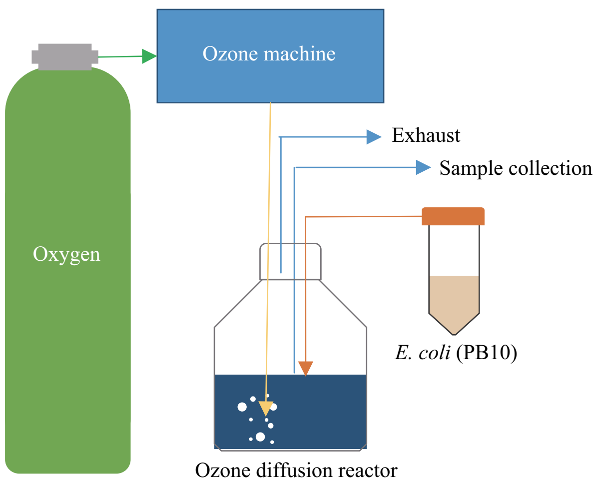

Fig. 1 Ozone generation system.

1.4 E-beam

An ELV-8 model electron accelerator was used for E-beam irradiation (EB-tech, Korea). An accelerated E-beam, electrons from a cathode of an electron gun placed in a vacuum accelerator and accelerated by high voltage, was irradiated through a window using a thin metal box. The E-beam energy used was 2.5 MeV with total absorbed doses of 0.5, 1, 2.5, 5, 10, and 25 kGy. The radiation doses were applied to triplicate samples with the doses controlled using conveyor speeds of 10 m/min. The absorbed dose was measured using a cellulose triacetate dosimeter. To minimize the variation in the disinfection effect of the samples, centrifuged *E. coli* DH5α cultures were re-suspended in phosphate buffer solution and then packed into a Whirl Pak with nitrogen purging to remove oxygen.

1.5 ARB evaluation

Samples were checked for bacterial colonies by a culture-based technique. Selection plates were prepared using LB and agar solution, then dosed with tetracycline to a concentration of 2 mg/L. Samples were then serially diluted with phosphate buffer (0.63 mmol/L) to achieve an approximate range of 30 to 300 colonies on the plate. A total of 0.1 mL of diluted sample was then placed into the agar plates in triplicate and then incubated at 37°C. After 16 hr of incubation, plate colonies were then counted and calculated based on number of dilutions.

1.6 ARG DNA evaluation

The pB10 plasmid after disinfection was evaluated by quantitative PCR (q-PCR) using an Eco Real-Time PCR System (Illumina, SD, USA). Plasmid DNA from the pB10-containing *E. coli* DH5α was isolated using a Nucleobond Kit PC100 on AX 100 columns (Macherey-Nagel), according to the manufacturer's supplied protocol. PCR was performed using a highly specific primer set (F5′-CAATACCGAAGAAAGCATGCG-3′, R5′-AGATATGGGTATAGAACAGCCGTCC-3′). The q-PCR conditions were similar to those employed in a previous study (Bonot, 2010). The concentration and purity of the DNA extracted was evaluated by ultraviolet absorbance spectrophotometry at 260 nm.

2 Results and discussion

Disinfection efficacy was evaluated by examining both ARB and ARG survival. A comparison of the three different disinfection techniques is shown in **Fig. 2**. For chlorine disinfection, more than 30 mg/L of chlorine was needed to remove over 90% of ARB and ARG. Ozone could reduce ARB and ARG by more than 90% using 3 mg/L ozone concentration, and E-beam required 0.5 kGy to disinfect ARB and 1 kGy to disinfect ARG. Concentration of 30 mg/L of chlorine is impractical in wastewater treatment (the typical concentration in Korean wastewater treatment plants are 6–15 mg/L). However, the applied doses of ozone and E-beam are within the typical range of doses of ozone (3–4 mg/L) and E-beam (1–2 kGy) in wastewater treatment processes (Metcalf and Eddy Inc., 2003). Therefore, ozone and E-beam are more effective ways to control antibiotic resistance compared to chlorination. However, E-beam treatment requires high energy and safety although it is a promising technology. Accordingly, an ozone process can be a more practical disinfection process for controlling antibiotic resistance.

With the aim of improving the disinfection capacity of ozonation for antibiotic resistance, the use of three different catalysts were also done in this study. Comparison of both ARB and ARG removal rates, at catalyst concentration of 1 mg/L, are presented in **Fig. 3**. ARB and ARG removal were monitored in separate experiments. As a result, most ARB and ARG were removed by 2-log within 10 min. In comparison to the disinfection of using ozone alone, processes with added catalysts showed better performance in removing both ARB and ARGs. Among the additives, MPS showed highest disinfection efficacy. Several previous works showed the enhancing effect of hydrogen peroxide when added to ozone. It aids by increasing the concentration of hydroxyl radical, which could prove to be more a potent oxidant compared to ozone. Persulfate ion is a weak oxidant, but heat or chemical activation can produce a strong oxidant, sulfate radical ion. The redox potential of persulfate, including activation, has been presented in previous studies (Huang et al., 2002) and was shown to be higher than that of

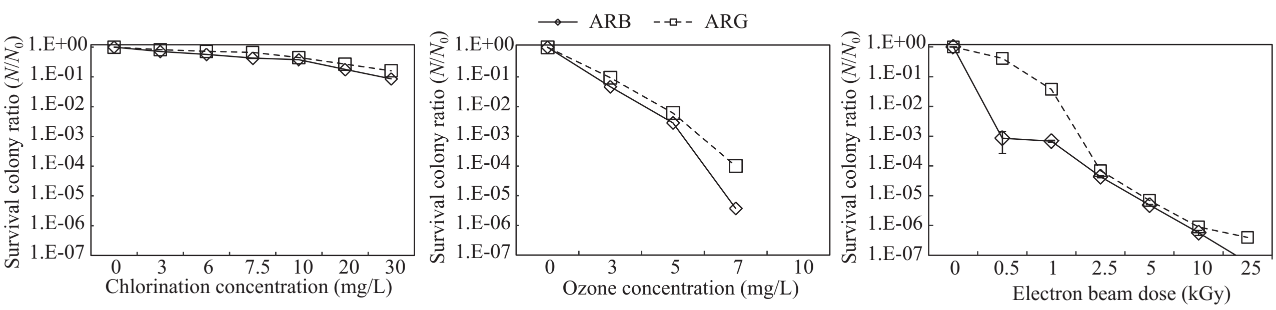

Fig. 2 Survival rate of antibiotic-resistant (ARB) and antibiotic resistance genes (ARGs) in different disinfection processes.

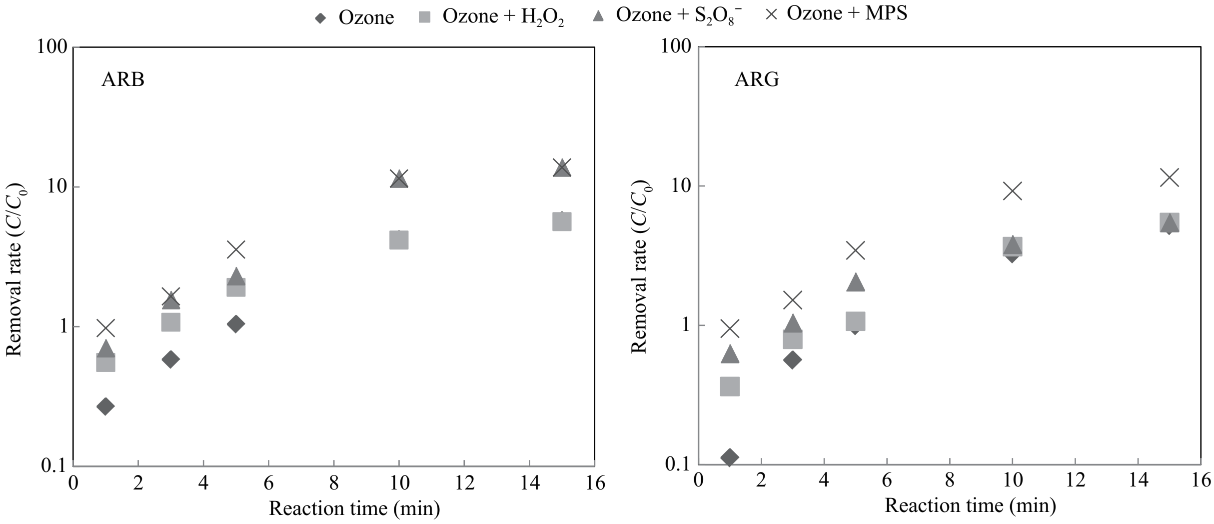

Fig. 3 Comparison of the ARB and ARG removal rates in different setups of ozonation experiments. Catalyst concentration: mg/L.

hydrogen peroxide. As for the case of MPS, since it is considered monosubstituted peroxide, part of its oxidative mechanism is similar to that of hydrogen peroxide. Also, it has a mechanism similar to that of the activated persulfate due to the monosulfate present.

MPS could be more potent than the persulfate, since the sulfate radical ion may be more readily available as compared to the case with the activation of persulfate. Though activation is required for persulfate, the data showed the combination of persulfate with ozone to be better than plain ozone and ozone with hydrogen peroxide, possibly because the activation energy may come from the high energy release from the ozone reactions.

CT (concentration × time) values for ozone alone and in combination with the different catalysts were compared based on the 2-log removal of ARB and ARG. Ozone yielded a value of 31 and 33 (mg·min)/L for ARB and ARGs respectively. On the other hand, ozone with persulfate yielded 15.87 and 18.47 (mg·min)/L while ozone with MPS yielded a value of 11.97 and 14.49 (mg·min)/L, and ozone with hydrogen peroxide yielded a value of 29.94 and 33 (mg·min)/L. This implies that the addition of these catalysts significantly reduces the contact time needed to achieve 2-log removal, thus enhancing the kinetics of the process.

Interactions between ozone and hydrogen peroxide have been discussed in previous studies (Glaze et al., 1987; Acero et al., 2001). However, the chemical interaction of ozone with persulfate and ozone with MPS has not received much attention. The ozone reaction possibly aids the activation of persulfate in producing the sulfate radical ion, thus significantly increasing its efficacy. On the other hand, ozone interaction with MPS may be similar to that with hydrogen peroxide, with the addition of a sulfate radical ion, which may be readily available upon addition (Huang et al., 2002).

The differences between the attributes of the MPS and the persulfate showed the higher potential of the hydroxyl and sulfate radical to inactivate antibiotic resistant bacteria. Due to this higher potential, the oxidants can penetrate more into the cells of the bacteria, thus becoming more effective in removing even in the presence of antibiotic resistance genes.

3 Conclusions

With the emergence of ARB and ARGs, there is a need for more powerful disinfection techniques. This study has compared several disinfection techniques including chlorination, E-beam and ozone for better ARB and ARG removal. In further study of ozonation, several catalysts were tested. It was found that it is possible to use persulfate and MPS as substitutes for hydrogen peroxide. These compounds increased the effectiveness of ozone in disinfecting ARB and ARGs. This experiment may also suggest the possible contribution of ozone in activating persulfate, making a strong oxidant. Further study of advanced oxidation processes is needed for the control of these emerging contaminants, ARGs, in the environment.

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