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A Review of Municipal Wastewater Disinfection using Advanced Oxidation Processes

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Summary: Microbial pathogenic microorganisms in wastewater have globally presented a substantial public health concern. The mainly applied conventional disinfection techniques are usually not able to achieve complete disinfection of bacteria in municipal wastewater. As a result, strategies for wastewater treatment and the development of next-generation water supply systems are needed. Although chlorination is the most used disinfection system, it presents various demerits such as the high doses required and the production of toxic by-products (trihalomethanes) that are harmful to human health. Advanced oxidation processes (AOPS) are being given a lot of attention for adequate disinfection. Compared with other conventional techniques, advanced oxidation processes have the characteristics of high oxidation efficiency and are environmentally friendly. However, a lot has to be done to optimize these processes for bulk wastewater disinfection. The scope of this review summarizes the current research findings on the application, performance and mechanisms of various AOPs for disinfection of wastewater. Furthermore, the basic principles of hybrid AOPs used to accelerate the oxidation efficiency of pathogenic pollutants are reviewed. Finally, the conclusion was that the main direction in the future of AOPs is the modification of the catalysts, coupled systems, and optimization of operating parameters which will ultimately translate to improved disinfection of wastewater.

Keywords: Advanced oxidation processes (AOPs), Natural zeolite, synergistic effect, Antibiotic resistance genes (ARGs), reactive oxygen species (ROS).

Introduction

Water pollution is a global concern amid the increasing demand for clean water required for the sustenance of domestic, agricultural and industrial activities [1]. The current stringent water quality protection measures are aimed at reducing the negative impacts of polluted water on ecosystems. Consequently, there are strict legislations regarding the disposal of treated municipal wastewater into natural water sources such as rivers [2]. The exponential population growth, rapid urbanization and intensified human activities have resulted in exponentially detrimental impacts on water sources, giving rise to water depletion and deterioration [3]. Even in developed countries with high sanitation levels, waterborne diseases are still prevalent [4]. These challenges have necessitated increased research on advanced wastewater treatment methods and water reclamation [5]. Water reclamation concepts have emerged as a response to the world's finite and frequently degrading freshwater sources. The reclamation concepts enables reuse of treated wastewater for various applications such as agricultural and industrial use [2].

Pathogenic microorganisms in water have been extensively studied since waterborne illnesses have been reported in both developing and developed countries [6]. Wastewater pathogens include; bacteria, viruses, and protozoa which originate mostly from fecal contamination from humans and animals. These pathogens cause many waterborne diseases [7]. The adoption of advanced oxidation processes (AOPs) for tertiary wastewater treatment is a promising approach for the abatement of pathogens [8]. In this regard, AOPs have emerged as promising technology because they are environmentally friendly, achieve a rapid oxidation rate and are highly efficient compared to other alternative disinfection methods such as chlorination [9].

The AOPs rely on the in situ generation of reactive oxygen species (ROS) which are very strong oxidants and highly reactive species [10]. An example of ROS produced include; hydroxyl radical ($HO^{\cdot}$), superoxide radicles (O_2^{-}), sulfate radicals, hydrogen peroxide, among others [10]. The types of AOPs that have been used with adequate degree of success includes, ozonation, electrochemical oxidation, UV light, photocatalytic oxidation process, Fenton and Fenton-like oxidation process and sulfate radical-based oxidation process. This review paper provides a critical analysis on the application of various AOPs including ozonation, electrochemical oxidation, Fenton and Fenton-like oxidation processes; sulfate based radical-based oxidation processes and photo-based process in

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the disinfection of municipal wastewater. This work further outlines the comparisons in the performance of the AOPs in different environments and their limitations when adapted to municipal wastewater. Additionally, the enhancement of AOPs disinfection using natural adsorbents like, natural zeolites and activated carbon are discussed.

Current trend and performance of various AOPs

Ozonation

Ozone (O_3) is a molecule that is extremely reactive and has an oxidation capacity of 2.07 eV [11]. Ozone can produce secondary oxidants such as hydroxyl radicals, which have a higher oxidation potential of 2.8 eV [12]. They rank second on the reactivity series after fluorine, which has an oxidation potential of 2.87 eV [10]. Due to these properties, ozone has the ability to react with various microbial cell components, including the cell wall and deoxyribonucleic acid (DNA) structures [13]. **Table** 1 shows the findings of recent studies on the disinfection of real wastewater using ozone. It can be seen from the **Table** that the required ozone dose is highly dependent on the wastewater microbial load and the microbial species.

The formation of hydroxyl radicals is usually not enough due to the low efficacious of O_3 decomposition, which crucially affects the capability of continuous disinfection [14]. Thus, ozone-based advanced oxidation processes (AOPs) have been evolved to enhance the production of free ROS radical during the disinfection treatment process [15]. Kim and Yousef *et al*. reported that the bacterial counts were reduced by 5-6 logs when (*E.coli, Pseudomonas fluorescens* and *Leuconostoc mesenteroides)* bacteria were exposed to O_3 concentration of 2.5mg/L for 40 seconds [16]. At ozone concentration of 1mg/L, 2mg/L and 3 mg/L, the deactivation rate constants of *bacillus cereus* bacteria were 0.3482, 0.3579 and 0.3761, respectively. This indicates that in order to enhance the efficiency of deactivation, a higher dosage of O_3 is necessary [17]. Jamil *et al*. studied the inactivation process of *Salmonella* and *E.coli* in drinking water [18]. The findings showed that using 2 mg/L of ozone effectively eliminated 5-6 log of *Salmonella* within a timeframe of 45-60 s. However, it was observed that *Salmonella* had a lower resistance to ozonation than *E.coli* during the disinfection.

The concentration of ozone significantly impacts its bactericidal efficiency. Higher concentrations of ozone for example, the 2.5 mg/L in Kim and Yousef [19] study result in more effective bacterial reduction compared to lower concentrations. This is evident in the differences observed in the deactivation rate constants in Ding et al., [20] study, where increasing the ozone concentration from 1 mg/L to 3 mg/L enhanced the deactivation rate of *Bacillus cereus*. The duration of exposure is crucial for achieving desired reduction levels. Kim and Yousef [16] study achieved a 5-6 log reduction within 40 seconds at a high ozone concentration, while Jamil et al., [21] observed similar reductions in *Salmonella* within 45-60 seconds at a lower concentration (2 mg/L). This indicates that shorter exposure times can be offset by higher ozone concentrations to achieve similar results.

Maniakova *et al*., [22] conducted a study to compare the effectiveness of H_2O_2 /sunlight, solar photo-Fenton and ozonation for inactivating target microbes (*Escherichia coli, Salmonella spp* and *Enteroccus spp*) in real secondary treated urban wastewater [22]. After 45 mins of treatment with an ozone (O3) concentration of 83 mg/L, complete inactivation was achieved. This method showed the highest rate of inactivation among all the tested bacteria. A similarity in the deactivation process was observed between the utilization of sunlight/H_2O_2 (50 mg/L) and solar photo-Fenton (0.1mM Fe^{2+} and 50 mg/L of H_2O_2). It was noted that *Enterococcus spp* showed a greater resistance to solar-driven disinfection when compared to *Escherichia coli* and *Salmonella spp*. The existence of carbonates had an impact on the effectiveness of sunlight/H_2O_2 and solar photo-Fenton methods.

Limitations of ozonation

The existence of dissolved organic materials in actual sewage wastewater hinders the disinfection capability in comparison with ozonation performance in synthetic effluent [23]. At a certain point, the organic compounds suppress some of the oxidant species. This reduces their availability for the microbial cell degradation [24]. Even if synthetic wastewater has a higher COD than real effluents, ozone disinfection efficiency is still lower for effluent. The ineffectiveness of disinfection on actual effluent may be due to the existence of other species in the secondary wastewater such as total suspended solids, cation and anions, which could compete with oxidants.

Table-1: The transferred and injected O_3 dose required for efficacious disinfection of pathogens.

Water type	Water properties	Microbes removal	O_3 dose	Reference
Secondary municipal wastewater effluent	***Escherichia coli* = 1.8 x10^3 CFU/ml** **TSS = 4.5-6 mg/L** **COD = 42-49mg/L**	***Escherichia coli* was totally removed**	**0.3mg/L** **Transferred dose**	[25]
Hospital wastewaters	***Escherichia coli*** ***Pseudomonas aeruginosa*** **pH values were in the neutral range (7–8)** **BOD=190 ± 35 mg/L** **COD=350 ± 67 mg/L**	**The concentration of 10^8 CFU/mL bacteria was reduced to an acceptable level by ozone treatment after a 5 min contact time, although the removal rate was much higher for concentrations of 10^6 CFU/mL and 10^4 CFU/mL bacteria**	**11 to 45 mg/L** **(TOD1 = 11 mg/L,** **TOD2 = 25 mg/L,** **TOD3 = 37 mg/L,** **TOD4 = 45 mg/L)** **Transferred ozone dose**	[26]
Real secondary urban wastewater effluents	**COD=25-50mg/L** **TSS = 5.2mg/L** **Fungi = 2.0 log** **Bacteria = 3 log**	**3-4 log bacteria** **3.9 log Fungi**	**225mg/L** **Injected dose**	[24]

Table-2: Summary of findings of various studies on the deactivation mechanism of pathogens by ozonation.

Name of the microorganism	Working condition	Deactivation efficiency	The deactivation mechanisms	Reference
Bacillus cereus	**Ozone (O_3)** **3 mg/L**	**Reduction to 3-log**	**Ozonation was successful in disinfecting chlorine-resistant spores by destructing both the microbial cell structures and genetic make-up**	[17]
Salmonella enteritidis, Escherichia coli	**Ozone (O_3), 20 mg/L of H_2O_2**	**Bacteria reduction to 5-log**	**Ozonation was due to progressive oxidation reaction of the vital cellular components of the microbial cells leading to complete destruction of the cells function**	[27]
Escherichia coli, Pseudomonas spp	**20 mg/L of total dissolved carbon, 1 mg/L Fe^{2+}**	**There was 3.3-log reduction**	**There was a significant reduction of adenosine triphosphate (ATP)**	[28]

Electrochemical oxidation

Electrochemical oxidation is the oxidation process that occurs at the anode of an electrolytic cell [29]. This process has been proven to be remarkably efficient in eliminating contaminants from wastewater[30]. The target contaminants are oxidized either through electron absorption from the anode or oxidized by anode-produced intermediates such as hydroxyl radicles [31]. Recently, researchers have been investigating the use of this technique for disinfecting wastewater and treating surface water [32]. When compared to disinfection methods like ozonation, photocatalytic oxidation and Ultra-violet irradiation, electrochemical oxidation is a more cost-effective option. This is because it requires minimal capital investment, has a simple set-up, can operate without catalysts and can perform multiple processes in a single set-up [33].

Cho et al., [34] carried out electrochemical oxidation disinfection of domestic wastewater containing coliform load of 0.8-7.8 x 10^5 CFU/100mL using a multiple metal-doped titanium anode and a supplied current density of 2.09 mA/cm^2 [34]. The study achieved total disinfection within 3 h. Lacasa *et al*. demonstrated that utilizing a boron doped diamond (BDD) anode with the current density of 25.5 mA/cm^2 and a chlorine concentration of 18.3 g/L resulted in a remarkable 6-log removal of *E.coli* within synthetic ballast wastewater in just 3mins [35]. The research revealed that the presence of chlorine in the electrolyte enhanced the efficiency of cell deactivation. Rajab *et al*. was able to realize a 6-log elimination of *Pseudomonas* after 15mins when using boron-doped diamond anode with an applied current density of 167mA/cm^3 without chlorine addition [36]. The study explained that the successful disinfection achieved was due to the production of adequate hydroxyl radicals.

The type of anode used in electrochemical oxidation significantly affects the efficiency of the disinfection process. The boron-doped diamond (BDD) anode is known for its high over-potential for oxygen evolution, which makes it highly effective for generating hydroxyl radicals. This explains the rapid disinfection achieved in the studies by Lacasa et al., [37] and Rajab et al., [38] compared to the multiple metal-doped titanium anode used by Cho et al., [39] which required a longer time to achieve total disinfection. Moreover, higher current densities

generally lead to higher production rates of reactive species (ROS) (e.g., hydroxyl radicals) that are responsible for bacterial inactivation. Lacasa et al., [40] used a current density of 25.5 mA/cm², achieving rapid 6-log removal of *E.coli* in just 3 minutes, while Rajab et al., [41] applied an even higher current density of 167 mA/cm³, resulting in a 6-log elimination of *Pseudomonas* within 15 minutes. In contrast, Cho et al., [42] used a much lower current density of 2.09 mA/cm², requiring 3 h for total disinfection. Lastly, the type of wastewater and the initial bacterial load also play crucial roles in the disinfection process. Cho et al., [42] dealt with domestic wastewater containing a coliform load ranging from 0.8-7.8 x 10^5 CFU/100mL, which may have required longer treatment times due to the complexity and variability of the organic and inorganic matter present. In comparison, Lacasa et al., [40] worked with synthetic ballast wastewater, which may have been less complex and more controlled, allowing for faster disinfection.

UV Light disinfection

This process uses ultraviolet (UV) light for water disinfection and it's the most used technique around the world [43]. The UV source can be artificial UV light or solar light irradiation. UV light can be used for disinfection because wavelengths between 200-300nm have a germicidal effect by absorbing the DNA molecules in cells. This enables the inactivation and distraction of different pathogens such as bacteria, protozoa and viruses [44]. The effectiveness of using artificial UV light for disinfection purposes varies depending on the specific type of pathogenic microorganism being targeted, as well as other relevant factors [45].

Hamilton et al.,[47] reported that bacteria spores and viruses are the most resistive to deactivation by artificial ultraviolet light irradiations, followed by protozoa found in the intestines such as *Giardia* and *cryptosporidium* and finally intestinal bacteria [47]. However, some types of microbial cells such as *Deinococcus radiodurans* [48] are resistant to low UV irradiation doses, hence, there is need for high dose irradiation for complete inactivation [49]. Although, artificial UV disinfection inactivates the microbial cells through the rupturing of their cell wall and also damages there DNA make-up [50], some of the microbes are able to repair their genetic make-up via photo-reactivation which enables the inactivated pathogens in water to re-contaminate [24]. Certain viruses, like human adenoviruses and polyomaviruses, possess a dsDNA genome that allows them to fix the machinery of their host cells during the viral replication process [51]. This ability helps them to overcome DNA damage caused by UV irradiation [52].

Guo *et al*. reported that an ultraviolet dose of 1.2 mJ/cm^2 was effective in inactivating *Escherichia coli* O157:H7 strain from 2-log to 6-log [54]. Medium-pressure and low-pressure UV lamps are commonly used for disinfection of water [55]. Medium-pressure lamps emit ultraviolet light at a wider range of wavelengths, typically around 200-400 nm. Light-emitting diodes have been successfully used for disinfection purposes in recent times [56]. Sholtes *et al*. conducted a study comparing the effectiveness of UV light-emitting diodes and low-pressure mercury-arc lamps in disinfecting water [57]. The study found that there was no significant difference in the ability to kill *Escherichia coli B* and coliphage *MS*-2 microbes. To attain a 4-log level reduction of *E.coli* B, low-pressure UV light of 6.5mJ/cm^2 was required while 59.3mJ/cm^2 was required for effective coliphage MS-2 disinfection. In comparison, the ultraviolet light-emitting diodes required 6.2mJ/cm^2 and 58mJ/cm^2 for *E.coli* and *coliphage MS*-2 inactivation respectively.

The effectiveness of UV treatment conditions varies significantly based on the type of UV source, the pathogen targeted, and specific experimental conditions. Guo *et al*., [58] demonstrated that a UV dose of 1.2 mJ/cm² was highly effective, achieving a 2-log to 6-log reduction of *Escherichia coli O157*. This suggests that the medium-pressure UV lamps, which emit light across a broad range of 200-400 nm, were highly efficient. In contrast, Sholtes *et al*., [59] compared low-pressure mercury-arc lamps and UV LEDs for disinfection of *E. coli B* and coliphage MS-2, finding no significant difference in efficacy between the two sources. For a 4-log reduction of *E. coli B*, low-pressure UV light required 6.5 mJ/cm², while UV LEDs required 6.2 mJ/cm². For coliphage MS-2, both low-pressure UV light and UV LEDs required much higher doses of 59.3 mJ/cm² and 58 mJ/cm², respectively. These differences underscore the variability in pathogen sensitivity to UV light and the influence of UV source characteristics. Medium-pressure lamps' broad spectrum may lead to higher efficacy at lower doses, while low-pressure lamps and LEDs, with their targeted wavelengths, require optimized doses for different pathogens. Experimental conditions such as water quality and UV delivery methods further impact these results.

Photocatalytic oxidation

It involves using a semiconductor catalyst exposed to light of an appropriate wavelength [60].

Artificial UV light, visible light, or solar light are all possible sources of illumination. Highly reactive hydroxyl radicals are produced when the photocatalyst is exposed to radiation in water. TiO_2 stands out as the most frequently utilized photocatalyst because of its exceptional photocatalytic activity, remarkable chemical stability, minimal toxicity and low production cost [61]. The effectiveness of Titanium dioxide (TiO_2) may be impaired due to the wide band gap, resulting in a high photoelectron-hole pair recombination rate and reduced light usage efficiency [62]. The photocatalytic activities of the semiconductors are primarily impacted by the surface properties (morphology) and energy band gap configuration [63].

Principle of photocatalytic oxidation.

The semiconductor possesses a band gap that divides the valence band (VB) from the conduction band (CB) [64]. When exposed to light with an appropriate energy level, the photocatalyst prompts the shift of its electrons from the valence band (VB) to the conduction band (CB). This leads to the creation of a positive hole (h^+) in the valence band (VB) and an electron (e-) in the conduction band (CB). When electrons (e^-) and holes (h^+) are produced and make their way to the surface of a semiconductor photocatalyst without recombining, they facilitate redox reactions with the compounds adsorbed on the catalyst [65]. During the transfer process, some of the electrons (e^-) produced by the semiconductor may recombine with holes (h^+) thereby decreasing the quantum efficiency of the reaction [66]. Contaminants are disintegrated by the holes (h^+) found in the valence band (VB), either through direct oxidation or a reaction with water, resulting in the production of potent hydroxyl radicals. In the same way, electrons (e^-) located in the conduction band (CB) lessen the amount of oxygen atoms that adhere to the photocatalyst. During the photocatalytic reaction, the reaction with moisture oxygen (O_2) leads to the production of superoxide radicals and other reactive groups. Photocatalysis relies on reduction and oxidation reactions as its elemental mechanisms. The main reactions in photocatalytic processes are as follows;

$$TiO_2 + hv \rightarrow TiO_2(e_{CB} + h^+_{VB}) \quad (1)$$

$$TiO_2(h^+_{VB}) + H_2O \rightarrow TiO_2 + H^+ + HO^{\cdot} \quad (2)$$

$$TiO_2(h^+_{VB}) + OH^- \rightarrow TiO_2 + HO^{\cdot} \quad (3)$$

$$TiO_2(e_{CB}) + O_2 \rightarrow TiO_2 + O_2^- \quad (4)$$

$$O_2^- + H^+ \rightarrow HO_2^{\cdot} \quad (5)$$

$$HO_2^{\cdot} + HO_2^{\cdot} \rightarrow H_2O_2 + O_2 \quad (6)$$

$$TiO_2(e_{CB}) + H_2O_2 \rightarrow HO^{\cdot} + OH^- \quad (7)$$

$$H_2O_2 + O_2 \rightarrow HO^{\cdot} + OH^- + O_2 \quad (8)$$

Methods of improving photocatalytic activity

To enhance the photocatalytic activities, the following modifications have been applied; Heterojunction method which involves the combination of two semiconductor materials with the same crystalline structures in contact with each other [67]. Current study findings have found that the heterojunction structure of TiO_2 with $Ni(OH)_2$, $NiTiO_2$ and other Ni elements exhibits higher photocatalytic activities and efficiency [68].

Elemental doping has been utilized in TiO_2 modification which is separated into cationic and anionic doping [69]. Doping TiO_2 with metal ions induces a new energy band near the valence of the band TiO_2 hence its activation by visible light [70]. Doping with noble metals has also been used in TiO_2 modification [71]. Noble metal doping entails the incorporation of a small amount of a noble metal such as silver into a material to change its properties. Noble metals have good electrical properties [71]. When noble metals are excited by a photon at a frequency that is similar to the inherent vibration rate of their surface free electrons, the free electrons of the metal resonate with the incident electrons leading to the creation of an electric field according to the (surface plasmon resonance phenomenon) which improves the photocatalytic performance [72].

Morphological control has also been used to improve semiconductor (TiO_2) activities. One way to enhance the photocatalytic efficiency of TiO_2 is to design the surface of the semiconductor with controllable surface defects. This can enhance the segregation efficiency of photogenerated electron-hole pairs[73]. It affects the photocatalyst surface area and porosity, which has an impact on the TiO_2 efficiency [74].

Mechanism of photocatalytic in disinfection

Photocatalysis produces hydroxyl radicals ($HO^{\cdot}$) and superoxide(O_2^-), which are highly reactive and naturally powerful oxidizing agents. In the presence of an oxidant such as hydrogen peroxide or O_3 photocatalysis yields additional hydroxyl radicals under UV irradiation [75]. For example, when exposed

to UV light, an H_2O_2 molecule undergoes the process of splitting into two $^{\bullet}OH$ [76]. Furthermore, when the wavelength falls below 242 nm, OH^- are generated via photolysis of H_2O. Semiconductors generate a variety of reactive oxygen species including O_2^-, $HO^{\cdot}$ and H_2O_2 during photocatalytic disinfection. Microbial cells can experience detrimental stress reactions from various ROS species, which causes damage to vital components like the peptidoglycan layer, genetic materials (DNA and RNA) and ribosomes [77]. ROS can change the permeability of pathogenic cells by attacking various components of their semi-permeable membrane, thus altering the integrity of the cells [78]. Therefore, the cytoplasmic contents are released [79]. The ROS attack on microbial cells starts on the outside of the organism, affecting the semi-permeable membrane. This can lead to damage to the genetic makeup and the inhibition of the metabolic processes [80]. Moreover, the produced species can inhibit various protein activities crucial for cellular physiological processes [81].

Bacteria are separated into two categories based on the structure and makeup of their cell walls. Gram-positive bacteria have a cell wall composed of a dense and thick peptidoglycan and phosphoric acid. Gram-negative pathogens have a moderately thin cell wall and a multi-layered structure made up of lipopolysaccharide, phospholipid/lipoprotein and peptidoglycan [82]. The protein and phospholipid bi-layer acts as a protective barrier and functions as a semi-permeable membrane, preventing the access of extracellular substances into the cell. Ideally, ROS produced in AOPs targets the cell envelope of microbes, which includes the lipopolysaccharide, peptidoglycan and phospholipid bilayers. The peptidoglycan layer exhibits porosity, allowing nanoparticles with a diameter of approximately ~2nm to effectively penetrate the cell. This phenomenon facilitates the unhindered passage of ROS [83]. Kühn *et al.* investigated and reported on the inactivation of *E.coli, staphylococcus aureus, pseudomonas aeruginosa* and *Enterococcus feacalis,* by employing TiO_2 under mild ultraviolet irradiation [84]. The disinfection efficiencies exhibited a decrease as the peptidoglycan layer thickness increased and as the microbial cell structure complexity escalated.

The phospholipid and lipopolysaccharide bilayers are made up of fatty acids that exhibit high susceptibility to peroxidation. As a result, these bilayers serve as the principal targets for ROS attacks [85]. ROS oxidizes unsaturated fatty acids, forming lipid-peroxyl radicles [86]. The generated radicals can initiate a chain reaction by abstracting hydrogen atoms from distinct diallyl methylene groups. This leads to the generation of a ketones group [87]. In addition, reactive oxygen species has also shown a tremendous effect on the protein function. During the deactivation process, cell proteins undergo structural modifications and aggregation. These alterations can lead to impaired cell metabolism, function loss, hindered DNA replications and mutations [88].

In the advanced oxidation process, the ROS preferentially targets the semi-permeable membrane of bacteria, which includes peptidoglycan, lipopolysaccharide and phospholipid bilayers. Due to its porous nature, the peptidoglycan layer in bacteria allows for unhindered passage of ROS, enabling them to penetrate the bacterial cell. Kühn *et al*. carried out a study on TiO_2 performance on *Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus faecalis* and *E.coli* [84]. The research findings indicated that as the thickness of the peptidoglycan layer and the intricacy of cell structure increased, there was a corresponding reduction in the effectiveness of disinfection efficiencies. ROS produced by AOPs with powerful oxidizing ability react with DNA to break a phosphodiester bond between the bases in a DNA molecule's double-stranded structure [89]. Pathogens contain regulators that control the imbalances of ROS in cells generated by enzyme autoxidation. The enzymes include co-enzyme A (CoA), catalase (CAT), hydroperoxidases (HPI), and glutathione reductase (GR) [90]. CoA is an important enzyme involved in cellular respiration in cells. The ROS generated directly participates in CoA oxidation by accepting an electron from CoA, limiting bacterial cell respiration and triggering pathogen destruction [91].

A great interest has been put into photocatalytic disinfection after effective inactivation of *lactobacillus acidophilus, E.coli*,[92] and *saccharomyces cerevisiae* using TiO_2-Pt supported catalyst [93]. Photocatalytic inactivation *Escherichia coli* is widely reported in literature [80]. Rincón & Pulgarin *et al*. investigated coliforms and *E.coli* inactivation under sunlight using TiO_2 and reported that *E.coli* was disinfected faster than other coliforms (*Enterococcus species*) [94]. Bogdan *et al*. reported a wide range of classifications of susceptible bacteria to modified semiconductors for photocatalytic disinfection as follows, viruses > prions > bacteria(gram-negative) > gram-positive bacteria >yeasts > molds [95]. Gomes *et al*. reported that the semiconductor TiO_2 doped and modified with noble metals showed a great effect on the total removal of *E.coli* from water within a dilution factor range of 10^3-10^4 CFU/ml. It was further noticed that TiO_2 doped with Pd (Pd-TiO_2) and Ag-TiO_2 did not depend

upon ultraviolet light for complete deactivation of *E.coli* [97]. The use of noble metals seems the most suitable since no or little energy is required.

To guarantee complete inactivation and zero regrowth after photocatalytic disinfection, the effective disinfection time has to be determined for the complete inactivation of microbes [94]. Effective disinfection time is the time required for the destruction or deactivation of the pathogens without post-treatment recovery when the treated samples are kept in the dark period referenced to 48 hours after photo-treatment ends. Photocatalytic disinfection has a prominent merit which is the residual disinfection effect. Residual disinfection effects lead to a decrease in bacterial count under dark conditions after the photocatalytic process [98]. Xiong & Hu *et al*. also reported on the residual disinfection effect after the photocatalytic oxidation process which could be due to H_2O_2 generated in water during the photocatalytic process which leads to the generation of more ROS that aids the photocatalytic process [99]. Dunlop *et al.* carried out a study on the efficiency of ultraviolet irradiated TiO_2 Degussa (P25) in the deactivation of *E.coli,* and the study found that the elimination of antibiotic resistance genes (ARGs) bacteria was lower in real wastewater than in synthetic wastewater [100]. This was a result of the presence of organic and inorganic components in the real wastewater hence ROS scavenging on those components.

The effectiveness of photocatalytic oxidation treatment varies significantly due to differences in photocatalyst composition, light sources, pathogen susceptibility, and environmental conditions. Rincón and Pulgarin [101] found that TiO_2 under sunlight disinfected *E. coli* faster than other coliforms, suggesting higher susceptibility of *E. coli* to this process. Gomes *et al*., [97] showed that TiO_2 doped with noble metals like Pd and Ag effectively removed *E. coli* without UV light, likely due to enhanced electron-hole separation and reactive oxygen species (ROS) generation. Xiong and Hu [102] reported a residual disinfection effect attributed to H_2O_2 produced during photocatalysis, further aiding the disinfection process. Bogdan *et al*., [103] classified microorganisms by their susceptibility to photocatalytic disinfection, with viruses being the most susceptible and molds the least, highlighting the influence of structural differences. Dunlop *et al*.,[104] observed lower elimination of antibiotic resistance genes (ARGs) in real wastewater compared to synthetic wastewater using UV-irradiated TiO_2, likely due to inhibitory substances present in real wastewater. These factors underscore the complex interplay between photocatalyst properties, light sources, microbial characteristics, and environmental conditions in determining the overall efficacy of photocatalytic oxidation treatments.

Fenton oxidation reaction and Fenton-like processes

Fenton oxidation is an oxidation method that utilizes a chain reaction of Fe^{2+} and hydrogen peroxide (H_2O_2) to generate the catalytic $HO^{\cdot}$. The Fenton reaction involved the activation of H_2O_2 by adding to Fe^{2+} solution under acidic conditions [105]. The main reaction in three stages as follows [106] ;

Chain initiation stage:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{2+} + OH^- + HO^{\cdot} \quad (9)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + H^+ + HOO^{\cdot} \quad (10)$$

$$HOO^{\cdot} \rightarrow H^+ + O_2 \quad (11)$$

Chain propagation stage:

$$H_2O_2 + HO^{\cdot} \rightarrow H_2O + HOO^- \quad (12)$$

$$H_2O_2 + HOO^{\cdot} \rightarrow O_2 + H_2O + HO^{\cdot} \quad (13)$$

$$H_2O_2 + O_2^- \rightarrow O_2 + HO^{\cdot} + OH^- \quad (14)$$

Chain termination stage:

$$Fe^{3+} + HOO^{\cdot} \rightarrow Fe^{2+} + O_2 + H^+ \quad (15)$$

$$Fe^{2+} + HOO^{\cdot} \rightarrow Fe^{3+} + OH^- \quad (16)$$

$$HO^{\cdot} + HO^{\cdot} \rightarrow H_2O_2 \quad (17)$$

$$2HO^{\cdot} + 2HO^{\cdot} \rightarrow O_2 + 2H_2O \quad (18)$$

$$HOO^{\cdot} + HOO^{\cdot} \rightarrow O_2 + H_2O_2 \quad (19)$$

$$HOO^{\cdot} + HO^{\cdot} \rightarrow O_2 + H_2O \quad (20)$$

Fenton systems have limitations in that they only operate in acidic conditions of about pH=3 and produce a substantial volume of iron-containing sludge, resulting in higher operational expenses [23]. To overcome these demerits coupled techniques such as the photo-Fenton process involving, UV light in conjunction with the Fenton process and the electro-Fenton process among others are utilized to enhance the Fenton/Fenton-like reactions for higher production of $HO^{\cdot}$ [107].

The Fenton/Fenton-like reaction decomposes H_2O_2 to produce hydroxyl radicals with a

high oxidation capacity, which can be used to destroy pathogens as indicated in **Table** 3. The Fenton system's effect on the microorganism cell is determined by the Fe^{2+} and H_2O_2 concentration used in the deactivation stages [108]. Diao *et al*. investigated the mechanism of Fenton reagents inactivation of *Escherichia coli* [109]. The results demonstrated that the interaction caused cell surface deformation and cell components leakage. This distortion may result in a loss of cell swelling, permeability and cells rupture.

Sulfate radical-based advanced oxidation processes.

Sulfate radical-based AOPs are applicable in a significantly wider pH range than Fenton reactions [110]. Sulfate radicals(SO_4^-) have attracted a lot of attention because the radicals have a very strong redox oxidation potential of about 2.5 – 3.1 eV plus the fact that the radicals are stable and adaptive to different pH conditions [111]. Sulfate radical-based advanced oxidation processes can use Sulfate radicals only or in combination with hydroxyl radicals to disinfect wastewater microorganisms. Peroxydisulfate (PDS) or peroxymonosulfate (PMS) activation produces sulfate radicals. PDS and PMS are inactive without being assisted by external energy [112]. Thermal treatment, metallic or non-metallic catalysis, UV-vis light, ultrasonic, microwave and photocatalytic activation are some of the methods that have been utilized to activate PMS and PDS [113]. Photocatalysis and electrochemical activation methods have been used, in which electrons are transferred between the metal and photo-generated electrons and the electrode for activation. Homogenous catalysts are preferred over heterogeneous catalysts due to their efficiency in the activation process [114]. Furthermore, some activation methods, such as ultrasound and UV light, employ their own energy to activate PMS and PDS. Activation of PMS and PDS proceeds as follows;

$$HSO_5^- + e^- \rightarrow SO_4^- + OH^- \quad (21)$$

$$HSO_5^- + e^- \rightarrow SO_4^{2-} + HO^{\cdot} \quad (22)$$

$$S_2O_8^{2-} + e^- \rightarrow SO_4^- + H^+ \quad (23)$$

$$HSO_5^- + h^+ \rightarrow SO_5^- + H^+ \quad (24)$$

$$HO^{\cdot} + H^+ + e^- \rightarrow H_2O \quad (25)$$

$$SO_4^- + H^+ + e^- \rightarrow HSO_4^{\cdot} \quad (26)$$

$$SO_4^- + OH^- \rightarrow SO_4^{2-} + HO^{\cdot} \quad (27)$$

Advanced oxidation processes (AOPs) hybrid systems

To solve the shortcomings of single AOPs in terms of ROS formation and operational parameters, a combination of multiple AOPs has been created and studied. The integration of several AOPs systems can accelerate the oxidation efficiency of pathogenic pollutants elimination compared to single treatment technologies due to the resultant synergy obtained in coupled systems [115]. Furthermore, hybrid AOP systems widen the operational parameters for each technology [116].

UV/ozone coupled process

Ozone (O_3) activity can be increased by combining it with other systems such as hydrogen peroxide and or persulfate [117]. Because H_2O_2 is a strong oxidant, it can enhance the generation of hydroxyl radicals ($HO^{\cdot}$). Persulfate also causes the formation of powerful oxidant sulfate ion radicals. The synergistic effect between ozonation and UV photolysis is a result of the improvement of radical generation[118]. Pathogen degradation can be enhanced by combining ozone technology with ultraviolet irradiation (O_3/UV).

Table-3: Performance and inactivation mechanism of Fenton and Fenton-like processes.

Pathogens	Working conditions	System deactivation efficiency	deactivation mechanisms	References
Escherichia coli	**H_2O_2, Fe^{2+} in a ratio of 1:10. UVA-VL lamp of 200W, For 120 minutes**	**There was 100% inactivation of the *E.coli* bacteria**	**Low irradiation which was dependent on intracellular photo-Fenton reaction which was enhanced by H_2O_2 concetration and Fe^{2+}.**	[119]
E.coli	**H_2O_2, Fe^{2+} in a ratio of 1:10 VL lamp of 200W, For 120 minutes**	**There was 100% inactivation of the E.coli bacteria**	**Irradiation was dependent on H_2O_2 activities. H_2O_2 and Fe^{2+} extracellular photo-Fenton**	[119]
E.coli	**H_2O_2 50mg, FeOx, 6762.W/m^2 irradiance**	**Bacterial removal to 5-log**	**Fe^{3+} and HO• destructed the microbial cell**	[120]
E.coli, S. aureus	**2mg of $FeSO_2$, 32ml H_2O_2 and MoS_2**	**99.96% 100%**	**Deactivation of *E.coli* in the MoS_2 co-catalytic system by hydroxyl radicals was very critical**	[121]

Microcystis aeruginosa	**Fe^{2+} concentration of 1 mg/L and H_2O_2 concentration of 1 mg/L, 20 kHz, 0.42 W/ml**	**Reduction from 4.19 x 10^6 to 0.45 x 10^6 CFU/ml**	**20 kHz sonication process generated holes on the bacteria cell wall by cavitation and acoustic streaming**	[122]
Microcystis aeruginosa	**Fe^{2+} concentration of 1 mg/L and H_2O_2 concentration of 1 mg/L, 800 kHz, 0.07 W/ml**	**Reduction from 4.19 x 10^6 to 2.33 x 10^6 CFU/ml**	**Low light intensity of 800kHz sonication induced H_2O_2 inside the cell via endocytosis process**	[122]
Escherichia coli	**Fe^{2+} concentration of 0.29 mg/L, at a pH=3-4,**	**The α-FeOOH@AC-H_2O_2 system achieved an effective inactivation efficiency of 4.5 log, which was much higher than the 1.6 log of the α-FeOOH-H_2O_2 system and 1.9 log of the AC-H_2O_2 system**	**Formation of hydroxyl radicals led to serious destruction and rupturing of the cells cell walls resulting in extensive cell lysis**	[123]
Escherichia coli	**pH=3.5-9.5, H_2O_2, Magnetic Fe_2O_3 deposited flower-like MoS_2**	**100%**	**Production of hydroxyl radicals led to serious distortion of the cells cell membrane and cell walls.**	[124]

Table-4: The recent performances of sulfate-based AOPs in microorganism inactivation.

Microorganisms name	Working conditions	The deactivation efficiency	The deactivation mechanisms	Reference
Escherichia coli	**T=25^{O}C, (PS)=2mM, (MHC)=200 mg/L, λ=420nm And pH=6.0**	**The cell reduction was to 8-log within 40 minutes.**	**Sulfate radicals generated damaged the cells cell membrane followed by genetic material destruction (DNA destroyed).**	[125]
Escherichia coli	**PS=0.5, ilmenite= 1 g/L**	**Cell 6-log reduction within 60 minutes**	**Reactive oxygen species (ROS) generated caused leakage and destruction of *Escherichia coli* intracellular makeup.**	[126]
Escherichia coli	**PDS=2mM, Na_2SO_4=50mM, GAC = 10 g/L**	**Cell 4.6-log reduction within 10 minutes**	**ROS generated (SO_4^- >HO^->H_2O_2) destroyed the cells cell membrane integrity and the microbe cell metabolism**	[127]
Escherichia coli	**SDBC=0.5g/L, PDS = 6mM**	**93% within 90 minutes**	**The bacterial cell membranes destroyed through electron-transfer mechanism**	[128]
***Escherichia coli* K-12**	**PDS=1g/L, magnetic pyrrhotite**	**7-log reduction within 15 minutes**	**ROS generated (SO_4^- >HO^->H_2O_2) caused the bacterial cell membrane and cell wall destruction followed by protein destruction and then lastly the genetic make-up breakdown**	[129]

Jung *et al*. investigated the integrated use of ultraviolet radiation/O_3 for the inactivation of *Bacillus subtilis spores* [130]. Ozone concentration at 2 mg/L was simultaneously introduced with UV rays into the reactor cell containing 2-4 x 10^6 CFU/ml of the *B. subtilis* spores. When UV light was combined with ozone, an overall inactivation efficiency of 4.5 log reduction was noted. However, when ultraviolet and ozone were employed separately, the inactivation profile appeared to be identical. To measure the level of synergy, the enhanced efficiency was evaluated on the basis of the ozone CT value, where the residual ozone was higher in the ozone process alone than in the combined O_3/UV process.

Fang et al., [131] studied the destruction of *Escherichia coli* and bacteriophage MS2 by UV photolysis, ozonation and a combination of UV/O_3 processes at pH = 7 and temperature of 22^{o}C [131]. The inactivation kinetics of *Escherichia coli* by UV, O_3 and UV/O_3 with an O_3 dosage of 0.05mg/L were investigated. During UV irradiation, the log deactivation of *Escherichia coli* displayed a linear trend until roughly 3-log reduction, followed by a lower deactivation rate. The 15 seconds of O_3 exposure led to a 1.2-log inactivation. Due to ozone depletion, the subsequent inactivation remained constant. O_3/UV exposure enhanced the *E.coli* inactivation.

The differences in treatment conditions using UV/ozone coupled process can be attributed to the synergistic effects between UV radiation and ozone. Jung *et al*., [132] demonstrated that the combined UV/O_3 process achieved a significantly higher inactivation efficiency (4.5 log reduction) for *Bacillus subtilis* spores compared to using UV or ozone separately. This synergistic effect is likely due to the combined action of UV radiation and ozone, producing more reactive oxygen species (ROS) such as hydroxyl radicals, which are highly effective in breaking down microbial cell walls and genetic material. The identical inactivation profiles for separate treatments suggest that each method alone has a limit to its effectiveness. Moreover, Fang *et al.,* [133] found that the combined UV/O_3 process enhanced the inactivation of *Escherichia coli* compared to using UV or ozone alone. The synergy between UV and ozone accelerates the generation of ROS, leading to more efficient microbial destruction. The linear inactivation

trend observed with UV alone until a 3-log reduction, followed by a decreased rate, indicates that UV alone may be less effective beyond a certain point. Ozone alone showed limited effectiveness due to its depletion over time.

Photocatalysis coupled oxidation process

The photocatalytic process can be coupled with other AOPs technologies like ozone and H_2O_2 which leads to the improvement of disinfection efficiency [134]. The coupling improves the production of $HO^{\cdot}$ due to the synergistic effect. Mecha *et al*. studied the synergy and bacterial regrowth in photocatalytic ozonation treatment of municipal wastewater [135]. The target microbes were *Escherichia coli, Shigella spp, Salmonella spp* and *Vibrio cholera* bacteria. The deductions made from the study showed that the working of photocatalytic ozonation was better than that of the separate individual unit process. It was noted that UV/O_3 of synthetic wastewater showed a complete inactivation of all the target bacteria within 15 minutes for all processes (99.9%). Furthermore, no recovery was detected after a recovery period of 48 hours. **Table** 5 summarizes some findings from the literature on the elimination of bacteria and ARGs from water using photo-coupled oxidation techniques from various sources. Dunlop *et al*. investigated TiO_2 (P25) efficiency under UVA light for *E.coli* inactivation [100]. The removal of antibiotic resistance genes was found to be less in real wastewater, which was related to ROS scavenging by inorganic and organic constituents of the effluent. It was noted that, in addition to pathogen regrowth, antibiotic resistance genes transfer may increase if the disinfection is not continued to the point of total pathogen deactivation prior to discharge.

Table-5: Antibiotic resistance genes (ARGs) bacteria degradation from water through photo-coupled oxidation process from different sources.

Hybrid systems	Working conditions	Significant findings	Pathogens	References
Photo-catalysis using natural sunlight. TiO_2/H_2O_2 Solar light/ H_2O_2	Effluent from urban wastewater treatment plant. H_2O_2=20mg/L TiO_2(P25) GO- TiO_2	TiO_2(P25)/ H_2O and solar light/ H_2O was the most effective hybrid system for elimination of ARGs	*Enterococci* and *Fecal coliforms*	[136]
Photo-catalysis/UVC/H_2O_2	UV-C lamp (254nm, 300W,800W) TiO_2 thin film Fluence=0-120mJ/cm^2 H_2O_2=10-100mM Phosphate-buffered saline solution (pH = 7.4) Natural water from drinking water source(pH = 7.2)	Increasing H_2O_2 concentration increased photo-catalysis efficiency	*Pseudomonas aeruginosa* *Staphylococcus aureus*	[137]
Photo-catalysis/UVC/H_2O_2	UVC (254nm, 300W,800W) TiO_2 thin film Fluence=0-120mJ/cm^2 H_2O_2=10-100mmol/L Phosphate-buffered saline solution (pH = 7.4) Natural water from drinking water source(pH = 7.2)	There was no significant difference that was noted between natural water and phosphate-buffered saline solution on bacteria inactivation. 4.7 log ampC and 5.8 log mecA reduction were achieved in the presence of TiO_2 for both matrixes	*Pseudomonas aeruginosa* ,*Staphylococcus aureus*	[138]

Photo-Fenton coupled oxidation process

This is a form of Fenton oxidation reaction that takes place under UV light irradiation utilizing a Fenton catalyst [139]. The photo-Fenton process is a sequence of Fenton reagents (H_2O_2 and Fe^{2+}) and UV-vis that gives rise to extra hydroxyl radicals by two additional reactions which are photo-reduction of Fe^{3+} to Fe^{2+} ions and peroxide photolysis [140]. In comparison with other AOPs systems like the UV/H_2O_2 system and the Fenton method, Photo-Fenton systems hold the merits of an enhanced removal rate of Fe^{2+} as well as improved use of H_2O_2 [141]. The efficacy of the Fenton system on cell inactivation depends on the Fe^{2+} and H_2O_2 concentrations used in the process [108]. The Fenton process can be of low efficiency toward H_2O_2 decomposition due to the slow kinetics of the Fe^{3+}/Fe^{2+} redox cycle. The $UV/Fe^{2+}/H_2O_2$ system can be a prospective technology for the degradation of bacterial contaminants present in wastewater. The application of UV light into the Fenton system accelerates the regeneration rate of Fe^{2+}. Additionally, there is a synergistic effect of UV light and Fe^{2+} on the catalytic decomposition of H_2O_2.

Through different studies, it has been shown that photo-Fenton using both solar or UV light, has significant effects on the inactivation of pathogens in wastewater. García-Fernández *et al*. carried out a

study on bacteria and fungi inactivation using Fe^{3+}/sunlight, H_2O_2/sunlight and solar photo-Fenton at near neutral pH, for the inactivation of *Escherichia coli* and *Fusarium solani* in water [142]. The results showed that bacteria inactivation with a high rate was noted using a photo-Fenton system with 5 mg/L of Fe^{3+} and 10 mg/L of H_2O_2 which gave a 5-log inactivation of *E.coli* in 10 minutes. *Fusarium solani* inactivation was also found using 2.5 mg/L of Fe^{3+} and 5 mg/L of H_2O_2, which resulted into a 3.4-log reduction in 3 hours.

Enhancement of disinfection using natural adsorbents

Natural adsorbents can be used to enhance disinfection by adsorbing contaminants present in wastewater [143]. Adsorbents such as activated carbon, silver nanoparticles, chitosan, natural zeolites, and other natural minerals can be used to adsorb pathogens (bacteria, protozoa, and viruses), organic compounds, heavy metals, and other pollutants that may be present in water, thus improving the overall quality of the water. When used in conjunction with AOPs systems, the adsorbents enhance the disinfection process since they have effective adsorption sites [144]. Zeolites are microporous crystalline materials made up of alumina-silicate elements widely utilized as adsorbents due to their chemical structure and surface morphology [145]. Zeolites enhance the disinfection process by reducing the levels of residual contaminants that interfere with disinfection [146]. Recently, more researchers have focused on improving the performance of AOPs systems by utilizing zeolites and activated carbons [147]. Different materials such as natural zeolite, activated carbon and clays have been incorporated with transition metals and have proven to have excellent catalytic activity in AOPs [148]. TiO_2-supported on natural zeolites combines the photocatalytic activities of TiO_2 with the adsorption properties of natural zeolites, inducing a synergistic effect and resulting in improved photocatalytic efficiency [149]. Combining TiO_2 and zeolites has been reported to enhance the degradation of pathogenic contaminants in water. The enhancement is explained by the adsorption of the bacterial pathogens on the zeolite surface which increases the concentration of the colonies in the proximity of the TiO_2 semiconductor hence improving the decomposition rate [150].

Muleja *et al*. studied the deactivation of *Escherichia coli* using cobalt-modified natural zeolite [151]. The observations revealed that the Co-Nat/Zeolite initiated the deactivation of *Escherichia coli* cells by adhering to the microbe's surface which was subsequently followed by oxidative distortion that resulted in *E. coli* inactivation. The study also reported that the cobalt-treated natural zeolite acted as a filter during the treatment of the same wastewater.

Conclusions

This review focuses on the application of AOPs as tertiary methods for the disinfection and degradation of microorganisms in municipal wastewater. The study analyzed the performance and challenges of frequently used AOPs. The mechanisms of destruction and inactivation of the microbial cell such as oxidation of the cell structural envelope, destruction of internal cell components and genetic materials (DNA and RNA) are comprehensively analyzed. Generally, different ROS have been discussed for pathogenic bacteria inactivation. The most difficult aspect of photocatalytic disinfection is producing ROS to overcome microbe defense systems and pathogen recovery or regrowth. A combination of different AOPs such as UV/O_3, $UV/TiO_3/H_2O_2$ among others has been created and studied to handle the shortcomings of single AOPs in terms of ROS (O_2^-, $HO^{\cdot}$) production and operating parameters which eventually leads to increased oxidation rates. The efficacy of the process and the extent of synergism are determined not only by increasing the number of free radicals, but also by changing the reactor conditions, which results in better contact of the generated free radicals with the pathogenic microbe and better utilization of the oxidants and catalytic activity. The integration of several AOPs systems can accelerate the oxidation efficacy of pathogenic pollutants elimination compared to single treatment technologies due to the resultant synergy obtained in coupled systems. Natural absorbents such as zeolites improve disinfection by lowering residual pollutants that interfere with disinfection.

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