Literature Review of Disinfection Techniques For Water Treatment

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Water treatment is a developing concern, both terrestrially and in spacecraft, as exploration missions extend in time and distance. Current biofilm control is limited for long-term applications. To optimize biocides for present and future space exploration vehicles, a thorough understanding of common and traditional disinfectant techniques is required. This review is focused on the three fundamental disinfection techniques: chemical, physical, and biological. Mechanisms, advantages, disadvantages, and specific properties of each major technique, as well as their studied effect on established biofilms, are also considered. This paper provides a general background on disinfectants and some information on effects on biofilms that can be useful to develop innovative ideas for state-of-the-art disinfection techniques for water treatment in specific environments, such as those currently posing mission risks as well as for future spacecraft water system development.

Nomenclature

ATP	=	Adenosine Triphosphate
DBP	=	Disinfectant Byproducts
DNA	=	Deoxyribonucleic Acid
EPA	=	Environmental Protection Agency
EPS	=	Extracellular Polymer Substance
ISS	=	International Space Station
HOI	=	Hypoiodous acid
MRSA	=	Methicillin-resistant *Staphylococcus aureus*
NASA	=	National Aeronautics and Space Administration
Nm	=	Nanometers
PI	=	Povidone Iodine
ppm	=	parts per million
PVP	=	Polyvinylpyrrolidone
RBC	=	Rotating Biological Contractor
ROS	=	Reactive Oxygen Species
SEM	=	Scanning Electron Microscopy
THM	=	Trihalomethane
TiO_2	=	Titanium Dioxide

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I. Introduction

Water treatment is very important to improve and sustain human life. It begins with wastewater, which is then filtered and treated through various processes to obtain potable and palatable water.[1] The physical quality of water pertains to the appearance, color, turbidity, taste and odor, which are determided by the chemical and microbiological composition of the water.[2] Pre 19th century, water treatment was relatively simple, as some of the stressors on water quality that are persistent today did not exist.[3] During this time, water treatment mainly existed to improve the taste, look, and odor of water. In order to achieve a better physical quality of water, ancient Greece and India would boil their water, filter the water through straining, such as through charcoal and gravel, and utilize sunlight as a disinfectant technique.

However, as populations grew, cities became larger and centralized drinking water systems were developed, which became sources of contaminated water easily distributed to people at a large scale, causing disease epidemics.[4] Sewage is the main source of infectious pathogen or microorganism contamination. Some noteworthy sewage pathogens include: rotaviruses, which are significant causes of gastrointestinal symptoms such as diarrhea and vomiting; cholera, a type of bacteria that can also cause severe diarrhea and dehydration; and a protozoan parasite *Giardia lamblia* that can inhabit the gastrointestinal tract and is considered to be a worldwide cause of human morbidity.[5,6,7] These are only a few examples of the types of pathogens found in wastewater. A longer list can be found in Table 1.

Table 1. Pathogenic microorganisms potentially present in wastewater.[8]

Bacteria	Viruses	Protozoa	Yeasts
Salmonella	***Hepatitis A***	***Cryptosporidium***	*Candida*
Shigella	***Enteroviruses***	***Entamoeba***	*Cryptococcus*
Yersinia	***Poliovirus***	***Giardia***	*Trichosporon*
Escherichia	***Coxsackie viruses***	***Balantidium***	
Pseudomonas	***Echoviruses***	*Toxoplasma*	***Fungi***
Clostridia	***Rotavirus***	*Sarcocystis*	*Aspergillus*
Bacillus	*Adenovirus*		*Phialophora*
Listeria	*Reovirus*	***Cestodes***	*Geotrichum*
Vibrio	*Astrovirus*	***Taenia***	*Trichophyton*
Mycobacterium	*Calicivirus*	*Diphyllobothrium*	*Epidermophyton*
Leptospira	*Coronavirus*	*Echinococcus*	
Campylobacter	*Norwalk*		
Staphylococcus		***Nematodes***	
Streptococcus		***Acaris***	
		Toxocara	
		Trichuris	
		Ancylostoma	
		Necator	
		Hymenolepsis	

Advances in bacteriology in the late 1800s awakened the transition period of water treatment with the exploration of various chemical disinfectant methods at the turn of the 1900s.[9] A plethora of disinfectant techniques, including physical and biological techniques, have been studied and discovered since then. The main concern of disinfectants today are: minimizing harmful chemical byproducts, preventing corrosion of water pipes, and bacterial resistance.

Disinfection is a technique that inactivates and reduces microbiological pathogens in water to an appropriate and safe level for human consumption. Growth inhibition and lethal action are the two different ways a disinfectant can act on a microorganism.[10] Microorganisms can deploy a form of resistance for growth inhibition, however, lethality ensures that there is no means of defense. Unlike antibiotics, which have a specific cellular target, disinfectants act non-specifically due to their broader spectrum of activity.[11] Therefore, it is helpful to be familiar with the structure and functions (primarily the cell membrane/wall) of types of microorganisms present in wastewater such as bacteria, viruses, protozoa, and yeast, to understand the mechanistic pathway of disinfectant inactivation.[12,13,14,15]

The mechanism of action for each disinfectant is an important factor to consider when choosing an ideal disinfectant, especially when the persistent microorganisms are known. The mechanism of each technique will be described further, but in general, they have similar trends of microorganism inactivation/lethality (Figure 1). Each disinfectant may directly or indirectly disrupt important components of the cell through reactions with proteins, lipids, and DNA. Primary disinfectants result in direct disruption of the cell, whereas secondary disinfectants result in the production of reactive oxygen species (ROS), which prompt the germicidal activity. The mechanistic pathways of

pathogen inactivation and death through such reactions result in: damage to cellular structural organization, interference with growth and biosynthesis, and interference with energy-yielding metabolism (Figure 1).[16]

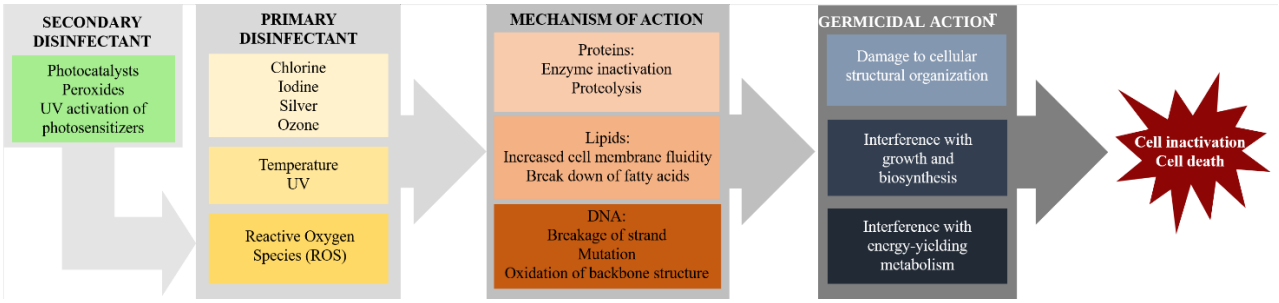


Figure 1. General overview of mechanistic action of each of the disinfectant categories.

This review provides an overview of common and traditional disinfectant techniques. It is broken up into three sections that highlight the three types of disinfectant techniques: chemical, physical and biological. In each section, mechanisms, advantages, disadvantages and specific properties of each will be described. This paper provides a general background on disinfectants and some information on effects on biofilms that can be useful to develop informed, insightful, innovative ideas for state-of-the-art techniques to address specific contamination problems.

II. Chemical Disinfection Techniques

Chemical disinfection techniques involve the addition of chemicals to water for germicidal effects. It is important to note that each chemical disinfectant exists in solution as complex formulations and that the environment will play a role in the reaction. Therefore, some knowledge of the composition of water to be disinfected is required to determine the best technique to implement. The composition of the water will determine the dosage, contact time, and persistence of the chemical disinfectant. Most importantly, composition of the water and nature of the chemical disinfectant will determine the formation of any byproducts. Herein lies the major problem the water treatment industry faces today: although microorganisms can be eliminated or inactivated, harmful disinfectant byproducts (DBPs) may be formed. Concern about DBPs began when trihalomethane (THM), which is the most common DBP, was demonstrated to be carcinogenic in rodents.[17] Later studies further demonstrated that there was association between the consumption of chlorinated drinking water and diseases such as cancer and reproductive effects.[18] Consequently, the U.S. Environmental Protection Agency (EPA) mandated that disinfection techniques for drinking water should control pathogens without increased health risks. Therefore, DBPs have to be monitored in a systematic way to determine the most effective disinfectant technique. With the risk of DBP formation in mind, the properties of each compound can be analyzed and the optimal disinfectant for a specific application can be determined. This section will cover the most commonly used chemical disinfectants, all of which are oxidizing agents (Table 2) or result in ROS. Oxidizing power determines the strength of the compound as a germicide since a stronger oxidizer is more likely to react with pathogens. However, it also increases the chances of reacting with other contaminants in the water and thereby losing efficacy.

Table 2. Half-cell reactants and products with their respective reduction potentials.[19,20]

Half-cell reactants/products	Redox Potential (V)
Chlorine (Cl_2)/Hypochlorous acid (HOCl)	-1.49
Chlorite (ClO_2^-)/Chlorine dioxide (ClO_2)	-0.95
Chloride (Cl^-)/Chlorite (ClO_2^-)	-0.76
Chloride (Cl^-)/Chlorate (ClO_3^-)	-0.62
Iodine (I_2)/Hypoiodous acid (HlO)	-1.44
Iodine (I_2)/Iodate (IO_3^-)	-1.21
Oxygen (O_2)/Ozone (O_3)	-2.07
Water (H_2O)/Hydrogen Peroxide (H_2O_2)	-1.78

A. Chlorine

Chlorine was one of the first chemical disinfectants to be utilized in the beginning of the 20th century to disinfect municipal water supplies in America.[21] Chlorine may be supplied in forms such as chlorine gas (Cl_2), hypochlorite

(OCl^-) salt, and other chlorine compounds. In solution, free chlorine will dissolve via hydrolysis as represented by the following reaction:

$$Cl_2 + H_2O \leftrightarrow HOCl + CI^- + H^+$$

This hydrolysis reaction, which occurs within seconds, forms hypochlorous acid (HOCl), which dissociates in water:

$$HOCl \leftrightarrow OCI^- + H^+ \quad \text{pKa 7.5 (25 °C)}$$

From the reactions above, it is apparent that pH determines the dominant species in water.

Many studies have claimed that HOCl is more effective as a disinfectant than its conjugate base (OCl^-) due to the charge associated with the ionic form, which is unexpected since there is no significant difference in reduction potentials. Some studies concluded that the electric charges that arise from the peptides is what constitutes the first barrier for oxidizing ion penetration.[16] In addition to polysaccharides in the cell walls of bacteria, proteins and glycoproteins are present to provide additional functionalities such as protective properties, virulence, and adherence.[12] However, the germicidal activity governing the efficiency of HOCl and OCl^- is the ability to diffuse through the cell membrane.[22] Because the cell membrane is a lipid bilayer, composed of hydrophobic fatty acid tails, it can be considered impenetrable by charged molecules. Therefore, the neutral HOCl can more readily passively diffuse through cell membranes than OCl^-, enabling attacks inside of the cell.

Once inside the cell, the damages induced by the chlorine (HOCl) involves the reaction with highly nucleophilic sites. These sites, or cellular components, include nucleic acid, bases, amines, amino acids, sulfhydryl groups, porphyrins, hemes and ferredoxin-like iron-sulfur centers.[10,23] Although OCl^- does not enter the cell as often, it still contributes germicidal activity such as oxidizing certain glycoproteins and disrupting the plasma membrane.

One factor to keep in mind is breakpoint addition of chlorine, where all of the oxidant demand has to be addressed before there is residual free chlorine in the system to act as a disinfectant. Some of the oxidant demand includes iron and manganese, which will precipitate out of solution when chlorine is introduced. Then there are the reactions with any organics present in the solution, which may result in toxic byproducts including organohalogens. Examples of toxic organohalogens include trihalomethane and haloacetic acid, which can increase the risk of developing serious diseases such as cancer and adverse reproductive outcomes.[24] There is also the possibility of corrosion and pitting of materials.[25] And finally, there is the presence of organic nitrogen compounds, which exert high chlorine demand. Once those demands have been met, free chlorine would be available as a disinfectant.[21]

Once the right amount for chlorine concentration in the water is achieved, the microbial growth stage requires further consideration. For instance, Lee et al.[26] studied the penetration of free chlorine into a mixed-culture nitrifying biofilm in a flow chamber in comparison to monochloramine (NH_2Cl) under the same conditions. In a biofilm of 2,000 µm thickness, free chlorine penetrated down to about 1,200 µm with evidence of sloughing within the first 24 hours. The chlorine continued reacting its way through the biofilm until reaching the substratum after about 16 days (84% penetration), with a final thickness of about 700 µm. Further characterization determined that there was an increase in the amount of suspended bacteria in the water, after chlorine application, and a linear relationship between the loss of biofilm thickness and time. In contrast, NH_2Cl penetrated much faster (within 2 days) but it took up to 16 days to have a fully nonviable biofilm. The interaction with biofilm was also different in that while chlorine caused sloughing, NH_2Cl increased the biofilm thickness by affecting its matrix, which created pockets where there were no cells present and led to density loss. Though at first glance NH_2Cl effects were more rapid, the less biased reactivity of chlorine with biofilm components led to more active decrease of biofilm density. Such trends have been noted across other studies as well.[27] A final phase of testing, in which NH_2Cl was applied prior to free chlorine, showed that the pattern of effect of chlorine did not change. This response could have been due to the characteristic interactions of NH_2Cl versus chlorine reactivity with biofilm components. Therefore, in consideration of its characteristics, chlorine application would require focus into the ideal disinfection time, concentration, and reaction-diffusion interactions for a more accurate biocidal design.

B. Chloramine

As mentioned above, HOCl can also combine with ammonia and other organic amines to produce chloramines. The pH of the solution will determine which oxidation state of the chloramine is present. The lower the pH, the more oxidized the chloroamine as shown in the following equations[21]:

$$NH_3 + HOCl \leftrightarrow NH_2Cl + H_2O$$

$$NH_2Cl + HOCl \leftrightarrow NHCl_2 + H_2O$$
$$NHCl_2 + HOCl \leftrightarrow NCl_3 + H_2O$$

These reactions compete with each other and the reaction that will dominate depends on the chlorine to nitrogen ratio, temperature, pH, and contact time.[28] Chloramines are less effective, as disinfecting agents, than HOCl due to their lower oxidation potential. This is due to their stability, which can consequently let the disinfectant persist in water as a residual for a longer time, a good quality for a secondary disinfectant.[29]

Chloramines are believed to be poor disinfectants and there are many studies on their chemistry. Only a few have demonstrated the germicidal role of chloramines. One study found that once NH_2Cl penetrated the bacterial cell, it could react with the amino acids cysteine, methionine, cystine, and tryptophan.[16] Other studies cite the interaction between cysteine and NH_2Cl, suggesting that thiol groups are required for the oxidation of the bacteria to be successful.[30] Thus, it is implied that chloramines inactivate pathogens by interfering and inhibiting proteins and mediated processes. Another study concluded that organic chloramines had very little to almost no disinfection potential, hence, not contributing to the disinfection process.[28] There was a study in which NH_2Cl was tested against biofilm penetration.[26] Under similar high free chlorine conditions, NH_2Cl reached 90% penetration of a thick biofilm in 2 days, versus the 16 it took for chlorine application to reach 84%. However, some cells were still viable and it took a full 16 days for all cells to be nonviable. The application of NH_2Cl also changed the outer layer of biofilm, creating a "fluffy slime" layer of loosely bound cell-less matrix, which led to an increase in biofilm thickness and a decrease in density over time. However, this effect was not noted in any other studies. Overall, though chloramines were shown to have fast penetration times, the cell viability within the biofilms decreased at a slower pace. Therefore further research into reactive interactions would be required to fully understand NH_2Cl effects on the biofilm matrix if desired as a biocidal choice.

C. Chlorine Dioxide

Chlorine dioxide (ClO_2) is a more powerful oxidizing agent than chlorine. The advantage is that the disinfectant is a longer lasting residual since it is less pH-dependent than chlorine and does not combine with ammonia to a significant extent. This disinfectant is more expensive than other chlorine-based techniques because it is unstable and has to be generated on site. ClO_2 can be generated by reacting chlorine or acid with sodium chlorite ($NaClO_2$)[31]:

$$2ClO_2^- + HOCl + H^+ \rightarrow 2ClO_2 + Cl^- + H_2O$$

ClO_2 can also be formed by reacting hydrochloric acid with a chlorite salt:

$$5NaClO_2 + 4HCl \rightarrow 4ClO_2 + 5NaCl + 2H_2O$$

ClO_2 will then decompose in water to ClO_2^- and ClO_3^- if there are no oxidizable compounds present.

Another advantage of ClO_2, as compared to free chlorine (HOCl), is that there is no formation of organohalides. This is due to the chemical tendency of ClO_2 to react as a free radical, as compared to the oxidative substitution and addition reaction of free chlorine.[32] Therefore, it would be best to use ClO_2 to treat water with a high concentration of organics.

ClO_2 reacts via free radical electrophilic abstraction and can therefore chemically disrupt the bacterial cell wall.[33] A previous study indicated that ClO_2 did not have any significant reaction with proteins or nucleic acids.[34] The study confirmed that ClO_2 additionally interfered with essential protein formation, though the mechanism was unclear. Bas et al.[35] suggested that ClO_2 biocidal ability was due to interaction with the protein synthesis pathway via inhibition enzyme interactions or through interference of nucleic acid-amino acid complexes. Efficacy against biofilms has also been shown in several studies. According to Simpson et al.,[36] ClO_2 is superior to chlorine in the penetration and dispersal of biomass as well as in slowing down re-establishment of the biofilm. This was corroborated by a study done by Gagnon et al.[33] comparing ClO_2^- and ClO_2 effects on heterotrophic bacteria grown from tap water taken from Nova Scotia. They determined that, regardless of the type of coupon the biofilm was grown on and with similar dosage, chlorine dioxide was more effective. However, for an improved log reduction, higher doses of chlorine dioxide seem to be necessary and require more time.[37]

D. Iodine

Iodine (I_2) is a cost-effective and simple disinfectant known to be fungicidal and virucidal as well as bactericidal.[38,39] However, there are concerns over the maximum safe I_2 dose and duration of use. Iodine disinfectant

products fall into either iodine solutions or iodine resins.[40] Iodine solutions include the solvation of compounds such as salts (potassium iodide), organic iodine compounds (bisglycine hydroiodide, tetraglycine hydroperiodide) and iodophores, which typically consist of iodine complexed with soluble compounds such as non-ionic surfactants. The second type of iodine systems are iodine incorporated resins, which release iodine at disinfecting levels.[41] An example of an iodophore is the iodine-polyvinyl pyrrolidone complex for the controlled release of iodine used by NASA. Specifically, NASA employs an iodine-poly (vinylpyrrolidone) (PVP) system in the US water recovery system of the International Space Station (ISS).[42]

Iodine chemistry is important when selecting it for specific disinfection applications. Iodine can rapidly hydrolyze in solution between pH 2 and 7 as follows:[43]

$$I_2 + H_2O \leftrightarrow HOI + I^- + H^+$$

Other iodine species, within the hydrolysis reaction, can undergo further reactions depending on the pH such as:[40]

$$I_2 + I^- \leftrightarrow I_3^-$$
$$HOI + H_2O \leftrightarrow H^+ + OI^-$$

Of the four iodine species in solution, I_2 and hypoiodous acid (HOI) are biocidal, with the toxicity ranking: I_2 > HOI > I_3^- > I^-. This is because the free molecular iodine is the main compound responsible for microbiocidal activity, where iodination of significant functional groups of cellular components can potentially inactivate the overall function.

One functional group targeted by iodination is the amine (-NH) groups of amino acids and nucleotides. The iodination results in the formation of N-iodo compounds, where H-bonding is blocked resulting in change of protein structure. Thiol (-SH) is another targeted functional group for iodination since it has a significant role in making disulphide bonds in proteins. Substitution of iodine prevents the formation of secondary protein structures. Iodination causes the amino acid tyrosine to form mono and di-iodo derivatives.[41] The products of this reaction prevent the formation of H-bonds due to steric hindrance of the iodo groups on the phenol functional group. Iodine can also react with unsaturated fatty acids by an addition reaction to olefinic groups.[43] The iodination of fatty acids results in a physical change of the cellular membrane bilayer leading to decrease in fluidity. Disinfectants can be adsorbed onto bacterial cell walls by electrostatic interactions, due to negatively charged cell wall and positively charged disinfectants, leading to membrane disruption. Thus, if a positively charged iodo-complex is chosen, the chances of bacterial wall disruption will be increased.[40]

Of the different iodine forms, povidone-iodine (PI) has seen more development in medical settings. For instance, a review by Bigliardi and colleagues[39] noted that the antibiotic-resistant strain of methicillin-resistant *Staphylococcus aureus* (MRSA) was killed by PI in 20-30 seconds, whereas chlorhexidine did not kill all bacteria and required much longer exposure for maximum effects. Another study by Whiteley et al.[44] found that PI (known as Betadine in this study) affected biofilms of 20 inoculated species in a way that correlated the concentration to effect. Interestingly, they did not encounter an enhanced survival effect on the biofilm. They also found that if isolated, some of the species in mono-cultured biofilms responded differently when Betadine was introduced. However, nutrient availability seemed to influence the biofilm population more than the introduction of Betadine. In a mixed-species biofilm from filtered tap water, run through dental unit water systems, Betadine was successful in reducing the biofilm total viable count 100% and achieved 97.3% reduction of biofilm coverage in 14-day old biofilms after 16 hours of contact time in the tubing. However, material compatibility, toxicity, and potential impracticality of having to take out the biocide (through flushing or filters) has overall made iodine a less desirable biocide for larger scale water systems.

E. Ozone

Ozone (O_3) is a powerful oxidant. It can react rapidly with organic and inorganic compounds. The limiting factors are its short half-life and the need to generate it on-site. It is generated by using a stream of oxygen and by passing an electrical discharge between electrodes.[2] The half-life of ozone in air is approximately 12 hours. When applied in water it depends on the pH, temperature, and presence of organic matter.[19] Ozone's mode of action relies on its ability to readily give up oxygen, which can then be inserted into bonds of organic compounds and result in the production of organic acids, ketones, and aldehydes.[2]

Ozone's mechanism of inactivation of microorganisms remains similar to that of other disinfectants, occurring via redox reactions of important cellular components. For instance, the oxidation of the cell wall causes the cell to rupture, resulting in immediate cell death.[45] The reactive ozone can also randomly react with lipids, proteins and nucleic acids within a cell.[46]

Several studies have shown that, even with the shorter span, ozone can be effective against various phases of bacterial growth. In a study done by Ding et al.[47], samples of municipal drinking water from a treatment plant in southern China were taken to test against several strains of chlorine-resistant bacteria, including spore-forming types. Overall, ozone was most effective at higher concentrations for longer exposure times (4 minutes was the maximum time) for chlorine-resistant bacteria. When effectiveness was compared between the chlorine-resistant bacteria and spores, low ozone doses were better at inactivating the bacteria. It also took a concentration of 3 mg/L (considered a higher dose) for one minute to cause inactivation in a representative sample of *Bacillus cereus* spores, with both structural integrity and genetic fragment damage verified. However, there were several spores, noted during scanning electron microscopy (SEM) imaging, which lacked damage likely due to their thicker coats, higher hydrophobicity, and particle shielding. Therefore, these could be indicative of reintroduction of microbes once ozone disinfection was cut off.

Biofilm removal effectiveness of ozone, according to Tachikawa et al.,[48] is influenced by the conditions of the biofilm structure. Their work utilized *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* to compare inactivation rates of their respective biofilms in a flow-through system. Tap water was dechlorinated prior to application of ozone at different concentrations, and was compared with chlorinated tap water. Overall, the effects of inactivation depended on concentration and exposure time but had a trend of slowing down regardless of concentration, time, or disinfectant type. For ozone, this effect was still noted even when the biocide itself was shown to still have about 50% the initial concentration after 10 minutes. It was suggested that these trends might not line up due to ozone interaction with biofilm components, and therefore less interaction with the bacterial targets. Furthermore, ozone did not affect the two bacterial strains the same. It was shown that for *P. fluorescens*, ozone application caused cell exfoliation, versus the more successful penetration deeper into the biofilm of *P. aeruginosa*. The difference in effect could be due to the different excretions by each bacterial strain, leading to altered surface interactions.

F. Nanomaterials

Nanomaterials are known for their large specific surface areas and high reactivity and therefore are used as catalysts, sensors, and adsorbents.[49] A variety of different nanomaterials have demonstrated antimicrobial properties such as: silver nanoparticles, photocatalytic titanium dioxide, chitosan, and carbon nanotubes. The advantages of these materials are that they are not strong oxidizers, inert, and do not produce harmful disinfection byproducts.[50] The inertness of the materials allows for the potential disinfectant to circulate as a residual in water systems for a longer time, preventing any microorganism recontamination. The proper utilization of nanomaterials requires that they be physically and chemically stable in aqueous solutions. Nanomaterials as disinfectants can fall into one of three categories: metal/metal oxides, naturally occurring biomaterials, and carbon-based engineered nanoparticles.

Silver is one of the nanomaterials that has been historically recognized and used as disinfectant. Although silver, the bulk metal material, has been previously used for disinfection, herein, the focus will be silver solely as nanoparticles. The mechanism of toxicity is still not well understood, as studies have demonstrated that silver ions inactivate various cell functional pathways. Some of the postulated mechanisms include: (1) degradation of lipopolysaccharide molecules by silver nanoparticles and accumulation of nanoparticles to cause large increases in membrane permeability; (2) transport of silver nanoparticles to inside the microbial cell to cause DNA damage; (3) release of silver ion from silver nanoparticles.[49] It has been demonstrated that the released silver ion reacts with thiol groups present in proteins and enzymes, which can have downstream hampering of the DNA replication process.[11,51,52] As mentioned before, the nanoparticles play a role in changing the structure and permeability of cell membranes. Specifically, their structure plays a huge role in their mechanism and efficacy. For instance, silver nanoparticles ranging from 1-10 nanometers (nm) can inhibit certain viruses and particles less than 10 nm and are more toxic to bacteria.

Research by Sheng and Liu[53] on biofilm collected from the Devon Wastewater Treatment Plant in Devon, Alberta, Canada indicated that silver nanoparticle efficacy was dependent on biofilm matrix thickness as well as rate of growth of bacteria. When comparing mature biofilms with an intact extracellular polymer substance (EPS), biofilms with their loose EPS layers removed, and planktonic cells, the fully intact biofilms showed the most resistance to silver nanoparticle application. They suggest that the slower growth rate of some bacteria has been shown to correlate with stronger resistance to antibiotics and could partially explain the biofilm robustness. Also, a mature biofilm might have matrix components which make it hardier than planktonic colonies against nanoparticles. This was supported by the EPS matrix ability of silver nanoparticle sorption detected in the intact biofilms.

Though a variety of nanomaterials are being developed, the more demanding and adaptable nature of biofouling microbes will have to be addressed in such a way as to not be damaging to materials, ecosystems, or humans. Silver shows promise to be effective in the nanoparticle form but requires a method of compensation when used in dirty

wastewater due to the high reactivity of the released ions with immediate environmental components. Furthermore, when Ag^+ is released and reduced to Ag^0, it can plate out on water system materials and further lower the available biocide.[54] If the concentrations of overall silver are raised to compensate, it could create toxicity issues.[55] Therefore, a synergistic approach with other biocides might offer optimized effectiveness, especially when trying to target the microbes protected by a thick EPS matrix.

G. Photocatalysis

This category of disinfectants is unique because it incorporates the combination of nanomaterials, a chemical disinfectant technique, with ultraviolet light (UV) radiation, a physical disinfectant technique. The photocatalysis process consists of using a solid semiconductor catalyst to generate ROS in response to light exposure with a specific wavelength.[46] The ROS species elicit the germicidal effects by oxidizing compounds that come in contact with the activated semiconductor surface where ROS species are generated. Most of the semiconductor metals used for photocatalysis are metal oxides but the heavily studied one is titanium dioxide (TiO_2). The general mechanism of photocatalysis begins with the excitation of an electron to the conduction band by applying sufficient energy (light) to overcome the band gap, leaving behind a hole where the electron once was in the valence band. The generation of the electron-hole pair is the initial step as summarized in the following equation:[46]

$$TiO_2 + hv \rightarrow e_{cb}^- \ (TiO_2) + h_{vb}^+ \ (TiO_2)$$

Where hv represents the energy, e_{cb}^- represents the electron in the conduction band and h_{vb}^+ represents the valence band hole. The following reactions involve the reaction of water or hydroxide ions with holes to produce reactive hydroxyl species due to the donation of one electron to the hole:

$$H_2O + h_{vb}^+ \rightarrow H^+ + \ OH\cdot$$
$$OH^- + h_{vb}^+ \rightarrow \ OH\cdot$$

The hydroxyl radical ($OH^{\bullet}$) then inactivates pathogens due to its highly oxidative nature. According to many researchers, the damage to the cell membrane (extracellular targeting) by photocatalysts may be the most significant step in the inactivation of pathogens. More specifically, lipid peroxidation.[56,46] However, destruction of the lipids correlating with effectiveness of photocatalysis has not been fully elucidated.

Foster et al.[57] did a review in which they state that TiO_2 has been shown to kill bacteria, viruses, bacteriophages, fungi, protozoa, and algae. Rajagopal et al.[58] also showed its efficacy against a biofilm created from stagnant pond water. The coating with subsequent application of polychromatic light was somewhat effective (five-fold decrease in bacterial count) against 45-day old biofilms. However, the presence of peroxide-resistant strains most likely helped prevent the biofilm from being fully destroyed. A study by Polo et al.[59] in which *Pseudomonas aeruginosa* biofilm persisted, after photocatalytic treatment with TiO_2 film or nanopowder, also suggested that the growth stages (from planktonic to sessile) could be changing something within the microbes themselves that increases resistance, since the planktonic cells were almost completely eradicated after 24 hours with TiO_2 thin films. Nonetheless, there is research which also supports photocatalysis as a more successful method of waste water treatment.[60,61]

H. Hydrogen Peroxide

Hydrogen peroxide (H_2O_2) is another strong oxidizing agent. However, it is unstable and difficult to prepare. It has a relatively high cost and concentration that is required to achieve disinfection in a reasonable time, which is why this disinfectant is not typically employed for drinking water treatment.[2] It has been determined that H_2O_2 works better synergistically with other disinfection techniques, rather than as the sole disinfection technique. Hydrogen peroxide can be advantageous because it does not produce any disinfectant byproducts as other disinfectants would, and degrades only into water and oxygen.

$$2H_2O_2 \ \leftrightarrow \ H_2O + \ O_2$$

The germicidal activity of H_2O_2 comes from the formation of hydroxyl radicals in the presence of a catalyst such as iron, via a Fenton's reagent redox reaction,[62] or UV light, via a hemolytic cleavage:[63,64]

$$H_2O_2 + \ Fe^{2+} \ \leftrightarrow OH^- + \ HO\cdot + Fe^{3+}$$
$$H_2O_2 + \ Fe^{3+} \ \leftrightarrow H^+ + \ HOO\cdot + Fe^{2+}$$
$$2H_2O_2 \ \leftrightarrow \ HOO\cdot + HO\cdot + H_2O$$

$$H_2O_2 + hv \leftrightarrow 2HO\cdot$$

These hydroxyl free radicals can then react with thiol groups in proteins, nucleic acids and lipids, and it has been proposed that double bonds are particularly targeted.[11] H_2O_2 may exert either bactericidal or bacteriostatic effects and concentration will depend on the bacterial strain and environmental factors.[65] It has been reported that dormant bacterial spores can be lysed at high concentrations of H_2O_2[66] and it was suggested that this was achieved by degrading the protein from the spore coat.[67]

In biofilms, the ability to induce catalase (such as in *P.aeruginosa*) has been cited as one of the areas of concern in avoiding resurgence of biofilm growth.[68] Overall, efficacy of biocides in destroying the EPS matrix can be considered the main obstacle for success in affecting the colonies protected within. This corroborates results from a study done by Shakeri et al.[69] in which H_2O_2 showed consistently higher kill rates of bacteria in a mixed biofilm, in comparison to sodium hypochlorite (NaClO) at a 1000 parts per million (ppm) dose for an hour (with refreshing of antimicrobial every 20 minutes) in microplate wells. Its ability for biofilm removal correlated with its oxidizing ability and dosage. However, even a 1000 ppm application into the well only led to a 50% reduction of biofilm in comparison to an about 93% removal of *Acinetobacter* single species biofilm.

III. Physical Disinfection Techniques

Physical disinfectation techniques pertain to those that do not involve addition of any chemical to the water to disinfect such as UV radiation, solar radiation, or temperature. These techniques were utilized before the use of chemical disinfectants. Increased concern over DBPs has sparked interest in the use of physical disinfectant techniques again. Due to technological advancements, these physical disinfectant techniques can be manipulated and utilized more efficiently and creatively than before.

I. Ultraviolet Radiation

UV radiation has been increasingly used as a drinking water disinfectant due to its ability to effectively inactivate various microorganisms in water. It has advantages over chemical additives as disinfectants such as no addition of chemicals, no introduction of disinfectant resistant bacteria, and no harmful DBP formation.[70] Some other advantages include: effectiveness at inactivating viruses, spores, and cysts; user-friendly operations; less space required for equipment; and shorter contact time as compared to other disinfectants.[71] The disadvantages of this technique are that the exposed organisms can sometimes repair the destructive effects caused by UV. The turbidity of the water can prevent effective application of UV as the suspended solids do not allow the light to reach microorganisms. Since UV is radiated, there is no residual disinfection and the disinfection can only occur inside the unit containing the UV source. It also does not help to improve taste, odor, or clarity since it specifically targets microorganisms, unlike other disinfectant techniques. The intensity of UV, contact time, and water quality are all factors in determining the percentage of microorganisms destroyed.

UV radiation consists of four wavelength zones: vacuum UV (100-200 nm), UV-C (200-280 nm), UV-B (280-315 nm), and UV-A (315-400 nm).[2] UV-C is the most effective as a disinfectant since nucleic acids absorb UV energy within the range of 240-280 nm. This wavelength range can be achieved by utilizing a low-pressure mercury arc lamp, which radiates a wavelength of 254 nm, falling within the desired range. Low and medium pressure mercury lamps are widely used in water treatment. However, there is still concern with the toxicity and fragility of the lamps. Thus, more recently, there has been an increase in the development and optimization of UV light-emitting diodes (LEDs) as a new source of UV radiation.[70]

The main disinfection mechanism of UV radiation can be explained in terms of the physiochemical changes in DNA.[72] Pyrimidines absorb UV radiation at around 254 nm and can form pyrimidine dimers, which can interrupt base-pairing dimers and block DNA replication. The replication machinery cannot tell which bases to insert opposite of the dimer and will proceed anyways and insert bases at random, inactivating the microorganism.

Elasri and colleagues[73] showed that a biofilm simulant could protect from UV light at various wavelengths by limiting the transmission into deeper biofilm components. And though UV-C and UV-B showed similar damage, the UV-C was better at damaging DNA. Application of UV-A did not show any indication of damage to the *P. aeruginosa* FRD1 strain in the simulant until photosensitizers were added, leading to cell death. Therefore, to overcome potential repair mechanisms for full genetic damage and interaction restrictions, applying UV synergistically with another biocide could increase efficiency.[74]

To overcome the ability of repair, studies are focusing more on the synergistic effect of silver interactions with UV. One study showed how dosing silver nanoparticles with UV-C for different time intervals revealed an unexpected relationship between UV exposure time and biocidal activity.[75] During dosage applications of 20 minutes or lower, biocidal activity was more effective than in samples which had received longer UV application times. Unfortunately this study was not conducted on biofilms, but highlights the importance of considering both the wavelength and dosage time to alter bacterial response. UV-C has also been applied synergistically with nanoparticles in studies aiming to destroy cancer cells. The effect of UV-C application after allowing uptake of silver nanoparticles into human lymphoblastoid TK6 cells was successful in decreasing viability, most significantly noted after 24 hours post-treatment, regardless of nanoparticle concentration.[76] They were also found to be successful against parasites when UV was applied, versus just the nanoparticles themselves.[77] And in consideration of other wavelengths, Zhao et al.[78] applied silver nanoparticles with either UV-A or UV-B against *E. coli* and found UV-A to be more effective, most likely due to the longer wavelengths being able to penetrate deeper. However their work also considered the order in which biocidal activity had the most effect and concluded that though the two could be applied separately, pre-treating the nanoparticles with UV-A was just as effective as adding the nanoparticles in first and then applying UV. Interestingly, they determined that nanoparticles treated with either UV-A or H_2O_2 had similar heightened biocidal activity, which could be attributed to the increased release of Ag^+.

J. Temperature/Distillation

Heating water has been practiced for centuries, as high temperature kills most microorganisms present in water.[79] Temperature plays a role in metabolic rate, hence higher temperature means higher growth rates (doubling every 10°C increase).[80] The optimal metabolic temperature range varies for microorganisms and can be grouped in one of three categories: psychrophilic (0-20 °C), mesophilic (20-42 °C) and thermophilic (42-75 °C).[81] The upper limits of the temperature range is when decay rates start exceeding growth rates. Thus holding water at boiling for 5-10 minutes may be necessary to inactivate all categories of microorganisms and their spores. Distillation is another form of heating where the water is boiled and the vapor escapes and condenses into a new container, leaving behind any solid contaminants.[2] The chemical mechanism of thermal disinfection involves the breaking of the cell membrane/wall constituents and complete degradation of vital cell components.

Thermal radiation (increased temperature) is a very effective technique. However, the power input makes it an inefficient technique to be applied in water treatment. Attempted application as a shock treatment instead has also been shown to not be as effective, therefore making it necessary to apply for longer durations or at higher temperatures for desired effect.[82,83] Newer techniques are looking to raise efficiency by designing with localization in mind, though research into such developments has been focused more on medical applications. Overall, the method utilizes nanoparticles and leads to two localized forms of destruction: hyperthermia (local increase of temperature) and thermoablation through application of optical excitation.[84] And though promising in the medical field, adaptation to water treatment systems would require further study.

K. Solar Radiation

Solar disinfection is an old technique that has been applied since nearly 2000 years ago and is still a powerful disinfection technique today.[85] Solar radiation is a source of thermal radiation as well as UV radiation. As mentioned previously, UV-C is the optimal wavelength to disinfect microorganisms in water due to the formation of pyrimidine dimers causing cascading deleterious reactions. Not mentioned before, is that UV-B wavelengths can play an important role in forming ROS in water.[86] Some of these species include superoxide, hydrogen peroxide, and hydroxyl radical. Formation of ROS occurs due to the absorption of UV-B by endogenous or exogenous photosensitizers such as chlorophylls and quinones. The electronically excited photosensitizer can then indirectly induce cellular damage.[87] This is done either by an electron transfer and hydrogen abstraction from water to form a free radical or energy transfer to free oxygen to form a singlet reactive oxygen.[88] These ROS can then react with cell components resulting in inactivation.

An approximate 1.12 kJ m^{-2} of thermal energy is received on a horizontal surface at ground level on the equator and this value reduces as the latitude increases away from the equator.[85] However, temperatures required to elicit germicidal effects are not achieved solely by exposure to the sun. Rather, thermal enhancement and modification to the container of the water is required and can be achieved by using solar reflectors or some type of black surface to absorb solar energy, where the combination of the two can result in an increase of 15 °C.[89]

Novel techniques to harvest solar energy and convert it to steam are currently being explored for water purification. Ideally, a direct liquid to gas phase change, without the process of temperature increase, would be desired for solar steam generation.[90] Thus, solar steam can be generated by optical absorption at the surface, photothermal conversion,

confinement of the heat at the evaporative surface, and transport of water from the bulk to the surface.[91] Although many materials have been explored such as, nanoparticles, nanowires, nanosheets, and gels, graphene oxide aerogels have demonstrated ideal properties for such an application.[92] Graphene oxide has properties such as superior thermal conductivity, excellent light absorption and low molar specific heat, making it an ideal candidate for efficient photothermal conversion.[93] The aerogel of graphene oxide demonstrates the optimal structure due to its abundant pores, ability to float, high light absorption, and the required capability of transporting water from the bulk to the surface.[90] The disinfectant ability of this technique was demonstrated by Zhang et al.[94] where they were able to purify clean water from seawater with a bacteria count below the national drinking water standard for the People's Republic of China (GB5749-85). Further work by Wang et al.[95] developed the material for longer term application through addition of zinc oxide nanoparticles for enhanced anti-biofouling characteristics. When compared to a double-layered graphene oxide-chitosan scaffold, their scaffold with zinc oxide nanoparticles did not show the same biofilm growth noted in the control material pores after 72 hours. Therefore, through further adaptation and optimization of the nanoparticles, this could become more widely used as a safer technique.

IV. BIOLOGICAL DISINFECTION TECHNIQUES

Biological disinfection techniques involve the manipulation of microorganisms in the wastewater to prevent pathogenic contamination. Although these techniques are still in the early stage of development, they have demonstrated potential for efficient removal of pathogens with further development.

L. Membrane Biofilm Reactors

Conventional activated sludge was patented in 1913 as a process that relies on microorganisms in water, with introduced air, to break down organic pollutants.[96] Activated sludge is a floc matrix, essentially a biofilm suspended in water rather than on a surface, and consists of EPS excreted by the microorganisms of the floc. The EPS is integrated with exo-enzymes and ecto-enzymes, which are found to be the rate-limiting step for the mineralization of organic matter.[97] Yu et al. investigated the distributions of enzyme activities in the sludge to understand the biological processes.[98] They determined that protease enzymes (which catalyze breakdown of proteins) were limited and bound to the cell surface. Therefore, protease is considered to be an ecto-enzyme. Whereas, α-amylase (which catalyzes the hydrolysis of polysaccharides) and α-glucosidase (which catalyzes complex carbohydrates) were determined to be freely immobilized within the EPS matrix, hence exo-enzymes.

The biosludge is important to be present as it decomposes organic material in wastewater. However, the disposal of this sludge could be another problem.[99] The grazing of predatory microorganisms such as protozoa on bacteria helps to maintain and reduce biomass in biosludge and prevents the contamination of downstream processing. The formation of a floc protects bacteria from being consumed by predatory microorganisms. Since the floc and predatory microorganisms are both required for activated sludge to result in an efficient technique, a two-stage system has been implemented.[100] The first stage consists of a mixed reactor, without biomass retention and short solid retention time, to promote growth of dispersed bacteria and prevent predator growth. The second stage comprises a biofilm process which can ensure the optimal conditions for the growth of predators.

A membrane biofilm reactor process consists of a biological reactor that is integrated with microfiltration membranes.[101] This technique combines the filtration of membranes, which prevent the passage of solids and microorganisms, and the disinfection process provided by microorganisms. The activated sludge in this particular technique is in the form of a biofilm that forms on the filters as microorganisms get trapped during passage. It is not until the biofilm is formed that increased virus filtration is observed, due to the ability of the biofilm gel to chemically adsorb or degrade via biological predation.[102] Sludge production by this method is decreased by 28-68% depending on the age of the sludge used.[99] Sludge age is a sensitive parameter, where a longer sludge age may limit oxygen transfer and a shorter sludge age may not be effective in filtering and removing smaller microorganisms such as viruses. However, inactivation of an already established biofilm is therefore not possible unless it is mechanically scraped off, detached, or reaches the stage of maturity in which it begins to slough off on its own.

M. Nutrient Removal

Organic matter is typically regarded as the primary limiting nutrient in water for bacterial growth but other factors can affect the growth of microorganisms in drinking water. For instance, phosphorus is known to cause eutrophication of surface waters which can lead to algal blooms and "dead zones."[103] Studies have demonstrated the effects of phosphorus on microbial growth in drinking water because it is an essential element and a vital component of DNA

and adenosine triphosphate (ATP).[104,105] Thus, as a preventative measure, rather than a disinfectant treatment, phosphorus can be removed from water. Phosphorus is currently removed from water by chemical precipitation and there is an increasing interest towards biological phosphate removal.[106]

Chemical precipitation of phosphorus has been implemented since the first use in Switzerland during the 1950s.[107] It involves the addition of divalent or trivalent metal salts to wastewater to precipitate phosphorus out, which then settles and gets removed from water via sedimentation. Some examples of precipitating metals include iron, calcium and aluminum. One advantage of this process is that it can be implemented at various steps of water processing. One disadvantage includes recyclability of the excess sludge after it has been removed from water because the water needs to undergo further treatment before it can be returned to the environment.[108]

Biological phosphorus removal was introduced in the later 1950s where it was found that activated sludge (as discussed briefly above) could uptake phosphorus in excess.[107,109] To apply this technique, anaerobic zones are required before an aerobic zone in the activated sludge. In the former zone, bacteria can process and remove available organic compounds and even release phosphorus. In the latter zone, bacteria can remove, process, and store the phosphorus.[110,111] An advantage of this technique is the bioavailability of phosphorus compared to that from chemical precipitation. For instance, one study proposed a method of releasing polyphosphate (a linear form of inorganic phosphate linked by high-energy phosphoanhydride bonds) from activated sludge simply by heating at 70 °C for 1 hour and further release of polyphosphate by precipitation via addition of calcium chloride.[109] However, the disadvantage of the biological removal is the inconsistency and lack of control of the process. But if understanding of the biofilm growth stages and responses to nutritional effects could be developed, processes could be optimized. For instance, it could be determined at which stages of growth biofilms are most vulnerable to enhance estimation of the timespan between biocidal applications.

Table 3 presents a summary of the above disinfection methods, their respective mechanisms, and their advantages and disadvantages. Chemical disinfection techniques are in purple, physical disinfection techniques are in blue, and biological disinfection techniques are in green.

Table 3. Summary of disinfection methods with respective mechanisms, advantages and disadvantages. Chemical disinfection techniques are in purple, physical disinfection techniques are in blue, and biological disinfection techniques are in green.

Disinfection Method	Mechanism	Advantage	Disadvantage
Chlorine	• Oxidizes cell components	• Reduction of microbial counts • Residual level of disinfection • Simple, versatile, and cheap	• Can react with naturally occurring acids to produce toxic byproducts • Pathogen resistance development • Can create objectionable smell and unpleasant taste
Chloramines	• Inactivation of energy-producing enzymes Inhibition of respiration processes	• Lower heterotrophic plate counts • Less taste and odor • Ability to penetrate biofilms • Does not originate THMs • Residual effect	• Ratio between chlorine and ammonia species at dosage step must be carefully controlled to prevent risk of nitrification (nitrification can increase level of nitrites and promotes microbial regrowth)
Chlorine Dioxides	• Inhibition of respiration processes • Adsorption and penetration of protein capsomeres in viruses	• Very persistent in water • Does not originate THMs • Reduces halohydrocarbon compounds • Can react with virus internal RNA	• Destroys phenols but not ammonia • Preparation and control operation are complex
Iodine	• Iodination of essential molecules in cell • Conformational changes in virus protein coats	• Simpler to use than chlorine • Less sensitive to pH, organic content, nitrogenous compounds, and high turbidity • More effective against viruses: enhanced iodination of tyrosine • Decreases risk of disease due to deficiency of iodine • Has better stability of chemical storage characteristics	• Allergic reaction sensitivity in humans • Recommended dietary allowance of iodine variation in humans • High cost • Difficult to apply to large water systems • Objectionable taste
Ozone	• Ozone is formed by exposing oxygen to high voltage source • Promotes oxidation processes	• More effective as disinfectant than chlorine for viruses • Particularly effective against spores and cysts • Avoids the production of THM • Strongest oxidants available • Efficiency independent of pH range	• No residual levels of disinfectant after ozone turns into oxygen • Changes surface charges of dissolved organics and colloidal suspended particles causing coagulation • Difficult to apply and control • Non-absorbed ozone needs to be destroyed or recycled • Byproducts may be more toxic than original contaminants • Can break down complex organic matter to form compounds that can enhance microbial growth
Ag Nanoparticles	• Interferes with metabolism	• More design freedom • Easily incorporated into ceramic membranes during water filtration	• Can cause argyrosis • General toxicity, ecotoxicity and unknown effects of metals to metabolism • Accumulation of silver in motor function organs
Hydrogen Peroxide	• Produce free hydroxyl radical	• Works well synergistically, such as with ozone	• Instability and difficulty of preparing concentrated solutions have limited use
Photocatalysis	• Generation of oxidizing species like hydroxyl radical or superoxide	• Method to treat water without adding chemicals • More controlled process	• More maintenance may be required
UV/Solar	• Causes irreparable damaging to microbial nucleic acid and makes them unable to replicate	• Able to inactivate protozoa but at a higher dose than other pathogens • No physiochemical features of water are changed • No chemical agents • No residual so no production of THM	• Photoreactivation effect • Can result in formation of ozone or radical oxidants • Requires electrical source to operate • Not suitable for water with high turbidity
Temperature/ Distillation	• Heating up from 80 °C to boiling: • Disruption of metabolic processes, plasma membrane, denaturation, inactivation of enzyme • Distillation to remove steam but leave behind solid remnants of microorganism	• Can be heated below boiling but for a longer time • Distiller materials can be sanitized and kept contaminant free • Allows storage without concern of recontamination • Distillation removes pathogens, dissolved minerals, and salts	• Needs to be heated for 5-10 minutes to inactivate spores • Expensive method for large-scale use or as an alternative to physical disinfection • Some contaminants such as volatile compounds that have boiling points close to water may still remain
Membrane Biofilm Reactors	• Consists of two facets of treatment: • Activated sludge (physically blocking membrane pores, allowing chemical adsorption, and biological predation of phages) • Membrane separation (filtration of microbes by size)	• Reduced chemical dosing requirements • Economic and environmental benefits • Membranes can further be functionalized with nanomaterials to render them antimicrobial	• Microbial regrowth • Membrane imperfections and breaching • Membrane cleaning • Optimal pathogen removal depends on the vitality of the membrane biofilm
Nutrient Removal	• Removes key nutrient from water	• Easy to implement • Can be versatile • Required nutrients can be determined for each type of microorganisms; tailored targeting	• Nutrient precipitation requires addition of chemicals • Removal of precipitate requires additional parameters • Recycling the nutrients is stressed by the industry and techniques to do so are required

V. Conclusion

This review focused on the three fundamental water disinfection techniques: chemical, physical, and biological. Mechanisms, advantages, disadvantages, and specific properties of each major technique, including their studied effect on established biofilms. Current methods of wastewater treatment can individually have various advantages and disadvantages, based on the water quality, local environment, and other parameters. Such approaches are relying more on the acute interactions between bacteria populations and biocides instead of overwhelming the target microorganisms with high concentrations alone. However, weakened biocidal abilities, due to impurities in the water interacting with the biocides, is a persistent problem. Furthermore, biofilm-protected bacteria tend to survive higher doses of a single biocide and can potentially adapt, making it less likely for one biocide to maintain efficacy for an indefinite period of time. Nevertheless it is possible to make up for the shortcomings of one biocide through synergistic approaches with multiple biocides. For instance the synergistic relationship between UV light and silver nanoparticles, or biofilm growth rate influenced via nutrient removal for optimal biocide application. Through several different microbial interaction mechanisms, a higher rate of disinfection in a system and therefore lower biofouling ability could be achieved.

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