Chapter 1 Introduction

1.1 Research background

1.1.1 Significance of wastewater reclamation and reuse

Water is a basic human need, whereas one in three people do not have reliable access to safe drinking water worldwide every year[1]. The contaminated drinking water has become one of the main transmission pathways of waterborne pathogens leading to diarrheal, malaria, and gastroenteritis[2-3]. Thus, waterborne illnesses remain a huge threat to public health, especially in the developing areas where sanitation facilities and gird power are not available or in developed areas that suffer from natural disasters (e. g. , earthquakes and tsunami)[4-5]. More specifically, as a representative of developing countries with the most population, China is a severely water-deficient country. The per capita water resource in China is only 2300 m^3, which is 25% of the world's average level[6]. At the same time, there are serious water pollution problems in China, which have caused poor quality water and further exacerbated water shortages[7]. In 2016, more than 23. 1% of rivers were polluted, including 9. 8% of severely polluted rivers. The pollution of lakes is even more severe, with 17. 8% of lakes severely polluted[8]. In light of the current severe water shortage problem, it is of great significance to develop unconventional water sources to support traditional water sources to relieve the shortage of water resources.

Wastewater reclamation has become an important way to solve the current challenge of water shortage in developing countries[9]. Given that urban sewage has the characteristics of easy access, stable water quality, and low cost of reuse, wastewater reclamation and reuse can be low cost and high efficiency, which has become an effective unconventional way to supplement water resources[10-11]. In addition, in the process of wastewater reclamation, the discharge of pollutants can be reduced effectively[12]. At present, wastewater reclamation and reuse have been widely applied in urban use, agricultural irrigation, industrial water, and drinking reuse in China[13-14]. At the same time, other countries have successfully applied the deep reuse of wastewater to direct drinking reuse. China's 13th Five Year Plan clearly stated that wastewater reclamation and reuse should be applied as an effective way to supplement urban water sources. The Plan pointed out that by the end of 2020, Beijing's wastewater reclamation rate should reach 68% and the wastewater reclamation rate of other provinces and cities should strive to reach 15%[13,15]. Wastewater reclamation has broad practical engineering applications, whereas the current large-scale and in-depth wastewater reclamation raises the concern for higher safety requirements.

1.1.2 Necessity of wastewater reclamation and reuse

Pathogenic microorganisms exist inevitably in municipal wastewater and may cause various diseases and pandemics[16-17]. Pathogens in the wastewater have the characteristics of multiple biological species, high concentration levels, and high infection risks. Thus, the control of pathogens has become a key issue that needs to be paid attention to during wastewater reclamation[18-19]. Table 1.1 summarizes the concentration and risk of various pathogenic microorganisms in the undisinfected effluent from municipal wastewater treatment plants.

Table 1.1 Concentration and risk of pathogens in effluent of municipal wastewater treatment plants before disinfection

Pathogens	Concentration	Potential risk	Reference
Bacteria/(count/100 mL)			
Enterococcus faecalis	$900\sim10^4$	Intra-abdominal infection, urinary tract infection	[20]

Continued

Pathogens	Concentration	Potential risk	Reference
Clostridium difficile	$50 \sim 10^5$	Diarrhea	[21]
Salmonella	$\approx 1.0 \times 10^2$	Food poisoning	[22]
Clostridium perfringens	$\approx 9 \times 10^3$	Enteritis, food poisoning	[23]
Pseudomonas aeruginosa	$\approx 1.1 \times 10^4$	Lung infection	[24]
Viruses/(copy/mL)			
Adenovirus	$10^3 \sim 10^5$	Respiratory tract infection, gastrointestinal infection, urinary tract infection	[25]
Norovirus	$10 \sim 10^3$	Acute gastroenteritis	[26]
Rotavirus	$0 \sim 10^3$	Diarrhea, gastroenteritis	[27]
Enterovirus	$10^3 \sim 10^5$ (PFU/mL) *	Gastrointestinal infection	[28]
Protozoa/(count/100 mL)			
Giardia	10～2500	Diarrhea, vomiting, fever	[29]
Cryptosporidium	0～100	Diarrhea, vomiting, fever	[30]

* A plaque-forming unit (PFU) is a measure used in virology to describe the number of virus particles capable of forming plaques per unit volume.

Based on the infection probability of one single exposure of pathogens, the World Health Organization (WHO) calculates the infection risk of pathogens in reclaimed water. Several pathogens showing a high infection risk exit in reclaimed water. Disinfection can inactive pathogenic microorganisms and control the biological risks of pathogens in the process of wastewater reclamation effectively. It has become an indispensable process to ensure the safety of the reclaimed wastewater[31].

1.1.3 Challenges of the existing disinfection technology

During wastewater reclamation, microbial disinfection has become the essential method to control the biological risks and ensure water safety. Table 1.2 summarizes the conventional disinfection technologies applied in wastewater reclamation. Chlorine disinfection has become the most widely used water disinfection technology attributed to its low operating cost, good microbial disinfection effect, and constant disinfection performance. However, the harmful carcinogenic disinfection by-products

(DBPs) produced in the chlorine disinfection process will involve new health and ecological risks inevitably[32-34]. In addition, the disinfection effect of chlorine disinfection on protozoa (i. e., giardia and cryptosporidium) is relatively limited[35]. Emerging disinfection technologies such as UV disinfection can effectively control the generation of DBPs during the operation process and effectively inactivate the pathogenic microorganisms in water. However, after UV disinfection, the inactivated bacteria will regrow or reactivate[36-37], and UV disinfection has a relatively low efficiency toward viruses[38-40]. Other disinfection technologies, such as ozone disinfection and chlorine dioxide disinfection, need to be prepared in-situ, which poses a potential hazard for chemical leakage[41]. In addition, ozone and chlorine dioxide can easily react with halogen and bromate substances in the water to generate carcinogenic DBPs[42]. Due to the strong oxidizing properties of ozone, macromolecular organic substances in wastewater can be degraded into small molecular substances that are easy for bioavailability, increasing the risk of harmful microorganism growth[43]. Membrane filtration technologies including microfiltration and ultrafiltration can effectively separate pathogenic microorganisms in water, whereas their high operation and maintenance costs and easy clogging properties hinder the large-scale application in actual situations[44-46].

Table 1.2 Technical and economic comparison of conventional wastewater disinfection methods[44]

Feature	Chemical-based disinfection			Physical-based disinfection		
	Cl_2	O_3	ClO_2	UV	Micro-filtration	Ultra-filtration
Bacterial disinfection performance	++	++	+++	+++	+++	+++
Viral disinfection performance	++	++	+++	+	+	+++
Protozoa disinfection performance	+	++	++	+++	+++	+++
Bacterial regrow	−	+	+	++	−	−

Continued

Feature	Chemical-based disinfection			Physical-based disinfection		
	Cl_2	O_3	ClO_2	UV	Micro-filtration	Ultra-filtration
Decolorization and deodorization property	++	+++	+++	−	−	++
Toxicity of DBPs	+	++	++	−	−	−
Constant disinfection property	++	−	+	−	−	−
Operation and maintenance costs	++	+++	+++	++	+++	+++

−no impact, + low, ++ medium, +++ high.

1.2 Electroporation disinfection

1.2.1 Electroporation for biomedical application

Electroporation is a physical process that relies on a strong electric field to damage the outer structure of microbes (bacterial membranes and viral capsids). During the electroporation process, the permeability of the microbial outer structure increases due to the strong electric field applied[47-50]. Electroporation can be divided into reversible and irreversible electroporation. With the relatively low external electric field ($10^4 \sim 10^5$ V/m), the reversible electroporation holes generate on the cell membrane which can automatically recover after the electric field disappears. Based on this phenomenon, biomacromolecules such as plasmids, proteins, and DNA that can not enter the cell through the membrane can be quickly involved in the cell during the reversible electroporation process while maintaining the cell viability[51-52]. As a result, reversible electroporation technology is widely applied in gene transduction and cell fusion[53-54]. On the other hand, when the external electric field strength is high enough ($>10^5$ V/m), the electroporation hole on the cell membrane increases and expands, causing irreversible cell damage and eventually leading to cell death[55-56]. Irreversible electroporation has the advantages of rapid and high efficiency and has been applied in cell inactivation and tumor therapy (Figure 1.1)[57-59].

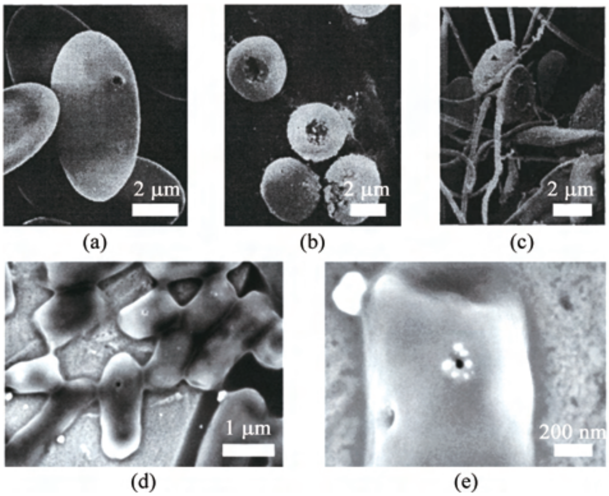

Figure 1.1 Scanning electron microscopy (SEM) images showing cell morphologies after electroporation operation

(a),(b) Red blood cells; (c) Sperm; (d),(e) *Escherichia coli* (*E. coli*)

1.2.2 Electroporation for water disinfection

Based on the phenomenon of irreversible electroporation, electroporation microbial disinfection in water has been demonstrated with bacteria, protozoa, and viruses (Table 1.3)[60]. Compared to traditional bacteria inactivation methods (e.g., thermal, chlorine, and ultraviolet radiation), electroporation disinfection has the following advantages: ① It is extraordinary fast (6 log inactivation can be achieved within 100 ms); ② No chemicals are added and no disinfection byproducts are generated during treatment; ③ It is effective to all bacteria, including "superbugs" that are resistant to multiple antimicrobials; ④ Bacteria do not develop resistance. Electroporation disinfection is also distinct from electrochemical processes where inactivation is due to in-situ generated antimicrobial chemicals (e.g., reactive oxygen and chlorine species) and/or direct oxidation on the electrode surface[61].

Table 1.3 Microbial inactivation using electroporation disinfection

Treated microbes	Energy consumption /(J/mL)	Disinfection performance	Reference
Enterococcus faecium	120～240	>4.0 log	[62]
Pseudomonas aeruginosa	84～190	>2.2 log	[63]
Escherichia coli	80～120	>4.5 log	[64]
Listeria	40～120	>1.5 log	[64]
Saccharomyces cerevisiae	40～80	>6.0 log	[64]
Bacillus	40～80	>3.5 log	[64]
Pseudomonas putida	120	>4.0 log	[65]
Giardia*	≈71	>2.0 log	[66]
Cryptosporidium*	≈78	>90%	[67]

* The electroporation disinfection operation toward Giardia and Cryptosporidium was assisted by chlorine.

Current electroporation disinfection methods can achieve pathogen inactivation in water within a short contact time under specific conditions, whereas to generate a strong electric field, voltages as high as 1～10 kV are normally required, which results in high running cost and safety issues concerns. In addition, it is necessary to reduce the electrode spacing for a sufficient electric field. In the actual situation, small electrode spacing increases the construction footprint of the reactor and the cost of the overall facility. It is more likely that the particulate matter will be blocked between electrodes, leading to short circuits and potential safety hazards[68-69]. Therefore, electroporation disinfection has not been successfully implemented so far and the technical limitations to achieve a high-strength electric field without the operational risk at a large scale have become the major obstacle.

1.3 Current research status of novel electroporation disinfection

1.3.1 Nanowire-assisted electroporation for water disinfection

The rapid development of nanomaterial technology provides new opportunities for solving the technical limitations in the electroporation disinfection. Nanomaterials are specific materials with dimensions (i.e., length, width, height, diameter, and/or thickness) less than 100 nm. Due to their small size, large specific surface area, and sufficient active sites, nanomaterials are widely applied in material science, life, chemistry, and environmental fields[70-71]. Among a variety of nanomaterials, nanowires are special materials whose length is in the micron level and the diameter is in the nanometer level[72-73]. Due to their extremely high aspect ratio, nanowires show unique optical, electrical, and biological properties[74-75]. When the nanowire is placed vertically on the surface of the electrode and connected to the power supply, its unique lightning rod effect can promote the formation of a strong electric field in the tip area[76]. Even when the external voltage decreases as low as 1 V, the electric field near the tip of the nanowire can still reach 10^5 V/m, which is sufficient for irreversible electroporation (Figure 1.2)[77].

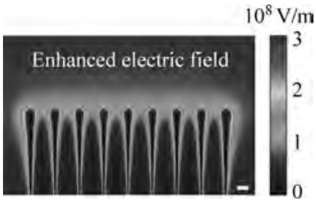

Figure 1.2 Simulation of electric field distribution near the nanowire tip structure (diameter: 50 nm; length: 5 μm) with 1 V external voltage, showing the enhancement of the localized electric field. The scale bar is 500 nm[78] (see the color figure before)

Electroporation-based microbial disinfection has been demonstrated with bacteria, protozoa, and viruses using different nanowire-modified electrodes (Table 1.4). However, the practical application of electroporation disinfection technology is still limited due to the following problems: First, the external voltage is still high. During the electroporation disinfection process, when the DC power supply voltage is higher than 10 V, the microorganisms in the water can be efficiently inactivated in a short time. However, the continuous 10 V direct current (DC) voltage can cause a significant electrolysis water reaction, leading to unnecessary energy consumption. In addition, for wastewater containing Cl^-, chlorine is generated during the electrolysis process, which increases the risk of generation of harmful DBPs. Second, the current studies commonly test the disinfection performance in synthetic water samples, and there is a lack of systematic research on the impact of water quality of reclaimed water in actual situations for electroporation disinfection. Third, some studies integrate nanowire-assisted electroporation disinfection with chemical electrocatalysis, where the mechanism and contribution of nanowire electroporation to microbial disinfection are still unclear. Fourth, during the electrode fabrication process, some nanowires are fixed on the electrode surface by adsorption, and the adhesion is weak, which is easily detached during operation. The detachment of nanowires from electrodes not only causes the release of nanomaterials to the effluent involving potential ecological risks but also affects the lifetime of the electrode.

Table 1.4 Applications using nanowire-assisted electroporation for water disinfection

Electrode materials	Power supply	Disinfection performance	Contact time	Reference
Ag nanowire-modified carbon fiber	DC supply 20 V	>98%	10 s	[79]
Ag nanowire-modified sponge	DC supply 10 V	>6.0 log	1 s	[77]
TiO_2 nanotube-modified electrode	DC and AC supply 2 V	>80%	15 min	[80]
CuO nanowire-modified cupper mesh	Triboelectric charge 1000 V, 1 μA	>6.0 log	1 s	[81]
ZnO nanowire-modified carbon cloth	Triboelectric nanogenerator 50 V, 1 μA	>6.0 log	10 s	[82]

In summary, the primary concern for the practical application of nanowire-assisted electroporation disinfection is to optimize the reactor design and electrode preparation method for a higher disinfection efficiency and longer electrode lifetime. A reasonable reactor design can significantly increase the disinfection performance of electroporation as well as reduce the external voltage required for microbial inactivation. When the external voltage is as low as 1 V, no electrolysis reaction occurs during the disinfection process, which can significantly reduce the potential for generating harmful DBPs. In addition, a feasible nanowire-modified electrode fabrication process can ensure a higher electrode stability for a longer lifetime and a trace nanowire release.

1.3.2 Current reactor for nanowire-assisted electroporation disinfection

During the nanowire-assisted electroporation disinfection process, commonly, the nanowire needs to be vertically loaded on the electrode surface and an external voltage is necessary to connect with two electrodes for the power supply. When the external power supply is turned on and the water flows through the electrodes, the bacteria in the water approach the surface of the electrode, and irreversible electroporation occurs in the area where nanowires exist. Due to the enhanced electric field only existing near the nanowire tip, unless the microbes approach the tip structure, disinfection won't occur[47,60]. Thus, the microbial transport process is the speed imitating step of nanowire-assisted electroporation disinfection. Rational reactor design can effectively accelerate the process of bacteria approaching the electrode surface. However, the studies for reactor design are limited in the current research for nanowire-assisted electroporation disinfection technology.

The reactor design can refer to the reaction device involved in the treatment of pollutants degradation in water using electrochemical processes. Common reaction devices show potential to be applied in nanowire-assisted electroporation disinfection mainly include the stirred reactor plug-flow reactor, and flow-through reactor (Figure 1.3)[68,83,84]. The stirred reactor can be classified into two groups based on the operation mode of applied materials: complete mixing stirred reactor with particle-sized nanomaterial complete mixed with microbes and electrode stirred reactor with nanostructures immobilized or grown on panel electrodes. In a stirred reactor, rapid stirring is commonly required to drive the microbes to approach the electrode surface. Considering the