Hybrid Disinfection of Sewage Using Chlorine and UV/Ozone in Series to Optimize the Process

This thesis is submitted as a partial fulfilment of the PhD programme in Science

by

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CERTIFICATE

This is to certify that the thesis entitled "**Hybrid Disinfection of Sewage Using Chlorine and UV/Ozone in Series to Optimize the Process**" submitted by **Mrs. Kavita Verma,** to the Malaviya National Institute of Technology, Jaipur for the award of the degree of **Doctor of Philosophy** is a bonafide record of original research work carried out by her. She has worked under our guidance and supervision and has fulfilled the requirement for the submission of this thesis.

The results contained in the thesis have not been submitted in part or full, to any other University or Institute for the award of any degree or diploma.

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DECLARATION

I hereby certify that the work which is presented in this thesis entitled "**Hybrid Disinfection of Sewage Using Chlorine and UV/Ozone in Series to Optimize the Process**" in partial fulfilment of requirements for the award of Doctor of Philosophy, in the Department of Chemistry, Malaviya National Institute of Technology, Jaipur, is an authentic record of my own work unless otherwise referenced or acknowledged. The thesis was completed under supervision of Prof. K. D. Gupta, Department of Chemistry, and Prof. A. B. Gupta, Department of Civil Engineering, Malaviya National Institute of Technology, Jaipur. The results presented in this thesis have not been submitted in part or full, to any other University or Institute for award of any degree. The content of this thesis has been checked using online software 'Turnitin' which shows 13% similarity index.

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Dedicated to my Parents and Husband

List of Publications

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4. K. Verma, K.D. Gupta, A.B. Gupta, "Optimization of Chlorination Process and analysis of THMs." *Journal Clean- Soil, Air, and Water of Wiley*, 2015. (Under Review)

Conferences

1. K. Verma, P. Shukla, A.B. Gupta, K.D. Gupta, "Biological analysis and effect of disinfection on secondary treated effluent of RBC (MNIT), Jaipur." *The 3rd Environment Asia International Conference on "Towards International Collaboration for an Environmentally Sustainable World",* Thai Society of Higher Education Institutes on Environment, Thailand, 17-19 June, 2015.
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Abstract

Reuse of treated sewage for irrigation is widely practiced to meet the ever growing demand for water across the globe. Sewage contains a range of pathogenic microorganisms and conventional treatment processes are insufficient to meet the existing WHO norms for wastewater discharge or reuse (1000 CFU/100 mL for TCs). Therefore disinfection is required.

The present study assesses the effectiveness of all the three conventional disinfectants such as chlorine, ozone and Ultraviolet (UV) radiations against dominant coliform species in the secondary treated sewage collected from rotating biological contactor of Malaviya National Institute of Technology (MNIT), Jaipur. Resistance to low chlorine dose was observed among *Serratia/Hafnia* and *Enterobacter*, which resulted in its excessive doses up to 80 mg-min/L for meeting the WHO standards, which leads to the formation of high concentrations of disinfection byproducts causing negative environmental consequences. Ozone was considered as an alternative to chlorine, which is highly effective disinfectant having lower concentration time for TCs (30 mg/L compared to 80 mg-min/L for chlorine) and reduced formation of total trihalomethanes (TTHMs). After ozone disinfection, reduction in TTHMs was by 80% as compared to chlorination. Thereafter, for disinfection using UV radiations, a dose of 150 mJ/cm^2 was found sufficient for meeting the norms as a standalone measure with reduction in TTHMs by 91%. Both ozone and UV have a further benefit that no residuals are left after treatment, hence do not pose any toxic risk to aquatic organisms of the receiving waters. However, due to their relatively higher cost as compared to chlorine, the process needs optimization, where low doses of disinfectant were used to achieve WHO standard. Different design methods of response surface methodology (RSM) was used for statistically obtaining optimum ozone dose.

Based on these observations, a hybrid disinfection strategy was evolved to avoid high doses of chlorine by adopting a two-step treatment. The first step brought down all chlorine susceptible bacteria to a low value with an optimum dose for its efficacy, while the second step employed ozone or UV in series to meet the TC norms. This resulted in substantial reduction in CD (about 47% reduction) and much lower CT values for the subsequent disinfectant (8 mg/L for ozone and 75 mJ/cm^2 for UV) compared to their standalone values. This could bring the overall cost of disinfection comparable, despite of using a costlier disinfectant in series and yields additional

benefits in terms of reduction in THMs. Where hybrid disinfection strategy 'A' (Cl_2/O_3) reduced TTHMs by 37% and Strategy 'B' (Cl_2/UV) reduced TTHMs by 44%. The novelty of the present research is the adoption of a reverse sequence of disinfectant such as Cl_2/O_3 and Cl_2/UV for wastewater to optimize the overall process in terms of cost as well as THM concentrations in the treated water. The hybrid doses reduced TTHMs formation by 37% and 44% when compared to chlorination alone exemplifying the overall superiority of the modified process.

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Nomenclature

List of symbols

$	US Dollar
$CHCl_2Br$	Bromodichloromethane
Br	Bromine
Br^-	Bromide
BrO^-	Hypobromite
BrO_3^-	Bromate
CaClO	Calcium hypochlorite
$CHBr_3$	Bromoform
$CHCl_3$	Chloroform
Cl_2	Chlorine
ClO^-	Hypochlorite ion
ClO_2	Chlorine dioxide
CO_2	Carbon Dioxide
$CHClBr_2$	Dibromochloromethane
df	Degree of freedom
F	F-test
Fe	Iron
Fe_2^+	Ferrous ion
H_2O_2	Hydrogen peroxide
H_2S	Hydrogen sulphide
HBrO	Hypobromous acid
HOCl	Hypochlorous acid
I	Iodine
i	Rate of interest
J/m^2	Joule per square meter
KI	Potassium iodide
mJ/cm^2	Millijoule per square cm
MLD	Million liters per day
Mn	Manganese
mW/cm^2	Milliwatt per square cm
N	TCC concentration after treatment

n	Duration
$Na_2S_2O_3$	Sodium thiosulphate
$Na_2S_2O_5$	Sodium metabisulfite
NaCl	Sodium chloride
NaOCl	Sodium hypochlorite
N_o	Initial concentration of TCs
NO_2^-	Nitrite
O_2	Oxygen
O_3	Ozone
OH	Hydroxyl
P	Probability
R^2	Coefficient of determination
SO_2	Sulfur dioxide
TiO_2	Titanium dioxide
UV A	Ultraviolet A (315-400 nm)
UV B	Ultraviolet B (280-315 nm)
UV C	Ultraviolet B (200-280 nm)
W	Watt
Wh/m^3	Watt hour per cubic meter

List of abbreviations

A	Annuities (yearly operating and maintaining cost)
ACS	American chemical society
AHL	Acylatedhomoserine lactones
ANOVA	Analysis of variance
AOPs	Advanced oxidation potential
APHA	American Public Health Association
ASP	Activated sludge process
BDCM	Bromodichloromethane
BOD	Biological oxygen demand
CCD	Central composite design
CD	Chlorine dose
CFU	Colony forming unit

COD	Chemical oxygen demand
Ct	Concentration time
CT	Contact time
DBCM	Dibromochloromethane
DBPs	Disinfection byproducts
df	Degree of freedom
DNA	Deoxyribonucleic Acid
DO	Dissolved oxygen
DOE	Design of experiments
DST	Defined substrate technology
EI	Electron impact
EMB	Eosin methylene blue
EPS	Extracellular polymeric substances
F.S.	Full scale
FV	Future value
GC/MS	Gas chromatography/mass spectrometry
GC-MS/MS	Gas chromatography-mass spectrometry and gas chromatography/tandem mass spectrometry
GC-ECD	Gas chromatography-electron capture detector
HAAs	Haloacetic acids
HANs	Haloacetonitriles
HRT	Hydraulic retention time
LPS	Lipopolysaccharide
MLD	Million liters per day
MNIT	Malaviya National Institute of Technology
MPN	Most probable number
MTBE	Methyl tertiary butyl ether
NMIMS	Narsee Monjee Institute of Management Studies
NOM	Natural organic matter
NTU	Nephelometric turbidity unit
ONPG	Ortho-nitrophenyl-β-galactoside
PAA	Peracetic acid
PCR	Polymerase chain reaction

PV	Present value
RAPD	Random amplified polymorphic DNA
RBC	Rotating biological contractor
RNA	Ribonucleic acid
RPM	Revolutions per minute
RSM	Response surface methodology
RT	Retention time
SEM	Scanned electron microscopy
SRM	Selected reaction monitoring
STPs	Sewage treatment plants
TC	Total coliform
TCC	Total coliform count
THMs	Trihalomethanes
TOD	Transferred ozone dose
TRC	Total residual chlorine
TSS	Total suspended solids
TTHMs	Total trihalomethanes
USEPA	United States Environmental Protection Agency
UV	Ultraviolet
UV A	Ultraviolet A (315-400 nm)
UV B	Ultraviolet B (280-315 nm)
UV C	Ultraviolet B (200-280 nm)
WHO	World Health Organization
XLD	Xylose lysine deoxycholate

Chapter 1

Chapter 1

Introduction

This chapter presents the background of the study and formulation of objectives based on the identification of problems. The scope of the work is followed by the description on the contribution to knowledge and novel elements of the research. The last section describes the structure of thesis presented here.

1.1 Context and Background

The developing countries like India are facing immense environmental problems with fast depleting natural resources and threatening the very existence of most of the natural sources out of which the most important is water [1]. During the past few decades, the number of countries experiencing water scarcity has increased as the total supply of freshwater on earth far exceeds human demand and the global water withdrawals increased by over six times-more than double the rate of population growth [2]. About 97% of the total water available is in oceans and out of the remaining 3%, only about one hundredth is the accessible freshwater that can be used for human demand. If this available water could be evenly distributed, still it is enough to support a population about ten times larger than today [3].

Available water resources in cites are becoming scarce because of increasingly urban population and usage, changing precipitation patterns, climate change, drought, scarcity of surface water, degradation of existing sources of water, unsustainable water use practices, competition for water between water users for domestic, industry, agriculture, and insufficient infrastructure for waste management [4]. The issue of water scarcity in India is expected to worsen as the overall population will be about 1.6 billion by the year 2050. With increasing population growth rate of 1.9% per year, the total availability of fresh water is expected to reduce 1,341 m^3 in 2025 and 1,140 m^3 in 2050 [5]. The demand for growing urban communities for both food and water requires the agriculture sector to increase food production under water-stressed conditions. On the other side, as the demand for water increases, making more efficient use of water becomes more important [6]. At the same time, due to increasing population, the

volume of sewage effluent is increasing, and its safe disposal is also difficult [7]. Hence, water reuse should be seriously considered before water availability is matched by its demand [8].

Wastewater reuse is an important approach for conservation of water resources particularly in areas suffering from water shortage [9]. To meet the demand-supply gap, reuse of treated effluents for non-potable purposes such as irrigation, industrial process water, cooling water, and environment-enhancement could be promoted as huge volume of wastewater is being generated and treated [10] [11]. But there are several hurdles in encouraging the reuse of treated effluents as most of the sewage treatment plants are designed to meet the discharge standards, where their main focus is to remove the organic load, and little consideration is given to the removal of microbes. As a matter of fact, municipal wastewater contains pathogenic microorganisms i.e. bacteria, viruses, and protozoa which are responsible for contamination of water bodies, that harms the aquatic environment resulting in several health issues posed due to severe diseases [12]. It has been reported that many thousands of farmers in India use sewage as their primary source of irrigation, thereby putting their health and that of the consumers at risk. Urban India generates more than 40,000 million litres of sewage each day (MLD), much of this contributes to the pollution load of water bodies [13]. It was estimated that out of this 20,000 MLD was used for irrigation every day and more than half of it is untreated [3] [13]. It may also contain bacteria and other organisms which are harmful to agricultural workers and those who handle, cook or eat the plant products. The highest risk is for crops that are eaten uncooked and grown in close contact with wastewater effluent as a few of the pathogens translocate to the edible parts of the plant [12] [13]. However, the direct and indirect exposure of populations to sewage is of primary concern. Hence, issues of both water quality and quantity are of major concern and the safe discharge of municipal wastewater back into the receiving water is very essential after its appropriate disinfection [8] [13].

The regulation of the wastewater treatment plants has been possible by imposing strict legislation on the discharge of treated water quality by the government authorities worldwide [14]. Reuse of wastewater after treatment is one of the main options being considered as a new source of water in regions where water is scarce [3] [15]. Different wastewater recycling and reuse case studies are carried out in India. In metro cities such as Bhopal, Hyderabad, Chandigarh, Madras, Patna, Pune etc. secondary treated effluent

from sewage treatment plants (STPs) is used in sewage farms, organized by farmers and irrigation department [16]. The biggest wastewater reclamation plant of capacity 48,000 m^3/d at Jamnagar in India is designed for the maximum reuse of the wastewater coming from the operational units of the refinery [17].

Wastewater generated at Narsee Monjee Institute of Management Studies (NMIMS), Maharashtra, India is treated to the standard to avoid the pollution of Tapi River which flows by the side of the campus. This treated wastewater is also reused for landscape irrigation and flushing of toilets after adequate disinfection [18].

In India, the estimation reveals that 38,354 million liters per day (MLD) sewage is generated in major cities of the country while the sewage treatment capacity is only of 11,786 MLD [19]. Though the wastewater treatment capacity in the country has increased by about 2.5 times since 1978-79 yet hardly 10% of the sewage generated is treated effectively, while the rest either sinks into the ground as a potential pollutant of ground water or is discharged into the natural ecosystems and causes large-scale pollution in downstream area such as rivers and ground waters [19] [20]. Hence, reuse of wastewater after proper disinfection will bring water back for use rather than disposing it and considering as a waste [19].

Domestic wastewater or sewage consists of approximate 99.9% water, 0.02-0.03% suspended solids and other soluble organic and inorganic substances [21]. As the composition of wastewater varies, it is expected that the type and numbers of organisms will also fluctuate [22]. The growing water demand has led to a global deterioration of surface water quality and in areas facing water shortage, more and more reclaimed water will be used in the future for irrigation. Still increased efforts are required for better wastewater treatment facilities so that treated water can be used for different purposes.

Wastewater treatment involves physical, chemical or biological processes. Primary treatment can reduce the biological oxygen demand (BOD) of the incoming wastewater by 20-30% and total suspended solids by some 50-60% [23]. The biological process is then followed by secondary sedimentation to remove more of the suspended solids. About 85% of the suspended solids and BOD can be removed by secondary treatment [24]. Whereas, the purpose of tertiary treatment is to provide a final treatment stage to raise the effluent quality to the desired level by removing the inorganic nutrients and pathogens.

Conventional treatment processes are known to remove up to 90-95% of some microorganisms, but their efficiency is not sufficient to meet the existing requirements for wastewater discharge or reuse [25]-[27]. Therefore, tertiary treatment which involves disinfection is a key step for reducing the number of pathogenic organisms to a level providing the highest degree of water quality. Tertiary treatment, can remove more than 99% of all the impurities from sewage, producing an effluent of almost drinking water quality [24]. Thus, to ensure the downstream protection of human health, any water discharged from a wastewater treatment plant must be disinfected to prevent the spread of disease causing pathogenic organisms [23]. The increasing concern for pathogenically water related diseases promotes the implementation of more and more stringent standards on microbiological pollution of wastewater effluents [28] [29].

1.2 Disinfection

The core purpose of disinfection is to protect the water in the distribution systems against microbial contamination and to prevent and control re-growth of the microorganisms in the water distribution system [9]. The objective of disinfection is the treatment of wastewater to substantially reduce the number of microorganisms in the water to be discharged back into the environment [30]. It is difficult to measure individual pathogenic organisms, therefore disinfection efficacy is most often measured using ‘indicator organisms’ that coexist in high quantities where pathogens are present. The most common indicator organism used in the evaluation of water is total coliform (TC) unless there is a reason to focus on a specific pathogen [31]-[33]. The coliform group of bacteria is used most often as an indicator of the presence of wastes of a warm-blooded animal [31].

Wastewater disinfection levels are determined by standards and recommendations that are specific to each country and region. In general, these standards are becoming more and more stringent to ensure the better public health and environmental protection [9]. The World Health Organization (WHO) recommends that treated wastewater for unrestricted irrigation should contain less than 1000 colony forming unit (CFU) per 100 mL (CFU/100 mL) fecal coliform [3] [8] [13]. An ideal wastewater disinfection technology should kill all potential pathogens in the water, adds no toxic compounds to the water, is safe, easy and inexpensive to use and meets current and upcoming regulations [34]-[36]. Short contact times, low doses and high flows all militate against

effective disinfection. Common methods of disinfection (Figure 1.1) include the use of chlorine (Cl_2), ultraviolet (UV) light, or ozone (O_3) [3] [9] [15] [34].

Chlorine is the most common and historical chemical disinfectant across the world for water and wastewater treatment due to its low cost and effectiveness [34]. Chlorination has made the greatest contribution to the prevention of waterborne diseases worldwide. It destroys target organisms by oxidizing cellular materials. It may be applied as chlorine gas, hypochlorite solutions and other chlorine compounds in solid or liquid form [37]. Chlorination is the most common form of disinfectant all over the world [22]. One major disadvantage of chlorination is that residual organic material can generate chlorinated organic by-products that are carcinogenic or harmful to the environment. Residual chlorine or chloramines may also be capable of chlorinating organic material in the natural aquatic environment. Further, because residual chlorine is toxic to aquatic species, the treated effluent must also be chemically dechlorinated, adding to the complexity and cost of treatment [38].

Ozone is a strong oxidizing agent. It has been proven to be one of the most effective disinfectants and is widely used to inactivate pathogens and to remove other organic compounds. It is effective in destroying microorganisms which are resistant to most other disinfectants [39] [40]. The germicidal effect of ozone consists of totally or partially destroying cell wall resulting in microorganism lysis. It also damages to the constituents of the nucleic acids. Ozone is highly effective and utilizes a short contact time. There are no harmful residuals and no regrowth of microorganisms reported but it is a complex technology, which is extremely reactive, corrosive and not economical. Ozone gas is extremely irritating and toxic [41].

UV light can be used as an alternative to chlorine, or other chemicals for disinfection, which relies on the transfer of electromagnetic energy from a source (lamp) to an organism's genetic material (DNA and RNA) by penetrating through the cell wall resulting in injury or death of exposed cells [39]. As no chemicals are used, the treated water has no residuals that can have an adverse effect on aquatic organisms that later consume it. The key disadvantages of UV disinfection are the need for frequent lamp maintenance and replacement and the need for a pre-treated effluent to ensure that the target microorganisms are not shielded from the UV radiation [27] [37].

1.3 Problem Identification

Out of the three disinfectants, Chlorine is a widely utilized disinfectant and is a leading contender for disinfection of reclaimed water. Calcium hypochlorite ($Ca(OCl)_2$) is widely used disinfectant due to its strong oxidizing capacity. This form of chlorine when comes into contact with water dissociates into hypochlorous acid (HOCl) and hypochlorite ion (ClO^-). Both these forms have a higher bactericidal property [42]. There are published reports that in the chlorination process, high doses of chlorine are required due to certain chlorine resistant species and excess of chlorine consumption may further give rise to excessive production of disinfection byproducts (DBPs) that are known to have negative environmental consequences [15] [43]. Typical chlorine dose for municipal wastewater disinfection is about 5-20 mg/L for a contact time of 20 min to meet the WHO norms for coliforms for its reuse in irrigation [15] [30]. But the major areas of concern that lies with the chlorination are the toxic effects of highly chlorinated effluent on aquatic ecosystem due to residual chlorine and the effects of the formation of potentially carcinogenic halogenated compounds such as trihalomethanes (THMs) and haloacetic acids (HAAs), many of which are proven human carcinogens and could contaminate downstream water sources [40]. This may be attributed to relatively higher chlorine demand exerted by certain microorganisms such as *Serratia/Hafnia*, *Enterobacter* which are resistant to chlorination (Table 1.1) and requires high doses of chlorine to bring them within standard norms i.e. 1000 CFU/100 mL.

Table 1.1: Resistivity of bacterial species against three disinfectant [37] [39] [41]

S. No.	***Chlorine***	***Ozone***	***UV Radiation***
1	*Serratia/Hafnia*	*Enterobactor*	*Shigella*
2	*Pseudomonas*	*Salmonella*	*Citrobacter*
3	*Enterobacter*	*Pseudomonas*	*Pseudomonas*
4	*Citobacter*	*Shigella*	*Salmonella*

In one of the recent research reports, a detailed analysis of the effect of chlorine on individual coliform species indicated that the counts of *Escherichia, Klebsiella* and *Citrobacter* in activated sludge process treated sewage could be brought down to below

1000 CFU/100 mL with only 7.5 mg/L of chlorine and *Enterobacter* offered some resistance at a chlorine dose of 10 mg/L. However, for *Serratia/Hafnia*, which were more resistant to chlorination, a much higher dose of 17.5 mg/L was required to meet the total coliform count (TCC) norms [15]. Therefore, from the previous studies, it was perceived that each disinfectant had some shortcomings and it could be overcome by the hybrid approach [3] [15] [22] [27] [34] [37] [40] [41]. Thus, it was postulated (Figure 1.1) that a combination of more than one tertiary treatment process may be used for better results, such as chlorine followed by UV radiations or ozone. This brought forth the importance of devising a hybrid disinfection strategy to avoid high doses of chlorine by adopting a serial step of another disinfectant that has the potential to remove chlorine resistant species and optimize the overall disinfection process. Such hybrid strategy may also possibly modify the DBP formation due to the prior chlorination step apart from comparably reducing the overall cost of the process.

Previous studies also gave the evidence of using the combined disinfection techniques. Several combinations such as chlorine dioxide (ClO_2)/HOCl, O_3/UV, UV/Cl_2, O_3/Cl_2 are utilized for drinking water for accruing the benefits of the synergistic inactivation of certain microbial species and the productive characteristics of the disinfectant byproducts have been reported in the studies [44]-[46]. Reports are also available on the sequential disinfection strategy for reclaimed water such as using combinations of UV/peracetic acid (PAA), UV/Cl_2, O_3/UV, UV/O_3 for complete inactivation of certain pathogenic microorganisms and to inhibit total trihalomethanes (TTHMs) formation in the effluent of wastewater treatment plants [14] [20] [47] [48].

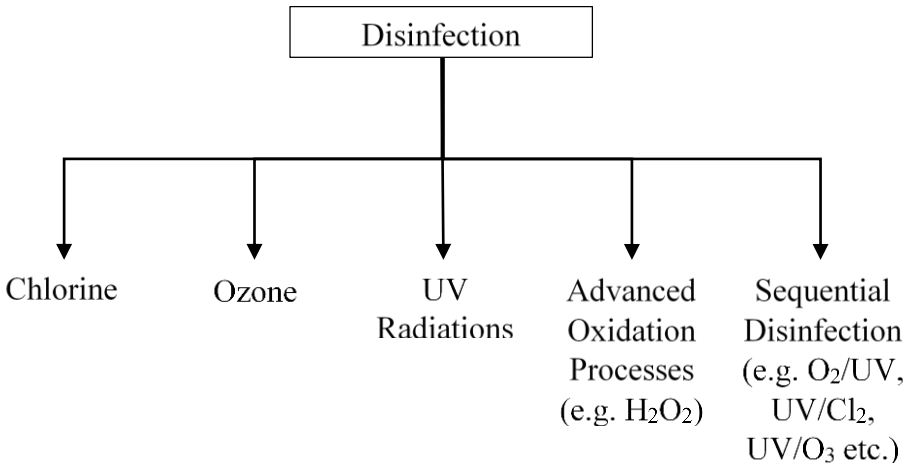

Figure 1.1: Different methods of disinfection

1.4 Contribution to Knowledge and Novel Elements of the Research

The novelty of the present research is the adoption of a reverse sequence of disinfection than what has been used for drinking water, where chlorine is given as a last step of disinfection process in series of other processes like UV or ozone in order to maintain certain residual to fight any infection occurring during the travel of water in the pipe network. Here combinations like Cl_2/UV, and Cl_2/O_3 for wastewater disinfection has been thought of to optimize the overall process of disinfection in terms of efficiency, cost and its byproducts. The novelty lies in the fact that the species-wise analysis of susceptible organisms to chlorination helps to minimize the chlorine dose, and the remaining disinfection can be achieved with the relatively more potent though costly disinfectants (O_3 and UV) at low doses. This may not only help to comparably reduce the overall cost but would also lower the THM formation. Hence, development of a new hybrid disinfection strategy, which can take care of chlorine resistant coliforms, as well as DBPs of chlorine and can go a long way in mitigating serious environmental consequences associated with current practices of sewage chlorination. While deriving the benefit of synergistic killing by the combination of disinfectants, another advantage of using hybrid mode of disinfection may be the relatively lesser reactivation of microorganisms due to dual modes of the destruction of cells, but it requires experimental validation.

1.5 Objectives of the Study

Based on the gaps identified (Table 2.2), the present study is focused on reducing the health risk associated with disposal of secondary treated effluent into water bodies and thus emphasizes on the importance of tertiary treatment i.e. disinfection. The main objective of the present study is the development of a new hybrid disinfection strategy that can help mitigate serious environmental consequences associated with current practices of sewage chlorination and may reduce the overall cost of disinfection despite using a costlier disinfectant in series, which is intended to remove only chlorine resistant coliform species. It is further perceived to have the benefit of lesser DBP formation and hence its transformations were tracked during the disinfection process. The research objectives of the present study have been highlighted in Figure 1.2.

To achieve these aims the specific objectives are:

- ***Objective 1***: Quantitative and qualitative analysis of wastewater samples drawn from different units (raw, primary, secondary treated effluent) of sewage treatment plant based on rotating biological contractor, located at the Malaviya National Institute of Technology (MNIT) campus.
- ***Objective 2***: Design and fabrication of laboratory scale experimental set up for chlorination, ozonation, and UV disinfection.
- ***Objective 3***: Chlorination of secondary treated effluent at specific doses, followed by physicochemical and microbiological analysis of treated effluent to assess species based inactivation of coliforms. Optimization of chlorination process using the design of expertiments (DOE) software as per parametric sensitivities.
- ***Objective 4***: Ozonation of secondary treated effluent at specific doses, followed by physicochemical and microbiological analysis of treated effluent to assess species based inactivation of coliforms. Optimization of the process to determine the optimum value of ozone for TCs.
- ***Objective 5***: Disinfection of secondary treated effluent at specific doses by UV radiations, followed by physicochemical and microbiological analysis of treated effluent. Analysis of the reactivation phenomenon shown by microbes in response to visible light.
- ***Objective 6***: Designing the hybrid disinfection strategies using chlorine as primary disinfectant followed by ozone (A) and chlorine followed by UV radiations (B) in series, respectively. Physicochemical and microbiological analysis of treated effluent to study the efficacy of hybrid disinfection.
- ***Objective 7***: Scanning electron microscopy (SEM) analysis to understand the mechanism of disinfection of each disinfectant.
- ***Objective 8***: Analysis of THMs using gas chromatography/mass spectrometry and gas chromatography/tandem mass spectrometry (GC-MS/MS) to assess the fate of four THMs, namely chloroform, bromoform, bromodichloromethane, and dibromochloromethane.
- ***Objective 9***: Comparative analysis of individual disinfection processes with hybrid disinfection strategy and cost analysis of all the treatment processes.

The research highlights have been presented in Figure 1.2.

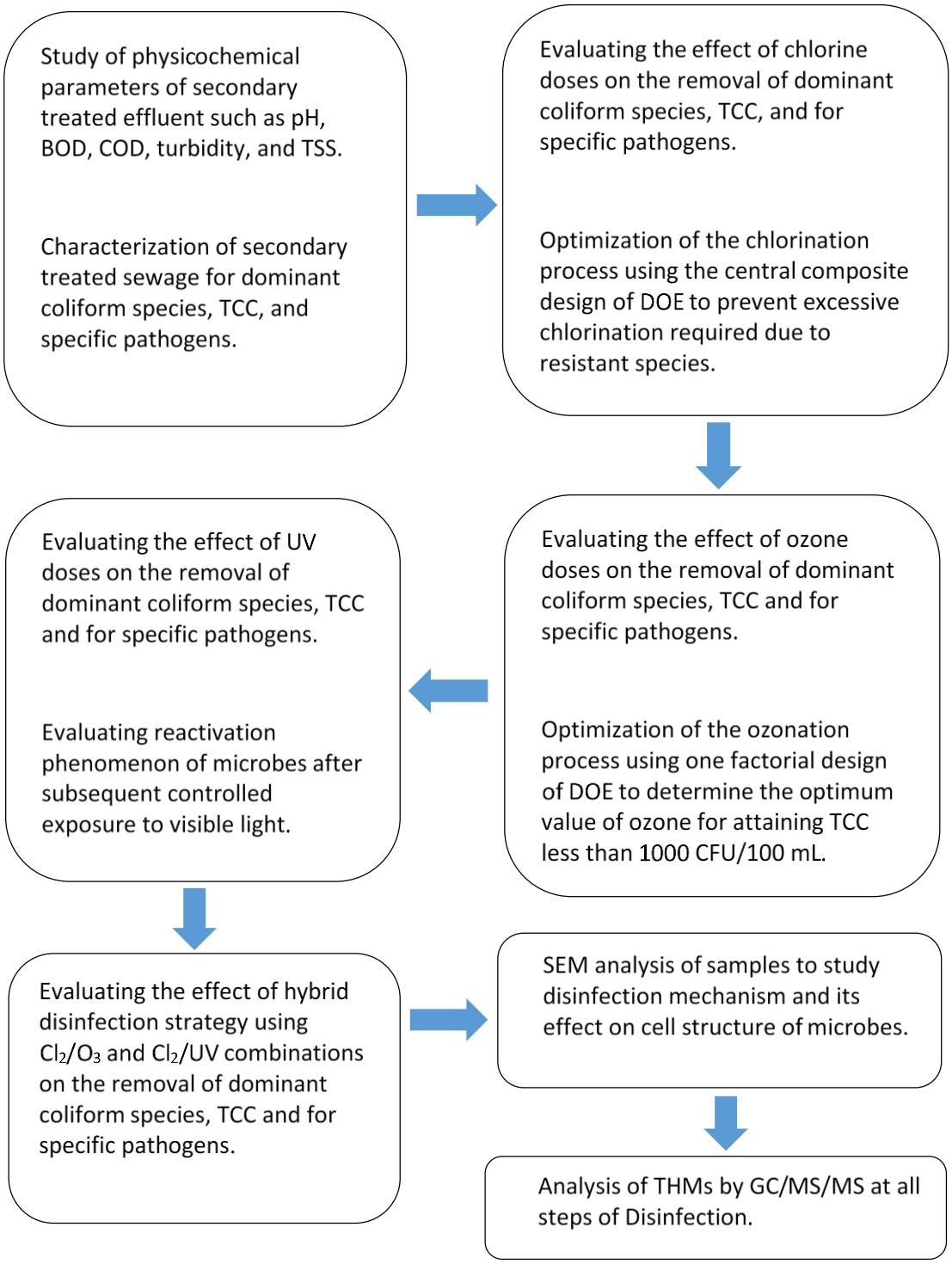

Figure 1.2: Research highlights

1.6 Organization of Thesis

The entire thesis is summed up in eight chapters. Presented below are the highlights of the chapters.

***Chapter 1*:** This chapter covers an overview of the environmental problems related to disinfection; the origin of the existing proposal; the need of current research and the specific objectives of the present approach to fill the identified gaps. It highlights the details of the proposed hybrid disinfection strategy to avoid the shortcomings of individual disinfectants and to derive the perceived benefits of reduction in cost as well as DPB formation.

***Chapter 2*:** This chapter includes a critical review of the literature, designed to provide a summary of the knowledge already available involving the issues of interest and research gaps. This chapter has been divided into six sections. The first and second section provides detailed information about the present approach of research in the field of disinfection. Third to fifth sections compile detailed information about the research carried out on chlorination, ozonation, and UV disinfection respectively. This review was useful in deriving the gaps in the present knowledge; developing an experimental protocol, and carving out the problem statement for the proposed hybrid disinfection strategy. The sixth section presents research reports on sequential or hybrid disinfection strategy to overcome the issues of individual disinfection processes, which helped to derive the present approach.

***Chapter 3*:** This chapter gives the detailed description of the methodology used for the research. It also introduces the analytical techniques and statistical software used for completion of the research.

***Chapter 4*:** This chapter represents the results of the physicochemical and microbial analysis of samples, which were drawn from different units of sewage treatment plant. Results of chlorine disinfection in terms of TC removal, pathogen inactivation, and species based removal are discussed, which form a baseline data for further research. Optimization of the process is carried out using central composite design (CCD) of DOE. Results of SEM shows the action mechanism of chlorination for inactivation of

microbial species. The concentration of four THMs formed due to chlorination are analyzed using GC-MS/MS.

Chapter 5: This chapter discusses the results of disinfection by ozone. The chapter includes results of the effect of ozone doses on five dominant microbial species, TCs, and three specific pathogenic species. The optimum value of ozone dose for bringing the TCs concentration within the standards (WHO) and for obtaining the corresponding value of chemical oxygen demand (COD) at this ozone dose is determined by one factorial design of DOE. Action mechanism of ozone is interpreted by analyzing SEM images. The effect of ozone on four THMs is also described using GC-MS/MS.

Chapter 6: The chapter includes results of the effectiveness of UV disinfection on five dominant microbial species, TCs and certain pathogens in secondary treated effluent of STP based on rotating biological contractor (RBC) treatment process. SEM images help in understanding the microbicidal effect of UV radiations. Effect of UV radiations on four THMs was discussed using GC-MS/MS. Photo-reactivation phenomenon of microorganisms as an effect of exposure to visible light is also studied.

Chapter 7: This chapter discusses the results of hybrid disinfection and brings out its importance. A reverse sequence of disinfection i.e. Cl_2/UV and Cl_2/O_3 for wastewater is used to optimize the overall process. SEM analysis helps to understand the effect of hybrid disinfection strategy on inactivation of microbes. Effect of hybrid disinfection strategy on concentration of four THM concentration is studied using GC-MS/MS.

Chapter 8: This chapter outlines the conclusions of the research findings and summarizes the key findings drawn from analysis of data including the contribution of the present research. At last, it discusses the scope for future research in this area on the basis of results and inferences derived.

Chapter Summary

Chlorine, ozone, and UV are the most common forms of disinfectants used all over the world. But all the three commonly used disinfectants have certain limitations. Thus, the present work proposes to evolve a hybrid disinfection strategy by adopting a two-step treatment to optimize the overall disinfection process. The present study focused on a combination of disinfectants such as Cl_2/UV and Cl_2/O_3.

The next chapter gives an overview of the various disinfection methods which are being practiced and brings out the importance of the present study in the current scenario.

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Chapter 2

Chapter 2

Review of Literature

The chapter begins by highlighting the background and need of the present research study and summarizing the process of disinfection. It further provides insight into the basic concepts and theory of three main disinfection processes chlorine, ozone and UV radiations, citing several investigations carried out in the recent past on disinfection of wastewater. The next part of the chapter gives an idea about the concept of hybrid disinfection on which the present work has been built.

2.1 Need as Well as Approach towards the Present Study

The foremost priority of the present modern society is to protect and conserve valuable natural resources. The most valuable resource in present time is water which is a basic requirement to human life [1]. During the past few years, there has been growing concern that the world is moving towards a water crisis. Increasingly inadequacy of water in dry climate regions (for example, Africa, India and South Asia) and issues of both water quality and quantity are of major concern [2]. At the same time safe disposal of sewage effluent is also challenging. Hence, the reuse of wastewater after adequate treatment is one of the main options being considered as a new source of water in regions where, water is scarce solving the problems of water scarcity and sanitation [3].

Reusing wastewater after proper and adequate treatment can significantly reduce the problem of environmental pollution and can save the irreplaceable water sources. It has been reported that the capacity to reuse wastewater could be equivalent to 15% of global water consumption [4]-[7]. Due to water scarcity, agriculture has to compete for water resources with industry and municipal users, and often there is no alternative for farmers but to use sewage horticulture, i.e., to use sewage from urban areas directly for irrigation [2] [3] [8]. Hence, it has become a national policy to gradually increase the fraction of reclaimed wastewater instead of fresh water for agriculture use. Studies reports that if agriculture maintains its present size then by the year 2020, approximately 50% of agricultural water demand has to be satisfied by reuse of treated wastewater [1] [8]. Thus, wastewater treatment seems to be an obligatory process. The

general purpose of any treatment plant is to remove organics from the water to be discharged back into locations so that the water will be reused in other daily applications. Reuse of wastewater has vast potential to reduce the pressure on the world's freshwater resources [3]. An estimation suggests that in India, 70% of total municipal sewage generation and effluent from over 900 cities and towns are discharging untreated sewage into rivers posing problems of water pollution. These rivers and water bodies, are a major source of drinking water and hence to protect them, 234 sewage treatment plants have been established recently in India [2] [9]-[11]. A typical wastewater treatment plant comprises of primary, secondary and tertiary treatment to remove suspended solids, organics, nutrients and pathogenic organisms. The core process of the sewage treatment plants is the biological treatment which is also known as the secondary treatment process. Although they offer proper primary and secondary treatment by reducing the contaminants but still contain a wide range of pathogenic microorganisms [11].

The conventional municipal STPs, which do not include disinfection process, reduce fecal microorganisms by 1-3 orders only [14]. Moreover, the discharge of treated wastewater effluent is found to contain microbial contaminants, which pose threat to the receiving streams and deteriorates the water quality of major rivers of India [14] [15]. As discussed in Chapter 1, thousands of farmers in India use sewage as their primary source of irrigation, thereby putting their health and that of the consumer at risk [3] [16]. The contaminated water is a main source of many waterborne diseases such as cholera, typhoid, giardiasis, amebiasis, etc. causing many serious public health crises, especially in developing countries with the low hygiene condition [17]. In a developing country like India, sanitary and hygiene conditions are unsafe due to the lack of sufficient wastewater treatment and disposal facilities which deteriorate the overall environmental quality [14] [18].

Table 2.1 represents concentration of some organisms which are generally present in wastewater. It was concluded from literature that in India, the need for tertiary treatment is found to be essential as the treated effluent does not meet the prescribed standards to reuse it for various purposes such as for irrigation, recreational and industrial purposes [3] [11]. Tertiary treatment includes disinfection which is the most crucial process and is the final barrier against bacteriological contamination [14] [15].

Table 2.1: Concentration of organisms in wastewater [19] [20]

Organism	***Number in wastewater (per liter)***
Thermotolerant coliforms	10^8-10^{10}
Campylobacter jejuni	10- 10^4
Salmonella spp.	1- 10^5
Shigella spp.	10-10^4
Vibrio cholera	10^2-10^5
Ascaris lumbricoides	1- 10^5
Ancylostoma duodenale	1-10^3
Trichuris trichiura	1-10^2
Cryptosporidium parvum	1-10^4
Entamoeba histolytica	1-10^2
Giardia intestinalis	10^2-10^5
Enteric viruses	10^5-10^6
Rotavirus	10^2-10^5

2.2 Disinfection

The process of killing or reducing pathogenic bacteria from the effluent is known as disinfection. It is the final step in the treatment process and is necessary to provide bacteriological safety to the public. [21]-[23]. Disinfection is now important for the wastewater systems and is necessary to reduce disease causing microbiological organisms in treated wastewater effluent to acceptable levels [15]. Some countries have banned the irrigation of crops with treated wastewater due to lack of proper disinfection facility [24] [25]. Several authors have shown that crops can be contaminated when irrigated with treated wastewaters [24] [26]. So, to control such problems there are specific national standard and international recommendations, such as those issued by the WHO, governing the irrigation of crops [24] [26] [28]. Standards are also set for reusing reclaimed water for non-potable purposes such as watering gardens, lawns, for bathing and flushing toilets [3]. The disinfection process is used as a control on the disease-producing bacteria, and it does not mean to sterilize the effluent. [29].

The effectiveness of disinfection depends on the following factors [18]:

- Quality of the water being treated
- The type of disinfection being used

- The disinfectant dosage (concentration (Ct) and contact time (CT))
- Other environmental variables such as number of microorganisms, resistant nature of microorganisms, biofilm etc.

Wastewater disinfection levels are determined by standards and recommendations that are specific to each country and region. In general, these standards are becoming more and more stringent in order to ensure better public health and environmental protection [21]. The current standard for TC under consideration is 10,000 most probable number per 100 mL (MPN/100 mL) in India. In 1989, the WHO issued guidelines setting faecal coliform limit at 1000 CFU/100 mL to be used for irrigation of crops. Stringent limits of faecal coliform in water were set at 200 MPN/100 mL for agricultural use [18] [30].

The isolation of pathogens from sewage is expensive and laborious, hence the disinfection efficiency of wastewater treatment unit is usually measured by indicator organisms. The thermotolerant coliform group has been considered as an indicator of fecal pollution because they are easy to detect and enumerate in water [31] [32]. The presence of coliform indicates that fecal pollution may have occurred and pathogens might be present as a result. Coliform are not considered to be a health risk. Thus, the absence of TC is generally evidence of a bacteriological safe water [33].

Desirable characteristics for a useful water quality indicator are listed below [34] [35]:

- They are universally present in the feces of warm blooded animals in large numbers.
- They are readily detected by simple methods.
- They do not grow in natural waters.
- Their persistence in water and the extent to which they are removed by water treatment is similar to those of waterborne pathogens.

2.2.1 Enumeration of Bacteria

Enumeration in microbiology is the determination of the number of individual viable microbes in a sample. It is necessary to estimate the number of bacterial cells during disinfection studies. For unicellular microorganisms, such as bacteria, microbial growth is considered similar to microbial reproduction as the reproduction of the cell reproduces the entire microbial colony [36] [37]. Some of the methods used to determine the number of microorganisms present in a sample are membrane filtration,

the MPN method, the standard plate count (pour and spread plate methods), and Colilert-18 Hr. method (defined substrate technology) methods. The best and most consistent results were obtained with the pour plate method [38].

2.2.2 Disinfection Methods

In general, disinfection can be achieved by any method that destroys pathogens. Different physical or chemical methods are capable of destroying microorganisms under certain conditions. However, the treatment of wastewaters for the destruction of pathogens, demands the use of practical measures that can be used economically and efficiently at all times on large quantities of wastewaters which have been treated to various degrees [13] [18] [21] [39].

The following methods and technologies are presented used for wastewater disinfection:

- Solar disinfection
- Chlorination
- UV radiation
- Ozone
- Alternative methods

In the past, wastewater treatment practices have principally relied on the use of chlorine for disinfection. The prevalent use of chlorine has come about because chlorine is an excellent disinfecting chemical and has been available at a reasonable cost [13] [15] [16] [18]. The use of chlorination has less been favored as a disinfectant of choice in wastewater treatment because of the fact that residual chlorine even at low concentration is toxic to fish and other aquatic biota and due to the possibility that potentially harmful chlorinated hydrocarbons may be formed [13] [22] [40]. As a result, ozone or UV light is also used as a disinfectant in wastewater disinfection. Both ozone and UV light are, effective disinfecting agents, leave no toxic residual and is economically competitive with chlorination at low doses as a disinfectant. Since chlorine continues to be used extensively as a disinfectant and has been used in the present study, the principles and practice of chlorination have been described in details in the subsequent sections [40]. As a part of the present study, it is also devoted to UV and ozone disinfection, so their mode of disinfection has also been described in details.

2.3 Chlorine Disinfection

Disinfection by chlorination was massively introduced worldwide in the early twentieth century and it has set off a technological revolution in wastewater treatment [22]. Although the pros and cons of disinfection with chlorine have been extensively debated, it remains the most widely used chemical for disinfection. It is an excellent bacterial disinfectant requiring short to moderate contact times, and its chemistry is very well understood [41] [42]. The keys to its success are its easy accessibility, reasonable cost, and capacity for oxidation, the mechanism to oxidize organic matter, and residual effect [21] [42]. All of this proves its benefit, not only for small systems, but also for large cities with extensive water distribution networks.

The different forms of chlorine related compounds available for disinfection are [22]:

- Gaseous chlorine
- Chlorinated lime
- Sodium hypochlorite (NaOCl)
- Calcium hypochlorite

The primary objectives of the chlorination process are disinfection, taste and odor control in the system and preventing the growth of undesirable microorganisms. The amount of disinfectant needed depends upon the wastewater flow to be treated, the required dosage according to the wastewater quality and the country's TC standards for wastewater disposal into the receiving stream or to be used for other purposes [18] [43]. Commercial chlorine products are obtained by different methods, which determine their concentration of active chlorine, and stability. Active chlorine is the percentage by weight of molecular chlorine. The word "active" means, this chlorine is ready to enter into action and "waiting" to attack the organic matter or any other substance that it is capable of oxidizing. For example $Ca(OCl)_2$ is a chlorinated lime, which contains about 25 to 34% of available chlorine by weight [42].

The amount of chlorine used in the reactions with substances that oxidize in the water can be measured by "chlorine demand test". It is the difference between the amount of chlorine added to the wastewater and the amount of chlorine residual remaining after a given CT [22] [44]. The chlorine dose (CD) is equivalent to the total demand for chlorine which is closely linked to the chemical and microbiological quality of the wastewater plus the amount of residual chlorine expected at the end of the water system [18] [21] [41] [42].

2.3.1 Chlorine Disinfection Mechanism

Chlorine in all forms hydrolyzes in the presence of water and forms HOCl [41] [42] [45]. The reactions of gaseous chlorine, NaOCl and $Ca(OCl)_2$ are represented by Equations 2.1, 2.2 and 2.3.

$$Cl_2 + H_2O \rightarrow H^+ + Cl^- + HOCl \quad (2.1)$$

$$NaOCl + H_2O \rightarrow Na^+ + OH^- + HOCl \quad (2.2)$$

$$Ca(OCl)_2 + 2H_2O \rightarrow Ca^{++} + 2OH^- + 2HOCl \quad (2.3)$$

When ammonia is present in the wastewater, chemical disinfection produces compounds such as monochloramines, dichloramines and trichloramines as shown by Equations 2.4, 2.5 and 2.6. The chloramines also serve as disinfectants, but they react very slowly and are considered weak disinfectants. They require longer contact times and higher concentrations.

$$HOCl + NH_3 \rightarrow NH_2Cl + H_2O \text{ (monochloramine)} \quad (2.4)$$

$$HOCl + NH_2Cl \rightarrow NHCl_2 + H_2O \text{ (dichloramine)} \quad (2.5)$$

$$HOCl + NHCl_2 \rightarrow NCl_3 + H_2O \text{ (trichloramine)} \quad (2.6)$$

HOCl and OCl^- are the two chemical species formed by chlorine in water. These two species are commonly referred to as "free available" chlorine as represented in Equation 2.7. The disinfecting agent is HOCl, which splits into hydrogenous ions (H^+) and OCl^- and takes on its oxidizing properties [22].

$$HOCl \rightarrow H^+ + OCl^- \quad (2.7)$$

Both segments of the agent have microbicidal nature and functions by inhibiting enzymatic activity and inactivating bacteria and viruses. HOCl and OCl^- are both present when the pH of the water is between 6 and 9. When the pH value of the chlorinated water is 7.5, 50% of the chlorine concentration present will consist of undissolved HOCl and the other 50% will be OCl^- [21] [23]. It is important to mention that the WHO recommends a pH < 8 for appropriate disinfection [46]. The different concentrations of the two species make a considerable difference in the bactericidal property of the chlorine, as these two compounds have different germicidal properties. As a matter of fact, HOCl efficiency is at least 80% greater than that of OCl^- [47].

The required degree of disinfection can be achieved by varying the dose and the CT for any chlorine disinfection system. Chlorine dosage will vary based on chlorine demand, wastewater characteristics, and discharge requirements. The dose usually

ranges from 2 to 20 mg/L for a CT of 20 minutes. Some of the physicochemical factors which affects chlorine disinfection are BOD, COD, total suspended solids (TSS), pH and turbidity. Hardness has minor effect on chlorine disinfection [14] [18] [48].

The germicidal action of the various forms of chlorine in solution appear to result from their oxidizing power on the chemical structure of the cell, denaturing cell protein and destroying the key enzymatic processes necessary for cell metabolism. Chlorine eliminates pathogens such as bacteria and viruses by breaking the chemical bonds in their molecules. When enzymes come in contact with chlorine, one or more of the hydrogen atoms in the molecule are replaced by chlorine [20] [45] [49]. This causes the entire molecule to change shape or fall apart. When enzymes do not function properly, a cell or bacterium will die as shown in Figure 2.1.

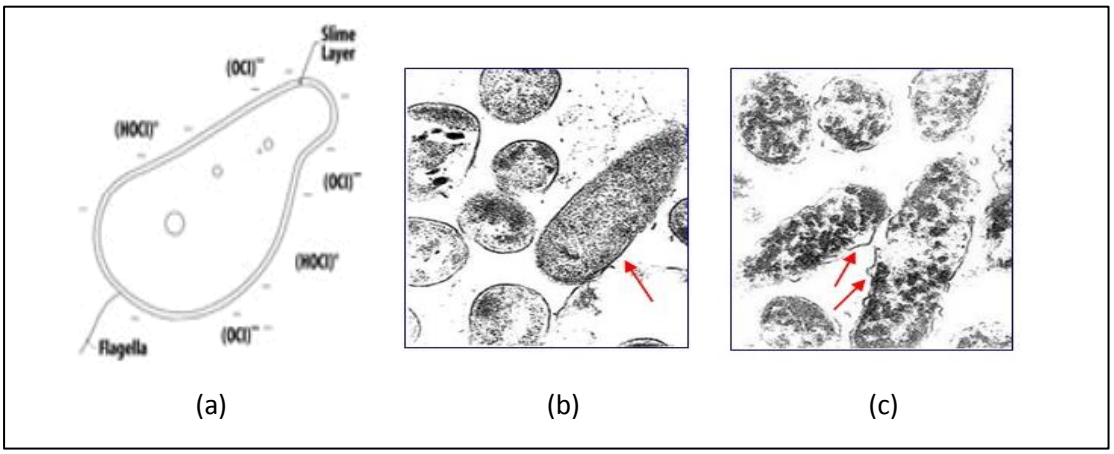

Figure 2.1: Disinfection reaction between chorine and microorganisms (a) bacterial cell wall, (b) untreated, and (c) treated [49].

The cell wall of pathogenic microorganisms is negatively charged by nature as shown in Figure 2.1 (a). The neutral HOCl can more easily penetrate cell walls of pathogenic microorganisms than the negatively charged hypochlorite ion (OCl^-). HOCl can penetrate slime layers, cell walls and protective layers of microorganisms and effectively kills pathogens as depicted in Figure 2.1 (b) and (c). The microorganisms will either die or suffer from reproductive failure [50].

2.3.2 Breakpoint Reaction

Breakpoint chlorination chemistry plays an important role in most chlorine disinfection systems [22]. The maintenance of a residual (combined or free chlorine) for the purpose

of disinfection is complicated by the fact that free chlorine not only reacts with ammonia but is also a strong oxidizing agent. As chlorine is added, readily oxidizable substance, such as iron (Fe) and manganese (Mn), hydrogen sulphide (H_2S) and organic matter reacts with chlorine and get reduced to chloride ion, point A on Figure.2.2.

After meeting the immediate demand, the chlorine continues to react with the ammonia to form chloramines, point A to B in Figure 2.2. Between point B and the breakpoint, chorine is reduced to chloride ion and some chloramines are oxidized to nitrogen trichloride and others. Continued addition of chlorine past the breakpoint will result in a directly proportional increase in the free available chlorine (unreacted hypochlorite). The amount of chlorine that must be added to reach a desired level of residual is called the chlorine demand [18] [42].

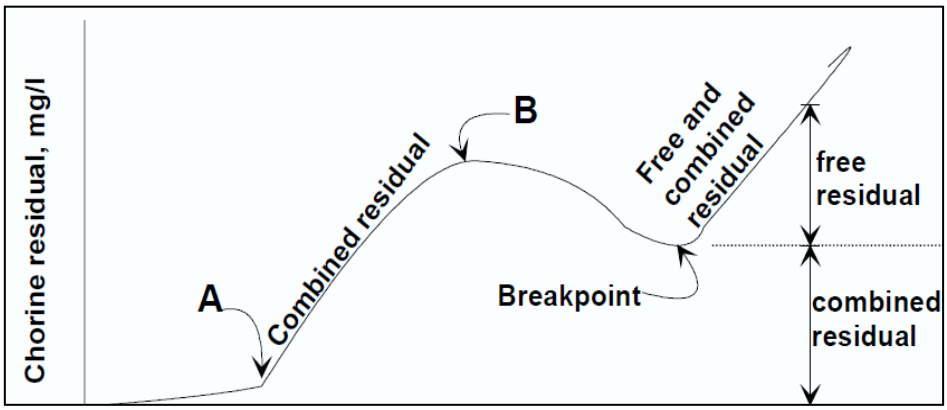

Figure 2.2: Chlorine break down curve [42]

When the physical parameters controlling the chlorination process are held constant, the germicidal effects of chlorine as measured by bacterial survival depend primarily on dosage (and form) and the CT. It has been found that increasing either dosage or CT, while simultaneously decreasing the other, can achieve approximately the same degree of disinfection [42].

Two factors of primary importance in disinfection are the concentration of the disinfectant residual (C) and the contact time (t). A low concentration of disinfectant with a long contact time accomplishes the same goal as using a high residual concentration with a short contact time [51].

2.3.3 Chlorine DBPs

Disinfection by chlorination of the secondary treated wastewater results in the formation of a wide range of organic compounds, called DBPs, which occur due to the reaction of chlorine with natural organic matter (NOM). The reaction creates diverse DBP such as THMs, HAAs and haloacetonitriles (HANs) as reflected in the following Equation 2.8 [52] [53].

$$Cl_2 + NOM \rightarrow THMs + HAAs + HANs + other\ DBPs \quad (2.8)$$

The byproducts formed are persistent, potentially toxic, and bio accumulative. The halogenated disinfection byproducts produced by chlorination came to the forefront of water research in the 1970's and are becoming a big issue. The organic materials present in water are known as "precursors," (organic matter, humic acids, etc.) of DBPs. Generally, water sample with higher content of NOM was found to form higher level of THMs during the chlorination process. The organic matter in water consists of humic substances and fulvic acid. A number of chlorinated byproducts are formed, out of which THMs are most often observed.

A THM is simply any carbon atom containing three halides. Common halides found in wastewater are Cl_2, bromine (Br) and sometimes iodine (I). The most common four THM compounds are chloroform ($CHCl_3$), bromoform ($CHBr_3$), dibromochloromethane (DBCM or $CHClBr_2$) and bromodichloromethane (BDCM or $CHCl_2Br$). The sum of these four compounds is referred to as TTHMs. It has been reported that prolonged exposure to the THMs may cause several types of cancers in humans. This is the reason that surface water quality standards have been developed and regulated at the discharge point of wastewater treatment plants. These finding highlight the concern for human health, which led to more comprehensive studies on monitoring and investigating the formation of DBP in chlorinated water. Due to the adverse health effects of THM, United States Environmental Protection Agency (USEPA) has set limits on acceptable TTHM concentration of 80-100 μg/L. With stricter regulation and guideline of THM and other DBP many water treatment plants are changing the disinfectant from chlorine to ozone, UV, chloramines etc. [53] [54]. Both gas chromatography – electron capture detector (GC-ECD) and gas chromatography/mass spectrometry (GC/MS) are adopted for detection and identification of DBPs.

The factors affecting the formation of DBPs are pH, temperature, CT, concentrations and speciation of disinfectant and its residuals, concentration and

characterization of NOM and bromide ions (Br^-) [55]. Generally, the greater CT and chlorine dosage will result in higher production of THM [56]. Several studies have found that there was a positive and linear relationship between chlorine dosage and the THM formation [17] [57].

2.3.4 Dechlorination

The growing concern regarding residual chlorine and its byproducts in wastewater effluents has resulted in the requirement to dechlorinate and to remove residual chlorine before it is discharged to the environment [41]. In most of the states the use of chlorination alone for wastewaters discharging to pristine receiving waters has come under criticism because of its effect on aquatic species [52]. High levels of total residual chlorine (TRC) in heavily chlorinated wastewaters must be removed by a dechlorination process to prevent toxic effects in the receiving waters [58]. Additional advantages of dechlorination are the removal of ammonia and COD from municipal wastewater. But it is a very expensive process, and difficult to operate properly.

Dechlorination can eliminate many of the problems associated with residual chlorine effects, but some drawbacks to this process require further study. Because the removal of chlorine will halt disinfection, adequate prior contact between residual chlorine and microorganisms must be insured. Sulfur dioxide (SO_2), sodium thiosulphate ($Na_2S_2O_3$), and sodium metabisulfite ($Na_2S_2O_5$) are the commonly used dechlorinating chemicals. Dechlorination reactions occur very rapidly, therefore no detention (contact) basin is required downstream from the dechlorinator [42] [52].

2.3.5 Advantages of Chlorine Disinfection

Although chlorine and chlorine related substances are not perfect disinfectants, they have a number of characteristics that make them highly valuable [23] [41].

- They have broad spectrum germicidal potency.
- They show a good degree of persistence in water distribution systems.
- Their easily measurable residual properties can be monitored in water networks after treatment.
- The feeding equipment is simple, reliable and inexpensive.
- Chlorine and chlorine based compounds are easily found, even in remote areas of developing countries.

- This method is economic and cost effective.

2.3.6 Limitations of Chlorine Disinfection

In sum, the major limitations of the chlorination process are as follows [22] [42].

- The chlorine residual, even at low concentration, is toxic to aquatic life and may require dechlorination.
- All forms of chlorine are highly corrosive and toxic. Thus, storage, shipping, and handling pose a risk, requiring increased safety regulations.
- Chlorine oxidizes organic matter in wastewater, creating more hazardous compounds (DBPs).
- Chlorine residual is unstable in the presence of high concentrations of chlorine demanding materials, thus requiring higher doses to effect adequate disinfection.
- Some microbial species have shown resistance to low doses of chlorine
- Long term effect of discharging dechlorinated compounds into the environment are unknown.
- Dechlorination increases the overall cost of the process.

2.4 Ozone Disinfection

Ozone is also an attractive disinfection alternative. It is a safe and powerful alternative to chlorination products which performs the same functions as the chlorine but without any undesirable side effects [59]. Owing to its oxidizing properties, ozone is currently known as one of the most efficient and fastest microbicides [60]. Ozone is not harmful to the environment since it is made from oxygen (O_2) and decomposes back into oxygen. The most common use of commercially produced ozone is in treatment of water and wastewater. Early application of ozone in the United States was primarily for non-disinfection purposes such as color removal or taste and odor control. But recently it is used in water treatment for disinfection and oxidation [59]. The increasing use of ozone in the treatment of municipal wastewater effluents has been stimulated by the need to achieve higher effluent quality and greater compliance with physicochemical and microbiological quality standards before discharge or reuse [61]. Pilot scale ozonation studies are being conducted in Austria, Germany, and Great Britain [62]-

[64]. The results which have helped STPs to explore the possibility of selecting ozonation for disinfection as well as for micro pollutant removal.

Ozone is a molecule composed of three oxygen atoms, temporarily existing in a very unstable and reactive state. It is so reactive that even a suitable container for storage probably does not exist. Since ozone cannot be stored or conveniently purchased, it must be produced on site as needed [59] [60] [65]. It is a pale blue gas at room temperature with a pungent odor and is highly corrosive and toxic. It is more than 10 times as soluble as oxygen, however only a small concentration of ozone dissolves in water in actual operating conditions due to its low partial pressure [66]. As compared to oxygen, ozone is an extremely active molecule, probably by a factor of 1,000 times and is sometimes referred to as activated oxygen [67]. The solubility of ozone in water or wastewater is an important property as the disinfection and oxidation of the micro pollutants depend on the amount of ozone transferred [13] [65]. It is produced when oxygen molecules are dissociated by an energy source into oxygen atoms and subsequently collide with an oxygen molecule to form an unstable gas, ozone [68]. The feed gas to produce ozone can be air or oxygen and the reaction is endothermic and requires a considerable input of energy as represented by Equation 2.9 [65] [66]:

$$3O_2 \leftrightarrow 2O_3 \quad (2.9)$$

There are many ways to produce ozone, such as [67] [69]:

- Electrical discharge – Corona discharge.
- Electrolytically – Electrolysis of an acid.
- Photochemically – UV radiation.
- Radiochemically

In most of the cases ozone is generated by imposing a high voltage alternating current (6 to 20 kV) across a dielectric discharge gap that contains oxygen gas. This process takes place within ozone generator and the generated gas was measured by an online ozone analyser [59].

Ozone is capable of oxidizing many organic and inorganic compounds in water. These reactions with organic and inorganic compounds cause an ozone demand in the water, which should be satisfied during water ozonation prior to developing a measurable residual [60] [61]. Basic chemistry research has shown that ozone decomposes spontaneously during water treatment by a complex mechanism that involves the generation of hydroxyl (OH) free radicals. The OH radicals are among the

most reactive oxidizing agents in water [21] [61] [65]. Ozone can react by either or both modes in aqueous solution [70]:

- Direct oxidation of compounds by molecular ozone.
- Oxidation of compounds by OH radicals produced during the decomposition of ozone.

The predominant oxidation reaction will depend on wastewater characteristics. The oxidation pathway that will dictate the transformation will depend on the reaction rate of ozone and the substrate, and the reaction products that may promote or inhibit ozone decomposition. The two oxidation pathways compete for substrate (i.e., compounds to oxidize). The direct oxidation with aqueous ozone is relatively slow (compared to OH radical oxidation) but the concentration of aqueous ozone is relatively high. On the other hand, the OH radical reaction is fast, but the concentration of OH radicals under normal ozonation conditions is relatively small as represented in Figure 2.3 [59] [60] [70].

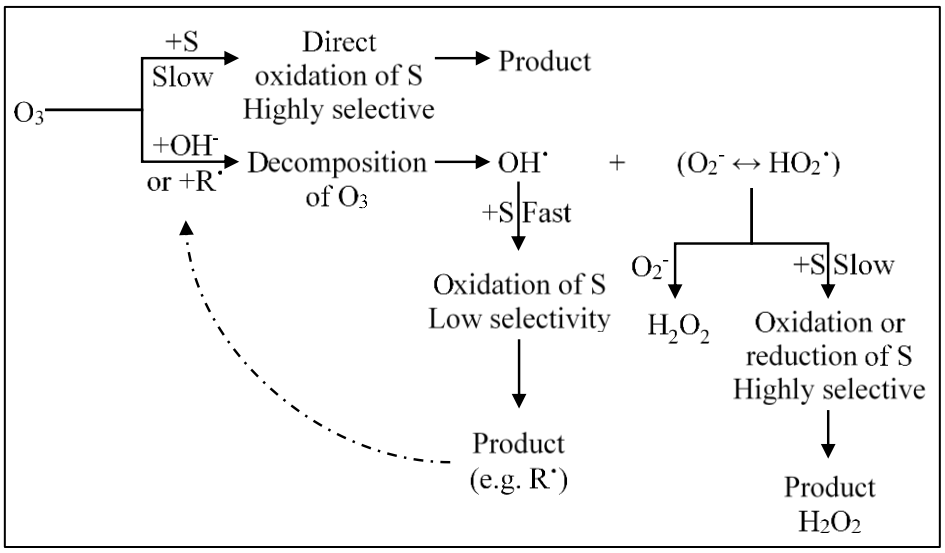

Figure 2.3: Oxidation of substrate during ozonation of water and wastewater

The main factors affecting the stability of ozone are the water or wastewater characteristics such as pH, alkalinity, and the organic matter content. The effectiveness of disinfection depends on the susceptibility of the target organisms, the CT, and the concentration of the ozone [71].

2.4.1 Ozone Disinfection Mechanism

A bacterium is composed of a cell wall surrounded by exopolysaccharides, then a cytoplasmic membrane, and finally the cytoplasm containing the genetic information carrying chromosome. Ozone is a powerful oxidant that destroys microorganisms through an irreversible physicochemical action, the action of ozone is instantaneous and irreversible [45] [66] [72].

Ozone inactivates bacteria by means of oxidation reactions. As can be seen in Figure 2.4, (a) the cell membrane is the first site under attack; then (b) the ozone attacks glycoproteins, glycolipids, or certain amino acids, and also acts upon the sulfhydryl groups of certain enzymes; (c) the effect of ozone on the cell wall begins to become apparent; (d) the bacterial cell begins to break down after being in contact with ozone; (e) the cell membrane is perforated during this process; and finally in (f) the cell "suffocate" to death or inactivation and disintegrates or suffers cellular lysis (see Figure 2.1). Ozone can destroy pathogenic and non-pathogenic microorganisms. The protective wall and the semi-permeable membrane are composed of molecules that are very rich in electron sites. This favours a very selective, and therefore efficient, action of ozone [60] [65].

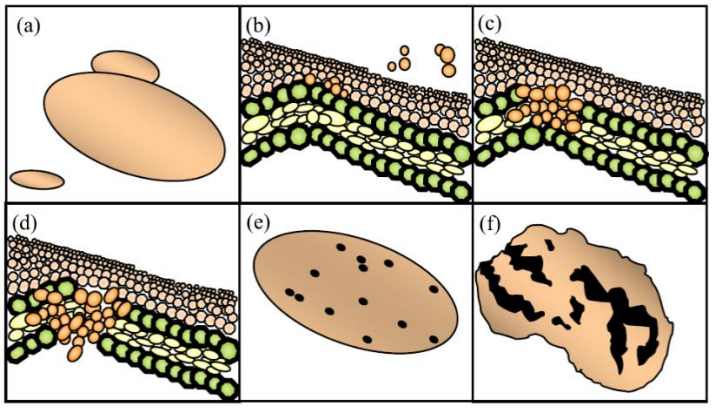

Figure 2.4: Bacteria undergoing lysis during disinfection with ozone [66]

Ozone is also capable of oxidizing organic matter in the effluent measured as COD [73]. It has been reported that microorganism reactivation after ozonation is unlikely to occur [60]. The disinfection dose is expressed as the transferred (or absorbed) mass of ozone per liter of effluent in mg/L. Another form of characterization for disinfection conditions is the concentration time (Ct) product, where C is the

concentration of dissolved (residual) ozone measured at the outlet of the contact chamber and t is the contact time between the residual of ozone and water [21] [61].

2.4.2 Ozone DBPs

Ozone reacts with a wide range of organic and inorganic compounds leading to the formation of reaction intermediates and stable byproducts. Ozonation leads to a change in the nature of organic matter (humic acid) in secondary wastewater effluent. The most common ozonation byproducts are aldehydes, fatty acids, alcohols, alkanes etc. A significant concern associated with ozone is the potential formation of halogenated substances such as bromate, a possible carcinogen and brominated organics arising from the reaction of ozone and bromide [74].

2.4.3 Advantages of Ozone Disinfection

Ozone has several advantages over chlorination which are listed below [21] [56]:

- Ozone is a very effective oxidant destroying viruses and chlorine resistant bacteria.
- The wastewater needs to be in contact with ozone for just a short time.
- Ozone decomposes rapidly, and therefore, leaves no harmful residual that would need to be removed from the wastewater after treatment.
- No regrowth of microorganisms is reported after ozonation.
- Ozone is generated onsite, and thus, there are fewer safety problems associated with shipping and handling.
- Ozonation increases the dissolved oxygen concentration of the discharged wastewater. The increase in dissolved oxygen can improve the oxygen content of the receiving body of water.

2.4.4 Limitations of Ozone Disinfection

Despite of several advantages of ozone it has few limitations also [21] [56] [75]:

- Low dosages may not effectively inactivate some viruses, spores, and cysts.
- Ozonation is more complex than other disinfection technologies.
- Ozone is very reactive and corrosive, thus requiring corrosion-resistant material, such as stainless steel.
- Ozonation is not economical for poor quality (poorly treated) wastewater.

- Ozone is extremely irritating and potentially toxic, so off-gases from the contactor must be destroyed to prevent worker exposure.
- The cost of treatment is relatively high, being both capital and power intensive.
- There is no measurable residual to indicate the efficacy of ozone disinfection.

2.5 UV Radiation Disinfection

Disinfection with UV radiation is a physical method of disinfection that has replaced chlorination in wastewater treatment due to the concern of DBPs [16] [75]. Disinfection by UV radiation requires a CT in the order of seconds to accomplish pathogen inactivation. The division of UV radiation may be classified as Vacuum UV (100-200 nm), UV-C (200-280 nm), UV-B (280-315 nm) and UV-A (315-400 nm) as represented in Figure 2.5. The most potent wavelength having germicidal efficiency is approximately 254 nm [75]. The lamps typically used in UV disinfection consist of a quartz tube filled with an inert gas, such as argon, and small quantities of mercury [75] [76].

The degree to which the destruction or inactivation of microorganisms occurs by UV radiation is directly related to the UV dose. The UV dose is calculated as product of intensity (mW/cm^2) and exposure time (s). In total, it is estimated that over 2,000 wastewater UV systems are in operation in the United States and Canada [77].

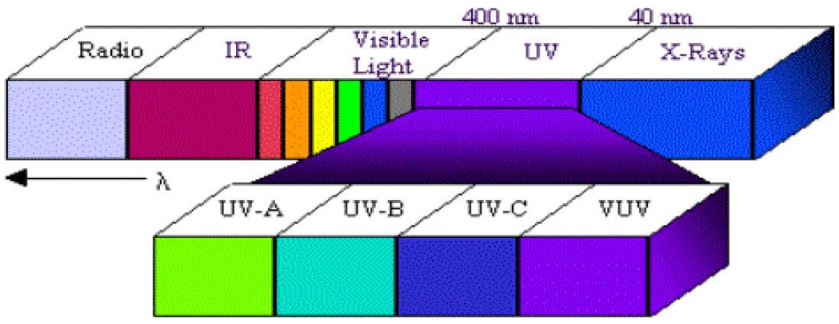

Figure 2.5: The UV spectrum

2.5.1 UV Disinfection Mechanism

The germicidal effects of UV light involve photochemical damage to nucleic acid (RNA or DNA) within the microorganisms [79]. UV radiation, generated by an electrical discharge through mercury vapour, penetrates the genetic material of microorganisms. DNA and RNA carry genetic information necessary for reproduction,

therefore, damage to either of these substances can effectively sterilize the organism. Damage often results from the dimerization of pyrimidine molecules. UV energy forms new bonds between adjacent nucleotides, creating double molecules or dimers. Dimerization of adjacent pyrimidine molecules, particularly thymine, is the most common photochemical damage. Cytosine (found in both DNA and RNA), thymine (found only in DNA), and uracil (found only in RNA) are the three primary types of pyrimidine molecules. Replication of the nucleic acid becomes very difficult once the pyrimidine molecules are bonded together due to the distortion of the DNA helical structure by UV radiation [80] [81].

UV radiation at low doses does not significantly change the chemistry of water nor does it significantly interact with any of the chemicals within water [77]. Therefore, no natural physiochemical features of water are changed and no chemical agents are introduced into the water. In addition, UV radiation does not produce a residual and as a result, the formation of THM or other DBPs with UV disinfection is minimal [82].

The source of UV radiation is either the low-pressure or medium-pressure mercury arc lamp with low or high intensities, an UV reactor is represented in Figure 2.6 [59] [60]. The optimum wavelength to effectively inactivate microorganisms is in the range of 250 to 270 nm. The intensity of the radiation emitted by the lamp dissipates as the distance from the lamp increases. Low-pressure lamps emit essentially monochromatic light at a wavelength of 253.7 nm [83].

Medium-pressure lamps are generally used for large facilities. They have approximately 15 to 20 times higher germicidal capacity than of low-pressure lamps. The medium-pressure lamp disinfects faster and has greater penetration capability because of its higher intensity. However, these lamps operate at higher temperatures with higher energy consumption [15] [77] [83].

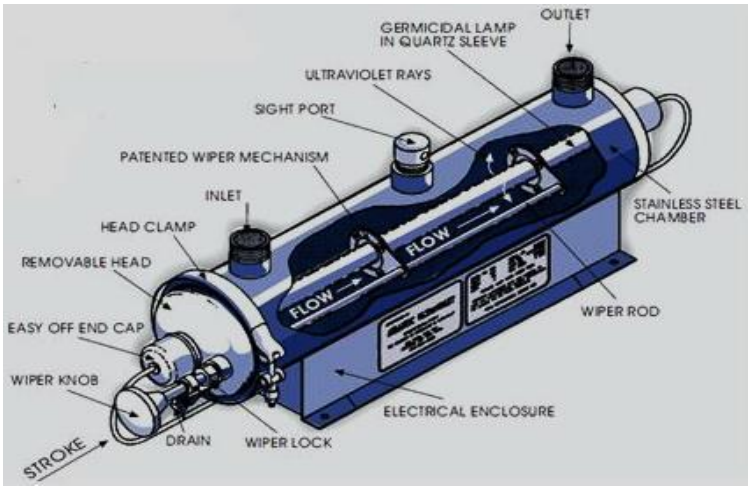

Figure 2.6: Closed vessel UV reactor [59] [60]

2.5.2 Repair Mechanism

Under certain conditions, some organisms are capable of repairing damaged DNA and reverting back to an active state in which reproduction is again possible. Studies report that the amount of cell damage and subsequent repair is directly related to the UV dose. For low UV doses the resulting minimal damage can be more readily repaired than for high doses where the number of damaged sites is greater [84]. Two phenomena are of key importance when using UV disinfection in water treatment, dark repair mechanisms and the capability of certain organisms to photoreactivate following exposure to certain light wavelengths especially visible light, also known as self-healing.

The details of these repair mechanism are as follows:

1. Photoreactivation occurs as a consequence of the catalyzing effects of sunlight at visible wavelengths outside of the effective disinfecting range. To minimize the effect of photoreactivation, UV contactors should be designed to either shield the process stream or limit the exposure of the disinfected water to sunlight immediately following disinfection. Therefore the wastewater stream is covered or somehow kept in the dark, immediately following the UV irradiation [84] [85]
2. Dark repair does not require light energy. It is an enzyme repair process involving the excision of dimers and may be similar to the repair of cell damage caused by non-photochemical agents. Dimer formation in cytosine is repaired by this mechanism [84] which is represented in Figure 2.7.

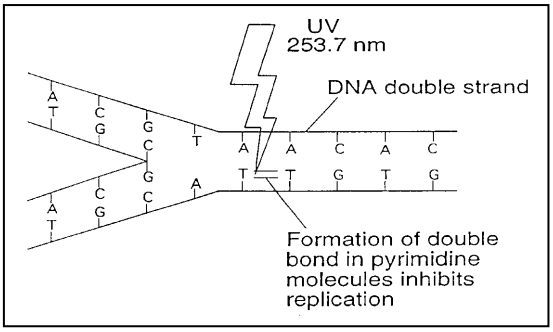

Figure 2.7: Repair mechanism by UV radiations

UV disinfection can be used in plants of various sizes that provide secondary or advanced levels of treatment. The wastewater should be highly treated and clear so that the UV light can pass through the water and strike the targeted microorganisms. Both the concentration of TSS and the concentration of particle-associated microorganisms determine how much UV radiation ultimately reaches the target organism [83]. The organic particles in the solids provide protection to microbes and thus higher UV doses are required to penetrate and kill all the bacteria. Figure 2.8 illustrates that the protection provided by particulates results in a high UV dose demand. Filtration results in a decrease in TSS levels, decreased particle sizes and numbers and also a decrease in the UV dose required to achieve a given disinfection target.

The UV dose required to achieve the desired level of disinfection will vary with the standard to be achieved. In general, a UV dose of 20 to 30 mWs/cm^2 or sometimes as high as 120 mWs/cm^2 is required to achieve this level of disinfection in secondary treated wastewater with a 65% transmittance and TSS < 20 mg/L [79].

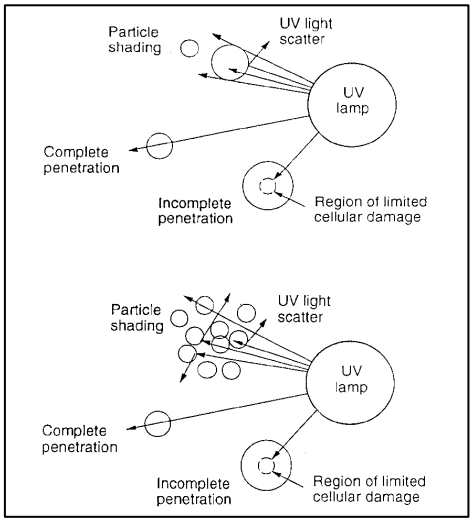

Figure 2.8: Particle Interactions that Impact UV Effectiveness [48]

2.5.3 Advantages of UV Disinfection

UV disinfection is a physical process of treatment that has some advantages over chemical treatments, which are listed below [13]:

- It is effective at inactivating most viruses, spores, cysts and species which are resistant to chlorination.
- It is a physical process rather than a chemical process, which eliminates the need to generate, handle, transport, or store toxic/hazardous or corrosive chemicals.
- It has no residual effect that can be harmful to humans or aquatic life.
- It is user-friendly for operators.
- It has a shorter CT when compared with other disinfectants (in order of seconds with low-pressure lamps).
- UV disinfection equipment requires less space than other methods.

2.5.4 Limitations of UV Disinfection

UV disinfection has some drawbacks which are listed below [15]:

- Low dosage may not effectively inactivate some viruses, spores, and cysts.
- Organisms can sometimes repair and reverse the destructive effects of UV radiation through a repair mechanism.
- A preventive maintenance program is necessary to control fouling of tubes.
- Turbidity and TSS in the wastewater can render it ineffective.
- It is not as cost-effective as chlorination, but costs are competitive when chlorination/dechlorination is used.

Various studies have been reported and the work done in this area of disinfection using the three disinfectants i.e. chlorine, ozone and UV radiations in the last ten years has been compiled and represented in Table 2.2.

Table 2.2: Summary of investigation (last 10 years) done on wastewater disinfection using the three disinfectants

S. No.	***Disinfectant***	***Investigator***	***Research Findings***
1	Chlorine	Chowdhury and Champagne, 2008	Effect of NOM, pH, time, dose and bromide concentration on DBPs [86]
2	Chlorine	RULE, D. B., 2006	Effect of chlorine on THMs and other DBPs [87]
3	Chlorine	Hong et al., 2007	THMs accounted for more than 85% of all DBPs [88]
4	Chlorine	Brown et al., 2011	Investigated that among THMs, chloroform is responsible for cancer. [89]
5	Chlorine	Li. D et al., 2013	Studied inactivation, reactivation, of indicators and pathogenic bacteria in reclaimed water after chlorine dose. [90]
6	Chlorine	Bashir et al., 2015	Reported optimization of wastewater treatment processes by RSM [91]
7	Chlorine	Kumar et al., 2011	Reported dose of 17.5 mg/L of calcium hypochlorite as optimum disinfection dose [18]
8	Ozone	Thanomsub et al., 2002	Studied ultrastructural changes and inactivation mechanism in bacteria by SEM [92]
9	Ozone	Xu et al., 2001	Reported that 2-15 mg/L ozone dose is sufficient. [65]
11	Ozone	Lazarova et al., 1999	Reported that 25-30 mg/L ozone dose is sufficient. [21]
12	Ozone	Subha and Muthukumar, 2012	Worked on optimization of ozonation using CCD. [93]
13	Ozone	Bustos et al., 2014	Reported 3 to 40 mg/L ozone dose to be sufficient. [73]
14	Ozone	Yasar et al., 2007	Reported that ozone improves effluent quality [94]
15	Ozone	Petalla et al., 2006	Reported ozone dose of 26.7 mg/L [95]

16	Ozone	Cho et al., 2010	Ozone inactivates microbes by causing damage to cell surface. [96]
17	UV	Brahmi and Haseen, 2012	Reported UV dose of 10.7-183 mWs/cm^2 [81]
18	UV	Sommer et al., 2000	Reported UV dose of 124 J/m^2 [97]
19	UV	Spiliotopoulou et al., 2000	Studied formation of DBPs during UV treatment and reported that post UV chlorine induced DBP formation. [82]
20	UV	Yu et al., 2006	Studied disinfection efficiency by UV exposure of microbes which increased with UV dose. [98]
21	UV	Das, 2001	Stated that UV disinfection depends on effluent quality. High TSS concentration in effluent will reduce the UV transmittance and results in a higher coliform count. [76]
22	UV	Abou-Elela et al., 2012	Reported 164 mWs/cm^2 as effective UV dose [13]

Table 2.3: Comparative performance of disinfection techniques [13] [14] [18] [23]

S. No.	***Attributes***	***Chlorine***	***Ozone***	***UV***
1	Disinfection capability	Good	Excellent	Excellent
2	Generation of DBPs	THMs and HAAs	Bromine products, Aldehydes, Ketones	None
3	Persistent residual	Good	None	None
4	Safety concerns	High	Medium	Low
5	Complexity of operations/maintenance	Minimal	Moderate	Minimal
6	Size applicability	All sizes	Medium-large	Small-medium
7	Relative cost	Low	High	Moderate
8	Long term applicability	Medium	High	High

2.6 Hybrid Disinfection Technology

The findings discussed above and in Table 2.3 proves that the three disinfectants; chlorine, UV irradiation and ozone are capable of producing a treated effluent free from pathogens. It is clear from the previous studies that chlorine is most cost effective and common disinfectant, but its residual value, even at low concentration is toxic to aquatic life and thus may require dechlorination. Also, all forms of chlorine are highly corrosive and toxic thus its storage, shipping and handling pose safety risks. UV irradiation, on the other hand, is clean and environment friendly but the main concern is that of regrowth or reactivation of microbes, high energy requirements and possible hazards to ill trained operators. In addition, the use of ozone as a disinfectant has no harmful residuals except for brominated residues that are of concern, but overall process is very expensive.

Hence, these findings indicate that individual disinfection methods are not totally safe and have some severe limitations especially in terms of environmental consequences making it difficult to meet the disinfection standards without harming environment. The recent research has shown the application of sequential, multiple, or simultaneous use of two or more disinfectants is more effective than the added effect of the individual disinfectants [17] [99] [100] [101]. Such a research where two or more

disinfectants produce simultaneous or sequential application to achieve more effective pathogen inactivation, is also termed as interactive or hybrid disinfection.

There are published reports [17] [99]-[106] from laboratory tests of synergistic benefits for using two or more disinfectants in water treatment which supports the basis that the overall inactivation of microorganisms is greater than the sum of the inactivation achieved for each disinfectant individually [104]. But not much could be traced on wastewater treatment except for a few reports, indicating that a combination of disinfectants such as ozonation followed by chlorination was found effective in reducing THMs and other halogenated DBPs [105].

Finch et al. [102] and Sobsey et al. [40] reported that the sequential combination of free chlorination followed by monochloramination showed better disinfection efficiency as compared to the sum of both disinfectants examined separately [40] [102]. Similar synergistic effects were reported for ozone and chloramines. Another synergistic study includes combinations such as UV/O_3, O_3/hydrogen peroxide (H_2O_2) etc. [105]. The combined performance of UV light followed by chlorine during disinfection of reclaimed water was experimentally assessed by some authors [101]. Gil Crozes [106] presented two case studies, outlining the benefits of using ozone and UV radiation. The synergies of the two treatment alternatives demonstrated the cost effectiveness and robustness of the treatments. Cecilia and Claudio [107] studied the results on the disinfection efficiency of the synergic combined treatment between UV and PAA. The efficiency enhances by using the UV/PAA treatment, but a much higher efficiency gain occurred by using PAA/UV treatment. The combination of UV irradiation as a primary disinfectant and free chlorine or monochloramine as a secondary disinfectant has shown to prevent better microbial regrowth by Ballester and Malley [108] and Shang et al. [109].

Sequential disinfection was proposed, to eliminate the inactivation lag phase [102]. Thus, a substitutive disinfectant technology that can supplement the insufficient Ct value rate and simultaneously optimize the removal of microorganisms during the conventional treatment processes must be considered for wastewater treatment also. Such technology will have a low risk of DBPs while having a strong Ct value to inactivate the microorganisms. It was reported that in a sequential disinfection scheme, a strong primary disinfectant is first applied to achieve a portion of the target inactivation level followed by the secondary disinfectant to attain further inactivation and to provide residual disinfection for water distribution [110].

2.7 Research Gaps

As it is discussed above that each individual disinfectant had some shortcomings, which could be overcome by the hybrid approach. Hence, the present research focused on the sequential/hybrid disinfection strategy for wastewater. A brief outline of the work reported by various researchers in last five years on hybrid disinfection has been presented here, which highlights the need of the present study.

Quiroz et al. [111] reported that advanced oxidation potential (AOPs) use different reagent systems for better disinfection efficiency, which include photochemical degradation processes (UV/O_3, UV/H_2O_2), photocatalysis (titanium dioxide (TiO_2)/UV, photo-Fenton reactives), and chemical oxidation processes (O_3, O_3/H_2O_2, H_2O_2/ferrous ion (Fe_2^+)), producing OH radicals. These radicals are very reactive, attack most organic molecules, and are not highly selective.

Kumar et al. [18] reported the efficiency of chlorine disinfection on secondary treated sewage. The results showed that, a dose of 17.5 mg/L in the form of calcium hypochlorite was found to be optimum for disinfection because this was the minimum dose required to bring the total coliform and pathogenic counts to less than 1000 CFU/ 100 mL, as per the desired USEPA standards.

Rojas-Valencia [66] reported that chlorine dose of 5 to 20 mg/L for CT of 15-30 min was required for up to 4 log reduction and ozone dose of 15 mg/L for CT of 5 min was required for achieving the same reduction. It states that in terms of cost, chlorination is more efficient (\$0.028 USD/m^3) than disinfection with ozone (\$0.043 USD/m^3). But ozone is 25 times more efficient than hypochlorus acid.

Abou-Elela et al. [13] investigated the efficiency and viability of chlorine, UV radiations and ozone as disinfectants for secondary treated wastewater. It was reported that for complete inactivation of total coliform the chlorine dose required was 32 mg/L for 15 min, ozone required was 15 mg/L for 15 min for removal of all pathogens and UV dose of 164 mWs/cm^2 was required to reduce the TCC by 3 logs. The economic analysis revealed that chlorination proved to be the most economical process followed by UV disinfection and then ozonation.

Wang et al. [100] investigated the effectiveness of UV and chlorination individually and sequentially in killing pathogenic microbes, and inhibiting the formation of DBPs in two different municipal wastewaters. Synergistic effect results

into the most effective option for complete removal of all the three tested bacteria. UV disinfection lowered the required chlorine dose which in turn decreased TTHM formation during chlorination. TTHM were reduced by 7.5 µg/L. The complete inactivation of bacteria in wastewater was accomplished by treatment with 15 mJ/cm^2 UV followed by 1.6 mg/L chlorine. This result could not be achieved with chlorine treatment alone, even with 5.5 mg/L chlorine.

Jang et al. [17] studied a sequential application of ClO_2 or UV as a primary disinfectant followed by HOCl as secondary disinfectant to evaluate the synergistic inactivation of *B. subtilis* spores and its effect on DBPs. It was observed that ClO_2/HOCl was more efficient in inactivation of bacteria as well as resulted in lower DBPs, when compared to UV/HOCl and HOCl alone. ClO_2/HOCl resulted into 20% decrement in THM formation than HOCl alone.

Souza et al. [112] studied the individual methods of disinfection by PAA and UV radiation and combined process PAA/UV in sanitary wastewater to verify the individual and combined action of these AOPs on the effectiveness of inactivation of microbial indicators. The results indicated that the combined method provided superior efficiency compared to individual methods of disinfection.

Rodríguez et al. [113] compared the inactivation of *Escherichia coli* in wastewater effluents using conventional treatments (chlorination) and AOPs such as UV irradiation, H_2O_2/solar irradiation, and photo-Fenton processes. In addition, an analysis of the operational costs of each treatment is carried out taking into account the optimal dose of chemicals used. Total inactivation of bacteria (7.5 log) was achieved by means of chlorination and UV irradiation. On the other hand, the combination H_2O_2/solar irradiation achieved a maximum inactivation of *E. coli* of 3.30 ± 0.35 log. The photo-Fenton reaction achieved a level of inactivation of 4.87 ± 0.10 log. The order of disinfection, taking into account the reagent/cost ratio of each treatment, was as follows: chlorination > UV irradiation > photo-Fenton > H_2O_2/sunlight irradiation.

Zhang et al. [75] investigated disinfection methods including chlorination, UV radiation and sequential UV/chlorination treatment for the inactivation of antibiotic resistance genes in municipal wastewater treatment plant effluent. It was observed that with sequential treatment log synergy removal values of target genes were higher.

Medeiros and Daniel [114] analysed individual disinfection with chlorine and UV and sequential disinfection (Cl_2/UV radiation). The test were conducted with anaerobic effluent in batch process with two dosages of chlorine (10 and 20 mg/L) and UV (2.5

and 6.1 Wh/m^3). It is possible to use smaller Ct for primary disinfection with chlorine, since sequential disinfection may be present inactivation similar to the one obtained with the use of only one disinfectant at higher Ct. Synergistic effect was noticeable for the two resistant species *G. perfringes* and *Giardia spp*.

In sum, the work done in this area in the recent past has been cited in the section 2.7, the information presented above indicated strongly that a hybrid disinfection strategy for wastewater involving reverse sequence of disinfectants i.e. Cl_2/O_3 and Cl_2/UV may result optimization of the overall disinfection process both in terms of economy and efficacy for disinfection. A further benefit may accrue in terms of reduced THMs both due to their lower formation (lesser CD) as well as possible scavenging of these during subsequent treatment.

Chapter Summary

Different disinfection techniques are available for wastewater which are listed in this chapter. The chapter highlights the need of an advanced disinfection technology that can overcome the limitations of the conventional disinfection technologies such as chlorine, ozone and UV. It presents a brief introduction about the most upcoming hybrid disinfection technique.

The next chapter marks the formal beginning of the thesis and discusses the materials and methodology used in the present study.

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Chapter 3

Chapter 3

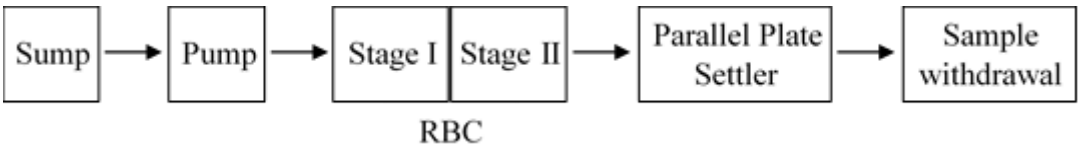
Materials and Methods

This chapter describes the research methodology adopted to accomplish the objectives delineated in Chapter 1. It presents the methods used to collect information and experimental protocols followed to generate data required for the study. The research work was carried out at PHE laboratory of Department of Civil Engineering at MNIT, Jaipur. The entire research work has been divided into six phases described below.

3.1 Phase 1: Sample Collection and Quality Analysis

The source water used in the study was collected from the nearby STP located in MNIT campus based on the RBC treatment process for carrying out secondary treatment. The plant consists of a series of the closely spaced honeycomb structured discs mounted on a rotating shaft, which is supported just above the surface of the wastewater keeping about 40% radial submergence. Microorganisms grow on the surface of the discs where biological degradation of the wastewater pollutants takes place. The capacity of this STP is 0.2 MLD. (Figure 3.1). The STP consists of preliminary, primary and secondary treatment. Samples were collected at a fixed time whenever required from the outlet of the STP between 9 to 9.30 am, which is the peak discharge period. The samples were collected in sterile glass containers and stored at low temperature (4ºC) in the dark before analysis.

(a)



(b)

Figure 3.1: (a) RBC plant and (b) Flow diagram of RBC plant at MNIT campus

3.1.1 Physicochemical Analysis

Some of the physicochemical parameters of secondary treated effluent, which affect the disinfection process, like pH, COD, BOD, turbidity and total suspended solids (TSS) were analysed in the laboratory. These selected parameters were analysed according to standard methods for the examination of water and wastewater analysis [1]. Samples were analysed before disinfection and after each stage of disinfection to study the effect of the specific disinfectants on the parameters.

***pH*:** The pH of a solution is a measure of the molar concentration of hydrogen ions in the solution and is a measure of the acidity or basicity of the solution. It was determined by using the pH meter of Hanna HI-n98128, with an accuracy of ± 0.02 pH. Electrodes were thoroughly rinsed with distilled water before being immersed in the solution.

***COD*:** The COD test was carried out to indirectly measure the amount of organic compounds in water. To determine COD, the closed reflux method [1] was followed. The COD analysis required digestion of samples using potassium dichromate and silver sulphate in sulphuric acid for 2 h at 145°C, which was carried out in COD digester (HACH DRB 200) and digested samples were analysed by UV-Visible spectrophotometer (UV- 1800 Shimandzu) at 600 nm.

***BOD*:** The BOD is the amount of dissolved oxygen needed by aerobic organisms in the water sample and BOD analysis was carried out for determining the organics in the sample. It was determined by Winkler's azide modification method as described in the standard methods for the examination of water and wastewater [1]. The analysis was carried out both before and after the disinfection step.

***Turbidity*:** Turbidity measure the water clarity, in terms of decrement in the passage of light through water due to presence of suspended material. A digital nephelometer

(Model 341 E, Electronics India) was used to measure turbidity with an accuracy of 2% F.S. ± 1 digit. The range of the instrument was 9 to 19.9 NTU and 0 to 199.9 NTU. A standard turbid solution was used to check the meter's calibration before each measurement to standardize the process.

***TSS*:** The TSS gives a measure of the turbidity of the water. The principle of TSS determination was that water samples were filtered through pre-weighed filters and the residue collected on the filter was dried to constant weight. The measured weight change gives the value of TSS in the sample as described by APHA [1].

3.1.2 Microbiological Analysis

Microbiological analysis of the wastewater samples before and after disinfection was conducted using two conventional methods, namely, the pour plating technique, and the Colilert-18 based on IDEXX's patented defined substrate technology (DST).

***Pour Plate Technique*:** It is an alternative method for using agar plates to obtain isolated colonies. Pour plates are used when it is necessary to know the number of organisms present per unit volume of a specimen or another sample. The sample was appropriately diluted and a small aliquot was transferred to an agar plate. The sample was then distributed evenly over the surface by a special spreading technique [2] [3]. All the microbiological work was carried out under sterilized conditions in Laminar Air Flow (Toshiba Kirloskar Electrodyne). Media require 24-36 h for the development of microbial colonies. Different specific media such as MacConkey Agar, Eosin Methylene Blue (EMB), Xylose Lysine Deoxycholate (XLD) Agar and Hekton Agar were used for morphological identification of specific bacterial species as described in Table 3.1.

The bacterial isolates are further identified by microscopy by observing the colony characteristics and spore formation as described in Figure A.1 (Appendix A). After colonies were grown, they were counted with the help of a microprocessor colony counter (Labtronics), and the number of bacteria in the original sample was calculated. The estimation of TC and specific pathogenic species were also carried out by summing up of individual counts of dominant species. The bacterial count is reported as colony forming units (CFU) and calculated by using the following formula [3].

CFU/100 mL = (Counts/volume of sample plated x dilution factor) x 100

Table 3.1: Colony characteristics of different bacterial species on specific media

S. No.	***Appearance of Colonies***	***Microorganisms***	***Media***
1	*Escherichia coli* colonies grow with a metallic sheen with a dark centre	*E.coli*	EMB
2	Brown, dark centred mucoid colonies	*Enterobacter*	EMB
3	Brown, dark centred colonies smaller than *Enterobacter*	*Klebsiella*	EMB
4	Yellow, surrounded by yellow zones, opaque	*Serratia/Hafnia*	XLD
5	Yellow, surrounded by yellow zones, opaque, sometimes with a black centre	*Citrobacter*	EMB, XLD
6	Blue and green with dark centre	*Salmonella*	Hekton
7	Red colonies with black centre	*Salmonella*	XLD
8	Light green colonies	*Shigella*	Hekton
9	Red colonies	*Shigella*	XLD
10	Pink, flat and rough colonies	*Pseudomonas*	XLD
11	Pink colonies	TC	MacConkey

Estimation of TC and total of some of the pathogenic species was also carried out by summing up of individual counts of dominant species.

Colilert-18 Hr. Method with Quanti-Trays: It was used for the simultaneous detection and confirmation of TC. It is based on IDEXX's patented Defined Substrate Technology® (DST®). When total coliforms metabolize Colilert-18's nutrient-indicator, ortho-nitrophenyl-β-galactoside (ONPG), the sample turns yellow. Colilert-18 can simultaneously detect these bacteria ranging between 1 to 2 million MPN/100 mL level within 18 hours [4] [5] [6].

The Colilert reagent was added to the raw or diluted sample for the test being run. The sample was shaken well to dissolve the reagent completely. The entire contents of the sample were poured into a sterile Quanti-Tray 2000, avoiding air bubbles. The Quanti-Trays were sealed with Quanti-Tray sealer. The sealed Quanti-Trays were placed in the incubator for 24 h with wells facing downwards. The number of positive

(yellow) wells was counted and referred to the most probable number (MPN) table as shown in Figure 3.2. The results are represented as MPN/100 mL of sample. If a dilution was performed, after obtaining the initial MPN result from the table, an appropriate correction was made to obtain the final counts of the sample.

Figure 3.2: Stepwise procedure for estimating TCs detection using Colilert-18 Hr. Method with Quanti-Trays

3.2 Phase 2: Disinfection by Chlorine

The disinfection of secondary treated effluent of sewage using controlled doses of chlorine was carried out in both batch and continuous processes.

3.2.1 Batch Process

Disinfection of secondary treated effluent was performed using calcium hypochlorite solution at different doses ranging from 1 mg/L to 4.5 mg/L for a contact time of 20 minutes for each specific dose. The specific doses were selected on the basis of previous studies and preliminary experiments [7] [8]. The secondary treated effluent (sample) of around two litre volume was collected in a glass beaker, and mixed thoroughly using magnetic stirrer as represented in Figure 3.3. The freshly prepared solution of calcium hypochlorite was added to the sample in required doses with continuous and complete mixing by the magnetic stirrer to dissolve the solution of calcium hypochlorite completely. The samples were then drawn after every 5 minutes, followed by the microbiological analysis as described in Section 3.1.2. The total disinfection time was 20 minutes. Afterwards, the dechlorination of chlorinated samples was conducted by 10% sodium thiosulphate solution [1]. Free and total residual chlorine was measured by using residual chlorine photometer (Hanna HI96711C) as shown in Figure 3.4.

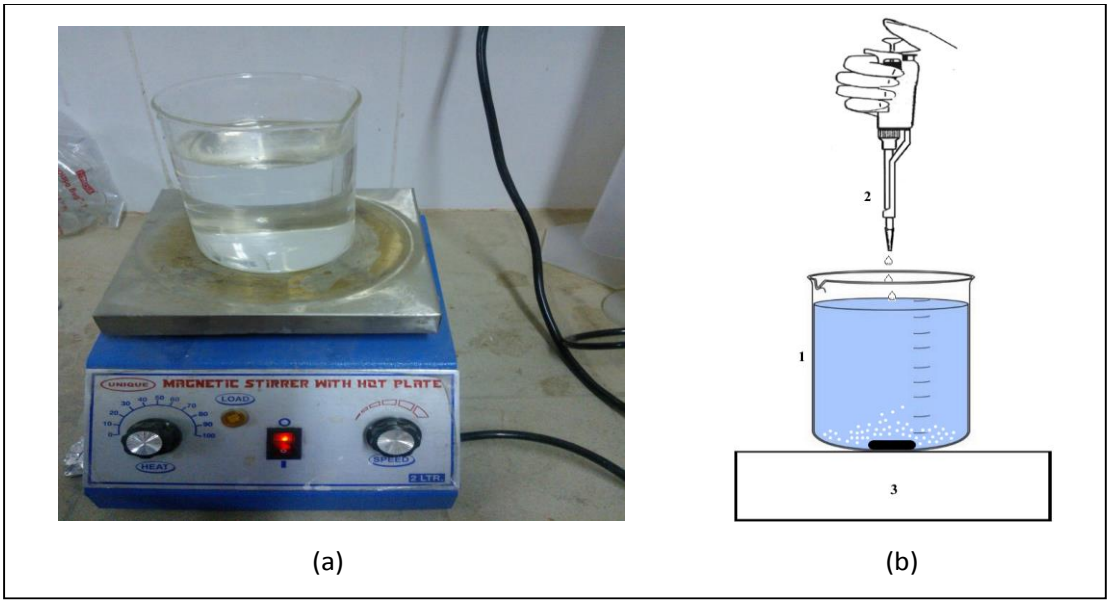


Figure 3.3: (a) Experimental set up for batch reactor for chlorination; (b) Schematic diagram of experimental set up – 1. Reactor vessel; 2. Pipette; 3. Magnetic stirrer

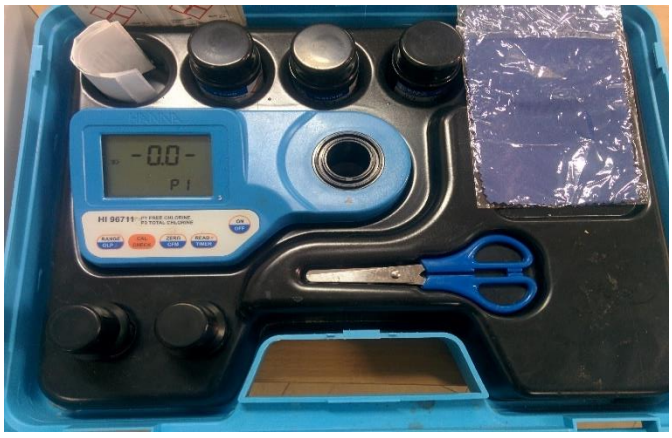


Figure 3.4: Residual chlorine photometer (Hanna HI96711C)

3.2.2 Continuous Process

The disinfection of secondary treated effluent was carried out using calcium hypochlorite solution in continuous mode at different doses ranging from 1 mg/L to 6.5 mg/L for a contact time of 20 min for each specific dose. The stock solution of chlorine was prepared by weighing bleaching powder according to the required dose and dissolving it in 2.5 L distilled water. The stock solution of chlorine was supplied to a chlorine reservoir bottle through the peristaltic pump and from this reservoir to the reactor vessel with the help of another peristaltic pump continuously. With another pipe and peristaltic pump, secondary treated effluent was passed through the reactor vessel

at a defined flow rate (6 mL/min) to maintain the contact time of 20 minutes. The reactor was continuously mixed with a magnetic stirrer (Figure 3.5). After 20 min, sodium thiosulphate was added to the mixture for dechlorination. Samples were microbiologically analysed and also for free and total chlorine residue after every 5 min duration.

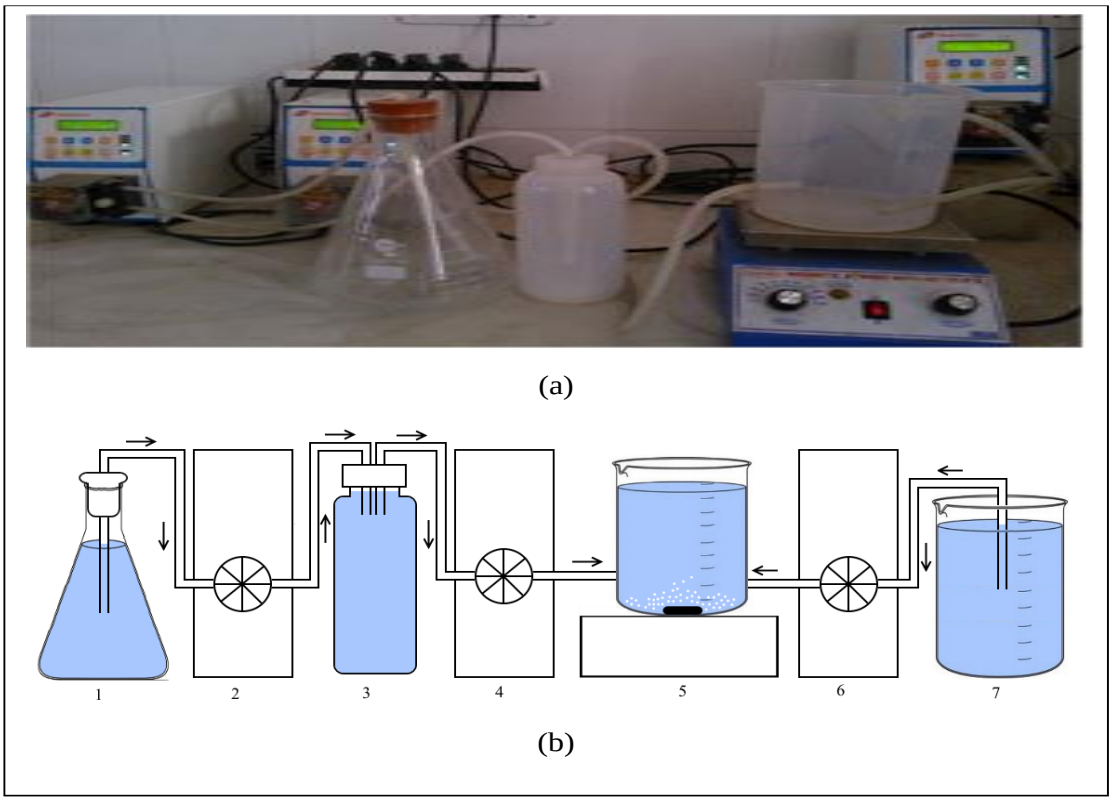


Figure 3.5: (a) Experimental set up for continuous reactor for chlorination; (b) Schematic diagram of experimental set up – 1. Stock chlorine reservoir; 2. Peristaltic pump; 3. Chlorine reservoir; 4. Peristaltic pump; 5. Reactor vessel; 6. Peristaltic pump 7. Sample container

3.3 Phase 3: Ozone Disinfection

The entire ozonation system consisting of the ozone generator, ozone analyser and assessor units were housed in the well vented hood. Ozone generation rate was monitored with the help of "Anseros" made "OZOMAT GM-OEM", which is associated with ozone generator as shown in Figure 1(a). Experiments were carried out in a 1000 mL capacity column glass reactor and the ozonation reactions were carried out in a batch process. The design parameters of reactor vessel [9]-[12] are listed in Table 3.2:

Table 3.2: Design parameters of ozone reactor

Parameters	***Value***
Column height	36 cm
Column diameter	6 cm
Water height	30 cm
Volume	1,000 mL
Air flow rate	150-200 L/h

A coarse bubble glass diffuser dispersed the air enriched with ozone at the bottom of the reactor due to which the water flowed counter current with the rising gas bubbles. A schematic sketch of the reactor set up is shown in Figure 3.6.

Ozone gas was produced by passing oxygen between two electrodes bearing an AC potential under electrical corona discharge through a prominent ozone lab generator of Anseros made "OZOMAT COM" which is based on the principal of the "Corona Discharge". The gas flow was maintained at 50-100 NL/h and the concentration of ozone in the generated gas was measured by an online ultraviolet gas ozone analyzer (OZOMAT GM-OEM of ANSEROS, Germany). Ozone is formed by combining an oxygen atom with an oxygen molecule (O_2). This reaction is endothermic and requires a considerable input of energy [13]. When ozone decomposes in water, the free radicals such as hydrogen peroxy (HO_2°) and hydroxyl ions (OH°) are formed that have a great oxidizing capacity [10] [13].

Knobs provided on the ozone generator (Figure. 3.6) were adjusted to achieve the desired gas flow rate, system operating pressure and ozone generation rate. After the ozone generation rate has been stabilized (within 2 minutes) as indicated by the constant reading on the ozone monitor/analyser, the gas was introduced into the reactor and a stopwatch was turned on to keep track of the reaction period (1-5 minutes). Specific doses of ozone were injected and a total of 5 minutes of residence was allowed before drawing out samples for further microbial and COD analysis. The outgoing ozone in the exit gas stream from the reactor was estimated iodometrically by titrating the iodine liberated in the Potassium iodide (KI) traps (having absorbed ozone) using sodium thiosulphate ($Na_2S_2O_3$) to calculate the total consumption of ozone in the reactor [13]. Hence, total ozone dose transferred in the reactor per volume of sample (mg/L) was calculated by subtracting ozone concentration absorbed in iodine

displacement in the outgoing gas stream of the reactor from the product of total ozone concentration applied in the reactor for a specific time.

3.3.1 Selection of Ozone Dose

Disinfection by ozone was carried out at different doses ranging from 15 to 42 mg/L to achieve the WHO standard for reusing treated wastewater. Different ozone doses were selected based on the literature review and preliminary experiments, as the ozone concentration ranging between 3 to 40 mg/L has been reported for the inactivation of total coliform [9] [10]. The criteria for selection were, the minimum ozone dose that meets disinfection target of 1000 CFU/100 mL and the ozone dose that consistently meets the disinfection target and at lowest COD value in the treated effluent [11].

The progress of ozonation was monitored by estimating the effect of ozone dose on removal of microbes (which are naturally present in wastewater samples) through bacteriological analysis and residual concentration of COD.

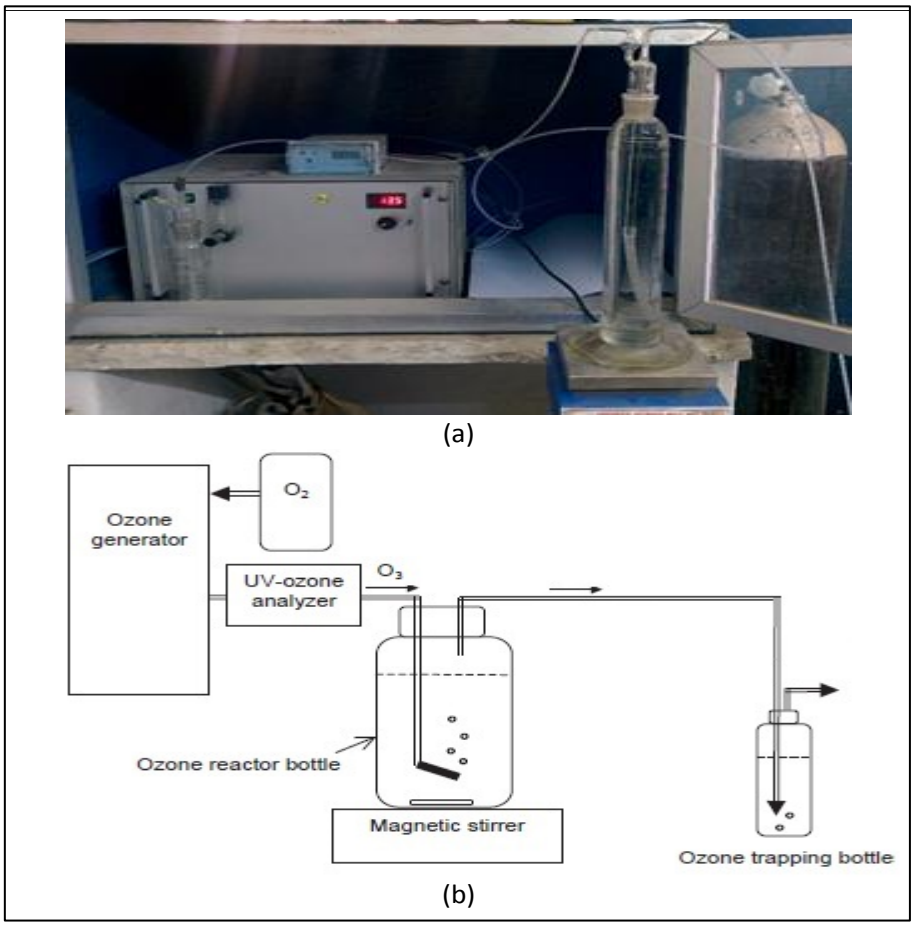


Figure 3.6: (a) Pictorial view of the experimental set up of ozone disinfection unit; (b) Schematic diagram of the semi-continuous ozonation set up

3.4 Phase 4: UV Disinfection

The Figure 3.7 represents the fabricated experimental set up of UV disinfection and to study the reactivation phenomenon of microbes due to the effect of visible light on the secondary treated effluents. The units were made of non-reactive aluminium metal (similar to "Aqua Guard") of volumetric capacities of 1000 mL. As shown in Figure 3.7, the left one is the visible light exposure chamber, and the right one is a closed vessel vertical UV reactor unit having 8 W UV lamp enclosed in a quartz tube which was used for disinfection (Table 3.3). Bulbs of UV as well as visible light were of 8 W capacity. Peristaltic pumps were used to maintain a constant flow of the treated effluents. For providing UV dose of 75 mJ/cm^2, 1,000 mL sewage sample was passed through the 8 W UV unit using a peristaltic pump (160 rpm) for a contact time of 94 s to find out the disinfection efficiency of the chlorinated effluent on the microbiological population [15].

In the same way, for providing UV dose of 112 mJ/cm^2, contact time provided was 140 s. For UV dose of 150 mJ/cm^2, 1,000 mL sewage sample was passed through the 8 W UV unit using a peristaltic pump (160 rpm) for a contact time of 188 s. To study the effect of visible light on reactivation of microbes, the tertiary treated effluent of UV disinfection was passed to the visible light chamber.

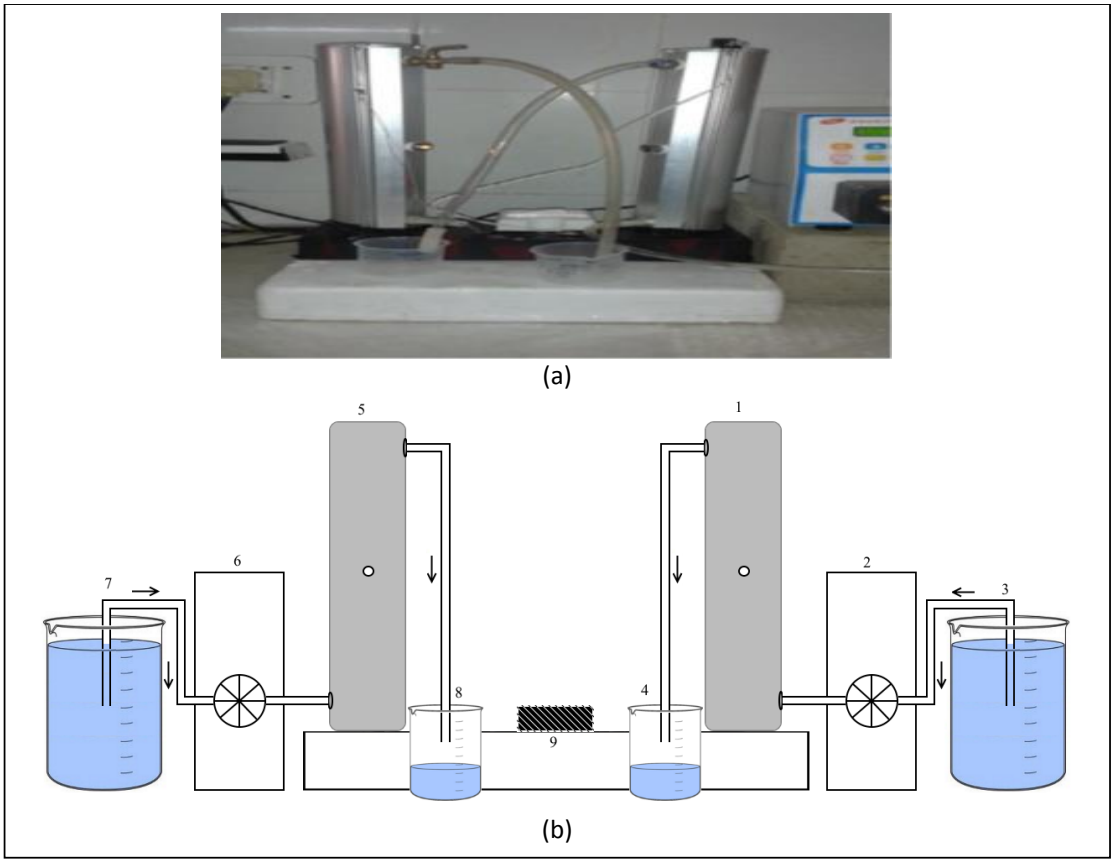


Figure 3.7: (a) Image of experimental set up for UV/Visible unit (b) Schematic diagram of experimental set up – 1. UV column; 2. Peristaltic pump; 2. Sample container; 4. Effluent container; 5. Visible column; 6. Peristaltic pump; 7. Sample container; 8. Effluent container; 9. On/Off switch

Table 3.3: Characteristics of the UV device [14]

S. No.	***Parameter***	***Value***
1	Length (cm)	38
2	Diameter (cm)	5.5
3	Lamp power (W)	8
4	Sample flow rate (rpm)	160
5	Volume (mL)	1,000
6	Ambient temperature (ºC)	25-35

3.5 Phase 4: Hybrid Disinfection

The hybrid disinfection strategy comprises of two methods. In the first method "A", the secondary treated effluent was first disinfected with optimised chlorine dose obtained

in a batch process in previous experiments. In the next step, the tertiary treated chlorinated sample was then sequentially disinfected with low ozone doses. In the second method "B", the secondary treated effluent was first disinfected with optimised chlorine dose in the batch process followed by sequential disinfection with UV dose. The procedure followed was same for the two disinfection studies as discussed above for individual disinfection studies (Figure 3.8). The effluent after hybrid disinfection was analysed for physicochemical properties and its effect on reduction of microbes was studied in detail.

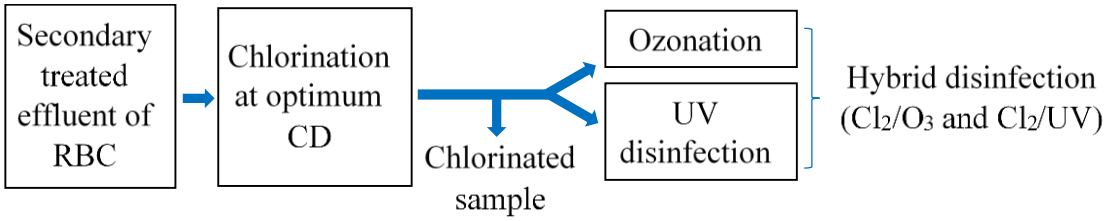


Figure 3.8: Schematic diagram of hybrid disinfection process

3.6 Phase 5: Optimization of Disinfection Processes Using DOE Software

The design of experiments (DOE) software has been used for experimental design, statistical data analysis, solving complex and multifactor problems. DOE assists in the planning, conducting, analysing and interpreting controlled tests to evaluate the optimal factors. Response surface methodology (RSM), a multivariate statistical tool of DOE, consists of a group of mathematical and statistical techniques that are based on the fit of empirical models to the experimental data obtained in relation to experimental design. The RSM has been used to optimize different types of wastewater treatment processes for industries such as textile, tannery, paint, and palm oil. In RSM category, generally, CCD and factorial design are used for optimization and carrying out the analysis of experiments with least experimental efforts [16].

The optimization feature can be used to calculate the optimum operating parameters for a process. Optimization helps in increasing the efficiency of the process without increasing the cost. In this study, CCD of the DOE (version 7) has been used as optimization software in chlorine disinfection. It helps to investigate the effects of input variables (operational parameter) on an output variable (response) at the same time. CCD has several design points, consisting of possible combinations of +1 and -1

levels of factor. In this case, the two independent variables were chlorine dose and contact time whereas dependent variable was total coliform counts. In the case of ozone, one factorial design was used that involves only one factor ozone dose as an independent variable and its effect on reduction of TCC and respective COD values was investigated.

Statistical analysis is necessary to verify the data, which is carried out by analysis of variance (ANOVA). Experiments suggested by software consist of a series of runs and data are collected for each run. The design of an experiment involves the following steps [17].

- ***Selection of the independent variables:*** The knowledge of the process under investigation is of prime importance before conducting the experiment. Factors that are likely to influence the outcome have to be identified. To compile the list of input factors for the present study; extensive literature review and parametric investigations were done.
- ***Deciding the number of levels:*** Once the input parameters are decided, the number of levels for each parameter is determined. The selection of the number of levels depends on how the performance parameter is affected due to different level settings. If the performance is a linear function of the independent variable, then the number of level setting are two. However, if the independent variable is not linearly related, then three, four or higher level settings can be selected depending on whether the relationship is quadratic, cubic or of greater order. In the absence of exact nature of the relationship between the independent variable and the performance parameter, one can decide whether the assumption of level setting is right or not based on the percent contribution and the error calculations.
- ***Selection of an orthogonal array:*** Before selecting the orthogonal array, the minimum number of experiments to be conducted is fixed. Once the minimum number of experiments is decided, the further selection of orthogonal array is based on the number of input parameters and number of levels for each parameter.
- ***Conducting the experiment:*** Once the orthogonal array is selected, the experiments are conducted as per the level combinations. It is necessary that all the experiments are performed.

- ***Analysis of the data:*** The analysis of the data is done to fulfil three objectives: to establish the optimum condition of a process, to estimate the contribution of individual factors, and to estimate the response under the optimum condition.
- ***Inferences:*** From the above experimental analysis, it is clear that the higher the value of the sum of squares of an independent variable, the more it has an influence on an independent variable. This ratio gives the percent contribution of the independent variable on the performance parameter. In addition to the above, near optimal solution to the problem could be found. This near optimum value may not be the global optimal solution. However, the solution can be used as an initial starting value for the standard optimization technique.

DOE included four phases as depicted in Figure 3.9.

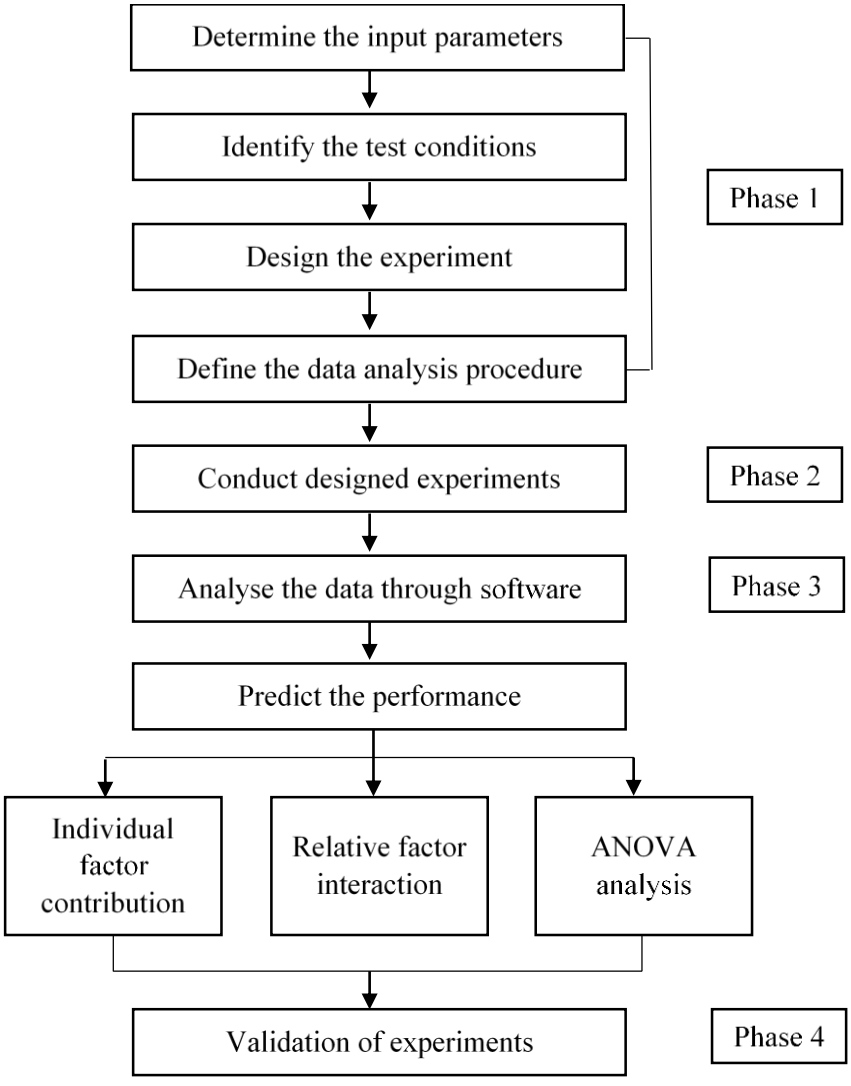


Figure 3.9: Flow chart representing different phases of DOE [17]

Hence, using RSM in wastewater treatment process optimization, could contribute to significant improvement in the removal efficiency and also the operational cost reduction.

3.7 Phase 6: SEM analysis

The scanning electron microscope (SEM) is a most suitable tool for morphological studies. SEM (FEI, Nova Nano SEM, 450) analysis was carried out after every disinfection process to study the effect of disinfectant on the morphology and outer membrane of microbes. The SEM is routinely used to generate high-resolution images of shapes of objects and to show spatial variations in chemical compositions [18]. It is a type of electron microscope that produces images of a sample by scanning it with a focused beam of electrons. It is a powerful magnification tool in which the electrons interact with atoms in the sample, producing various signals that can be detected and that contain information about the sample's surface topography and composition. The SEM images have a characteristics three-dimensional appearance and are useful for judging the surface structure of the sample. The SEM is capable of performing analyses of selected point locations on the sample. It gives characterization of solid materials. (Figure 3.10).

Figure 3.10: Scanning electron microscope

3.7.1 Sample Preparation

Secondary treated sample was centrifuged at 1,500 rpm for 5 min, and the supernatant was discarded. The process was repeated two times with the addition of distilled water. The pellet was then carefully homogenized and transferred to the nutrient broth, the tubes containing broth were kept at 37ºC for 18 h. On the other hand, the slides were prepared by using 0.8% agar. Samples from broth were spread on the agar film. Dehydration of agar slides was carried out in the oven at 37ºC for 2 h. Staining of the samples was performed by using glutaraldehyde. In the next step, dehydration of slides was carried out in 10, 25, 50, 75, 90 and absolute 99.99% ethanol solution each for 30 min. Final drying was done at 37ºC for about 1 h [18]. Prepared samples were coated with the thin gold film (210 nm) and analysed by SEM (Figure 3.10).

3.8 Phase 7: Analysis of THMs by GC-MS/MS

The four THMs i.e. $CHCl_3$, $CHBr_3$, $CHCl_2Br$ and $CHClBr_2$ were analysed with Triple Quadrupole GC-MS/MS (GC: Thermo Scientific Trace 1300, MS/MS: TSQ 8000) to study the effect of different disinfection processes on formation and concentration of THMs. The procedure used for the extraction of THMs was adopted from USEPA method 551.1 [19] [20].

It is an analytical method that combines the features of gas chromatography and mass spectrometry to identify different substances within a test sample. It is a highly sensitive and accurate multiplex gas chromatography coupled to tandem mass spectroscopy technique [19]. The GC-MS/MS using a triple quadrupole analyser is a powerful technique for the determination of trace residues due to its robustness, excellent sensitivity and selectivity (Figure 3.11). The advantages of SRM include high efficiency and rapid data processing. This method gave cleaner baselines, had few interfering peaks and made use of a short run time without compromising the analyte results in comparison to gas chromatography-electron capture detector (GC-ECD). The limit of detection was less than 1 µg/L. [20].

Figure 3.11: GC-MS/MS (Thermo scientific)

3.8.1 Sample Preparation and Extraction Procedure

The technique used for the extraction of THMs from water was liquid-liquid extraction. All samples were collected in duplicate. Sample bottles were filled in such a manner that no air bubbles passed through the sample as the bottle was filled. Homogenous phosphate buffer/dechlorinating agent mixtures (1 g) was added to the base of vials of 60 mL. Phosphate buffer was added to lower the sample matrix pH to the range of 4.8-5.5 to standardize the pH of all samples. Ammonium chloride was added as a dechlorinating agent which converts free chlorine to monochloramine. The vials were filled to the brim with chlorinated sample and samples were stored at 4ºC. Before extraction, samples were removed from storage and allowed to attain room temperature. 10 mL of the water sample was removed from the vials and pH was again monitored. In the next step, 3 mL of a non-polar solvent i.e. methyl tertiary butyl ether (MTBE) was added to the vials which was miscible with water to extract the target compounds. 10 g muffled sodium chloride (NaCl) was added to each sample vial as an addition of salt will increase extraction rates. Adding salt to an aqueous sample decreased the solubility of target compounds. Vials were recapped, shaken for 4 min on an orbital mixer (until most of the salts were dissolved) and kept for 15 min for separation. The 2

mL of the top organic layer was removed with a pasteur pipette and added to GC vials [20].

Samples can be stored for 14 days following extraction. In the present study analysis of samples was carried out at CEG Laboratory, Jaipur after one day of sample preparation. Stock standard solutions were used which were of high purity grade (Sigma products) to prepare the calibration curve. All reagents used were of analytical grade (Sigma Aldrich) with no interference. Samples were analysed by GC-MS/MS, and configuration of the instrument is listed in Table 3.4 and 3.5.

Table 3.4: Analytical condition of GC

S. No.	***Parameter***	***Specification***
1	Column type	TSQ 8000
2	Injector	Split/Splitless
3	Injector temp	280ºC
4	Oven temp	40ºC (4 min), 100ºC @10ºC/min (2 min)
5	Carrier gas	Helium
6	Flow rate	50 cm/s, constant linear velocity
7	Detector	MS/MS

Table 3.5: Analytical condition of MS/MS

S. No.	***Parameter***	***Specification***
1	MS temperature	300ºC
2	Ion source temperature	250ºC
3	Total scan time	0.500 s
4	Ionisation method	EI
5	Timed scan type	SRM
6	Scans per peak	6

Chapter Summary

This chapter describes the experimental methodology adopted for performing the study. It gives the detailed insight of experimental setup, protocols and equipment used in the present study to achieve the objectives. A detailed explanation of the use of software for the research methodology is also described.

The next chapter describes the results and discussions.

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Chapter 4

Chapter 4

Chlorine Disinfection of Secondary Treated Effluent

After conducting the experiments according to the test plan and methodology as described in Chapter 3, the results from the study are presented for analysis and are discussed in details in this chapter. The quantitative analysis of the microbial population of different treatment processes of the STP inside the MNIT campus based on RBC process was carried out before conducting extensive studies on disinfection. The efficacy of disinfection for secondary treated domestic wastewater in batch process using chlorine was examined for contact time of 5, 10, 15, and 20 minutes against five different coliforms, total coliforms and few of the pathogen species for chlorine doses (CD) of up to 5 mg/L. To simulate situations analogous to those found in actual practical applications, experiments were repeated under continuous flow conditions for CD up to 6.5 mg/L. During these experiments, the effect of chlorine on physiology of bacterial cells was also examined through SEM analysis to get a clue to their inactivation mechanism. Optimization of the results of chlorination was attempted by statistical analysis using DOE software. An attempt was also made for analysis of four THMs with the help of GC-MS/MS to assess their concentrations due to chlorination of sewage.

4.1 Quantitative Analysis of Microbial Load in the STP

The experiments were carried out to evaluate the microbial population of the sample collected from STP, during different stages of operation. Samples were drawn from different units and appropriate dilutions were prepared in order to obtain colony counts of individual species as per the statistical requirements as described in Chapter 3. The results of the quantitative analysis of microbial load during different treatment stages are given in Table 4.1.

Table 4.1: TCC during different treatment processes

S. No.	***Treatment stage***	***TCC (MPN/100 mL) n= 10***	***TCC (CFU/100 mL) n= 10***
1	Raw sewage	$(36.8 \pm 19.04) \times 10^7$	$(685 \pm 39.34) \times 10^7$
2	Primary treated effluent	$(20.2 \pm 15.31) \times 10^7$	$(514 \pm 28.16) \times 10^7$
3	Secondary treated effluent	$(16.9 \pm 12.08) \times 10^5$	$(155 \pm 36.22) \times 10^5$

It is evident from the Table 4.1, that there was no significant reduction in the TCC after primary treatment of sewage. But after secondary treatment more than 99% reduction was achieved in TCC. However, the secondary treated effluent still showed higher presence of coliforms indicating the need for disinfection to fulfil the WHO standards for reuse of water for irrigation purposes. The results of TCC in the secondary treated effluent are comparable to the results presented by Bustos et al. [1].

4.2 Characteristics of Secondary Treated Effluent

The results of the various physiochemical characteristics of the secondary treated effluent sample were measured and presented in Table 4.2 as they affect the disinfection process.

Table 4.2: Characteristics of secondary treated effluent samples of RBC and their effect on chlorine disinfection

S. No.	***Quality parameters***	***Secondary treated effluent (n= 10)***	***Effect on chlorine disinfection [1]-[3]***
1	pH	7.7 ± 1.20	Affects distribution between hypochlorous acid and hypochlorite ions and among various chloramine species.
2	BOD	15.83 ± 2.10 mg/L	The degree of interference depends on their functional groups and chemical structures of organic compounds being present.
3	COD	105.35 ± 1.73 mg/L	The degree of interference due to organic matter.
4	Turbidity	41.10 ± 1.50 NTU	Disinfection efficiency is negatively correlated with turbidity. Particles can shield microorganisms from chlorination.
6	TSS	15 ± 5 mg/L	Shielding of embedded bacteria and chlorine demand.

4.3 Quantitative Analysis of Dominant Bacterial Species

The quantitative load of bacterial species isolated from the secondary treated effluent of RBC is presented in Figure. 4.1. It was observed that the average TCC in the secondary treated effluent was 155 x 10^5 CFU/100 mL. Two species, namely, *Enterobacter* and *Serratia/Hafnia* were the most dominant coliform species besides *E.coli*, *Klebsiella* and *Citrobacter* in the effluent of RBC. Different units (i.e. physicochemical and biological) of wastewater treatment plants attempt to remove pathogenic microorganisms to some extent however, they do not provide qualified effluent of category A, defined by World Health Organization [4] [5].

The final effluent released from the STP did not confirm to the WHO standards for TC [5] designated for reuse of wastewater for irrigation and is used for maintaining greenery and irrigation inside the lawns of the MNIT campus. This may result in the direct contact of people with the pathogens and further has the potential to contaminate

the ground water, if it is not disinfected before its discharge. The presence of a large number of coliforms and pathogens after the secondary treatment is a matter of concern as discussed in section 2.2 of Chapter 2. Therefore, the disinfection treatment of the effluent is indicated as an obligatory step in wastewater treatment for the intended use of maintaining green belt [4]. The results are represented in Figure 4.1 as the average of 10 independent experiments along with the error-bars.

As discussed in the Chapter 2 several physical and chemical processes of disinfection are available, however, chlorination is commonly practiced chemical method of disinfection due to its low cost [3] [6]-[11].

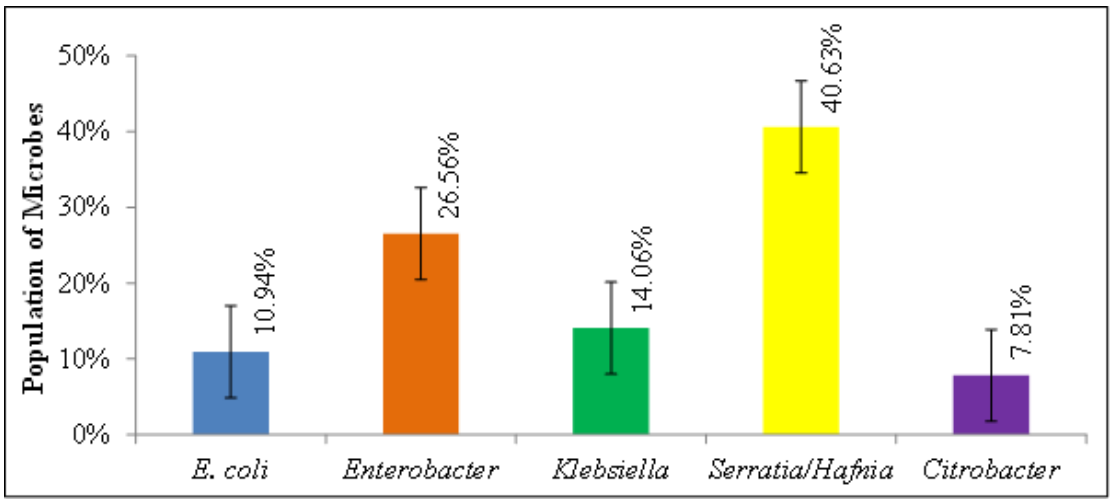


Figure 4.1: Bacterial distribution in the isolates from secondary treated effluent of STP.

4.4 Chlorine Disinfection Profile for Batch Mode

The samples collected after the secondary treatment from the STP was disinfected with different CD in batch reactors. Batch reactors are the simplest types of experimental disinfection vessel and generally the most efficient in terms of the amount of inactivation observed [12]. The basic principle of good disinfection practice is effective mixing of the disinfectant at the point of dosing and close approximation to plug flow in the contact zone [13]. If any system satisfies these conditions, then the results of batch disinfection studies can be used in designing disinfection facilities for full scale operations.

The goal of this study was to determine the CD required to meet the disinfection standard of WHO i.e. 1000 CFU/100 mL for the reuse of secondary treated effluent for

agriculture and other non-drinking purposes. An attempt was carried out to find out those species, which offer relatively higher resistance to chlorination in order to design a hybrid disinfection strategy for optimizing the overall disinfection process. Thus the study was carried out to investigate the effect of chlorine disinfection on five dominant coliform bacterial species (*E.coli*, *Enterobacter*, *Klebsiella*, *Serratia/Hafnia* and *Citrobacter)* and TCs in the secondary treated effluent sample from STP. TCs is generally expected to have a similar sensitivity to a given disinfectant dose as the pathogens do, however, to confirm this fact few pathogen species were also considered for observing the effect of chlorine disinfection on their removal [14] [15].

Disinfection was carried out at different doses of chlorine ranging between 1 to 5 mg/L in a batch process for a total contact time of 20 minutes. Samples were collected at intervals of every 5 minutes during the total contact time to analyse the effect of CD on microbial counts and several physicochemical parameters. Table 4.3 presents the results of chlorine disinfection effect on different physiochemical parameters.

Table 4.3: Effect of chlorine doses on physiochemical characteristics of secondary treated effluent of RBC

S. No.	***Quality parameters***	***Secondary treated sample (Influent)***	***Chlorinated sample (Effluent)***	***% Reduction***
1	pH	7.7± 1.10	8.1 ± 1.5	-
2	BOD	15.83 ± 1.50 mg/L	10.12 ± 2.8 mg/L	36.07
3	COD	105.35 ± 1.73 mg/L	58.88 ± 5.3 mg/L	44.76
4	Turbidity	41.10 ± 1.50 NTU	28.52 ± 1.5 NTU	30.60
6	TSS	15.00 ± 2.6 mg/L	10.00 ± 2.2 mg/L	33.33

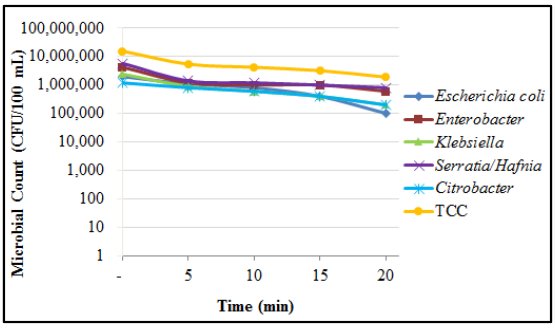
Table 4.3 represents the average values of different physicochemical parameters before and after chlorination and their reduction in terms of percentages. The value of pH showed a slight increment in the final effluent due to the presence of sodium hypochlorite and calcium hypochlorite which themselves have high pH due to hypochlorite ions. The BOD and COD reduction was observed to be 36% and 45%, respectively. This may be attributed to the good oxidation capacity of chlorine, due to which organic matter present in the effluent is oxidised and reduced to sub parts, as a result of which reduction was observed in BOD, COD, turbidity and TSS concentration [1]–[3] [10] [11]. It has also been stated that reduction in BOD and COD is

accomplished by oxidation of organic compounds present in wastewater [16]. The response of BOD and COD to chlorination is a function of CD and the organic content of wastewater [18]. The overall reaction for oxidation of organic molecules is represented in Equation 4.1 [17].

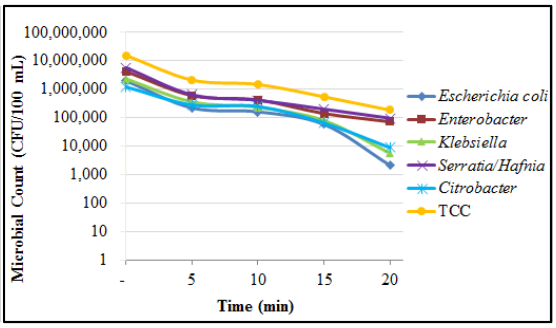
Organic molecules → Intermediate oxygenated molecules → End products (4.1)
(BOD, COD etc.) (CO_2, H_2O etc.)

4.4.1 Chlorine Disinfection Profile for TC in Batch Mode

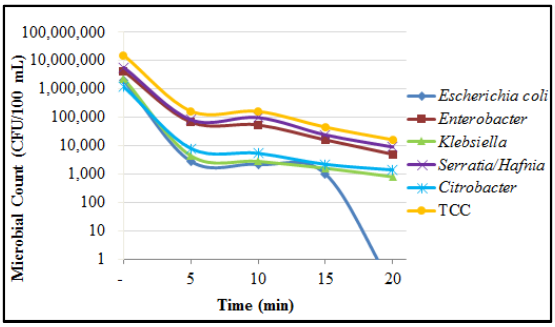
Different set of experiments were carried out for 1, 1.5 , 2, 2.5, 3, 3.5, 4, 4.5 and 5 mg/L of CD and for contact times of 5, 10, 15, and 20 minutes. Samples were collected at a regular time interval of 5 minutes and were analysed for different microbial counts and TCC. The results of TC removal are graphically represented in Figure 4.2.



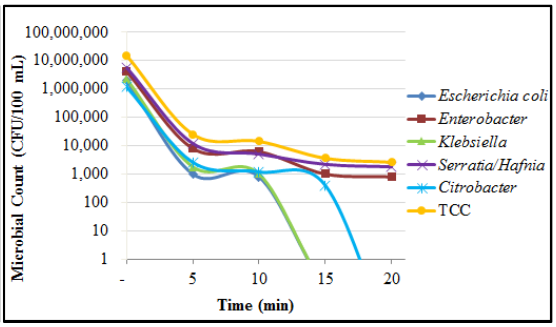
(a) 1 mg/L



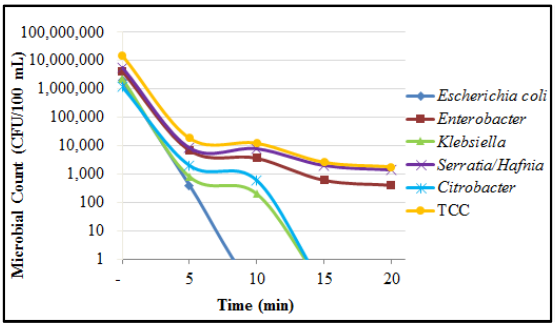
(b) 1.5 mg/L



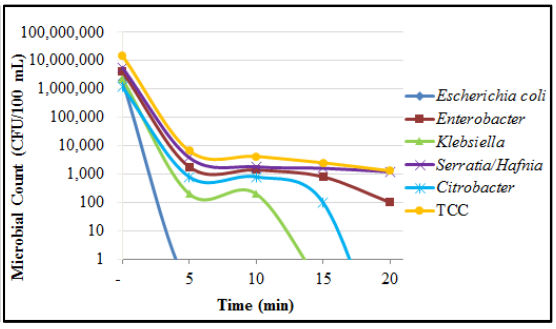
(c) 2 mg/L



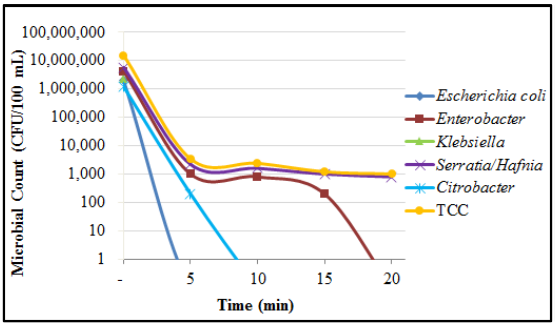
(d) 2.5 mg/L



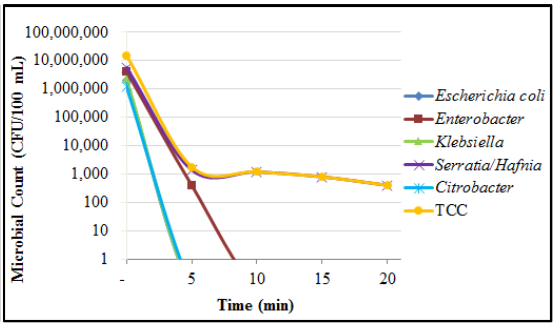
(e) 3 mg/L



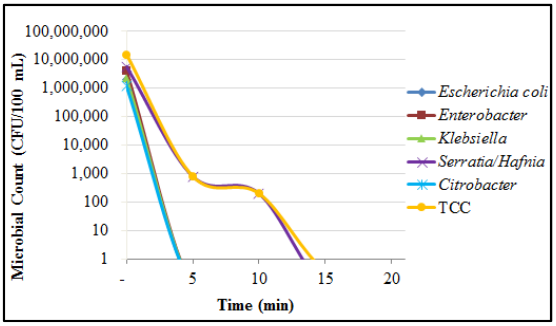
(f) 3.5 mg/L



(g) 4 mg/L



(h) 4.5 mg/L



(i) 5 mg/L

Figure 4.2: TC removal profile for CD of (a) 1 mg/L, (b) 1.5 mg/L, (c) 2 mg/L, (d) 2.5 mg/L, (e) 3 mg/L, (f) 3.5 mg/L, (g) 4 mg/L, (h) 4.5 mg/L, (i) 5 mg/L

It is evident from the Figure 4.2 (a) that at 1 mg/L of CD typically zero log reduction was observed and at 1.5 mg/L of CD (Figure 4.2 (b)) only one log reduction was observed for most of the species after 20 min of CT. This might be due to chlorine demand of several organic and inorganic substances in initial stages due to which very less amount of free chlorine was available for disinfection purpose [19] [20].

It is represented in Figure 4.2 (c) that at 2 mg/L of CD typically two log reduction was observed for most of the species in the first 5 min and thereafter the period between 15 to 20 min was observed to be effective. This may possibly be due to the high initial disinfection efficiency because of the presence of free chlorine forms, which later on reduced as the combined forms were produced. The combined forms of chlorine are expected to require larger contact time for disinfection [3] [19]. As observed from the Figure 4.2 *Enterobacter* and *Serratia/Hafnia* seemed to be the sturdiest among the above species against chlorination [21] [22].

Figure 4.2 (d) represents the results of 2.5 mg/L of CD. It was observed that a continuous decrement in the value of microbial count was observed in *E.coli*, *Klebsiella* and their counts reached zero at 15 min of contact time followed by *Citrobacter*, which

reached to zero at 20 minutes of contact time. From the previous studies, it was reported that *Enterobacter* is one of the chlorine resistant species due to the presence of higher phospholipids and neutral lipids in its outer membrane, which increases resistance at low doses [23]. At this stage counts for *Enterobacter* also reached around 800 CFU/100 mL. On further increasing the CD up to 3 mg/L of CD (Figure 4.2 (e) a similar trend in microbial reduction was observed to 2.5 mg/L of CD. Hence, there was as such no additional benefit of increasing the CD from 2.5 mg/L to 3 mg/L. The counts for *Serratia/Hafnia* were still high.

At 3.5 mg/L of CD and 20 min of CT (Figure 4.2 (f)) the microbial counts for *E.coli, Klebsiella, Citrobacter* reached zero. Counts for individual *Enterobacter* also got reduced and reached within the standard limit. *Serratia/Hafnia* dominated the total count after 10 min of disinfection at this CD, with its curve almost coinciding with that of total coliforms (TC) and there was no additional benefit of increasing contact time from 10 to 20 minutes. *Serratia/Hafnia* did not respond to this CD after 15 min of contact period and the reduction in microbial count was very less.

It is observed from Figure 4.2 (g) that at 4 mg/L CD and 10 min of contact time almost all the microbial counts reduced and were within WHO standards except for *Enterobacter* and *Serratia/Hafnia* which seemed to be the sturdiest among all species. At this stage, 15 min of contact time was very effective as at this time the count for *Enterobacter* also reached a negligible value. At 20 min of contact time the TCC also reached within the WHO norms due to reduction in the counts for the most resistant species i.e. *Serratia/Hafnia*.

It is evident from the Figure 4.2 (h) that at 4.5 mg/L of CD and 10 min of contact time, the microbial count reduced to zero for all the species except for *Serratia/Hafnia*. Hence, higher contact time was provided and at 15 min of contact time the TCC was also within the WHO standard of less than 1000 CFU/100 mL. At 5 mg/L (Figure 4.2 (i) and 5 min of contact time almost all the species along with TC were within the standard limits. On increasing the contact time further there was more reduction in counts of *Serratia/Hafnia.*

Hence, it was concluded from chlorine disinfection profile that at 2.5 mg/L of CD most of the microbial species reached within WHO norms, except for *Serratia/Hafnia*. Because of *Serratia/Hafnia* high doses of chlorine up to 4 mg/L were required, as these were resistant to low doses of chlorine.

The resistant nature of *Serratia/Hafnia* might be due to the fact that chlorine disinfection is based on its reaction with the cell constituents and *Serratia/Hafnia* have higher lipid content than other coliform species. In addition, the lipopolysaccharide (LPS) layer is attached to the outer membrane of these gram negative bacteria which makes them difficult to obliterate, thus possibly resulting in the consumption of a high amount of chlorine [24] [25]. It is also reported that *Serratia*/*Hafnia* is resistant to many antimicrobial agents due to certain characteristics such as their ability to survive in aerobic and anaerobic conditions (unique membrane), and their motility, as they have 100 – 1,000 flagella per swimmer cell and also secrete AHLs (acylatedhomoserine lactones), which are involved in swarming motility [25].

It has been reported in the literature also that due to microbial resistance to low doses of chlorine, a much higher dose of chlorine was required to achieve TCC within WHO standard of 1000 CFU/100 mL [5] [21] [26]. This will consequently increase the formation of Disinfection by-products (DBPs) as formation of DBPs is directly linked to higher CD. The details of DBPs are discussed at the end of this chapter.

In a similar study with ASP based secondary treated effluent [21], the CD required to achieve TCC within standards was very high (17.5 mg/L), which was entirely governed by the presence of *Serratia/Hafnia* and *Enterobacter*. Such a high dose was required due to presence of high organic content, high BOD and high suspended solids in secondary treated effluent of ASP [22]. It has also been reported that microorganisms associated with particulate matter in water have greater resistance to inactivation by chlorine compared to dispersed microorganisms [15]. Such high doses of chlorine may lead to very large THM concentrations which may result into several environmental health issues.

The TCC obtained before and after disinfection at the different pre – determined CD are presented in Table 4.4 in terms of CFU/100 mL and in terms of MPN/100 mL in Table A.1 (Appendix A). Exposure of treated effluent to the CD (4 mg/L for 20 minute) was capable of reducing TCC from 155 x 10^5 CFU/100 mL to 1000 CFU/100 mL. Almost 99.99% removal and 4 log reduction of TC was obtained at this CD. Pour plate results of inactivation of TC at different CD are presented in Figure A.2 (Appendix A) and colilert results are shown in Figure A.3 (Appendix A), indicating removal of microbes in terms of MPN/100 mL.

Table 4.4: Removal of microbial organisms (TC) at different CD in terms of CFU/100 mL

S. No.	***CD (mg/L)***	***TCC before disinfection***	***TCC after disinfection***	***% Removal of TC***	***Log reduction of TC***
1	1.0	155×10^5	191×10^4	87.74	0
2	1.5	155×10^5	18×10^4	98.83	1
3	2.0	155×10^5	16×10^3	99.89	2
4	2.5	155×10^5	26×10^2	99.98	3
5	3.0	155×10^5	18×10^2	99.98	3
6	3.5	155×10^5	13×10^2	99.99	4
7	4.0	155×10^5	10×10^2	99.99	4
8	4.5	155×10^5	1×10^2	99.99	4
9	5.0	155×10^5	0	100.00	5

4.5 Chlorine Inactivation of TC

The inactivation kinetics of TC in secondary treated wastewater with different CDs are represented in Figure 4.3. The log survival of the TC and the CD used for inactivation showed a strong first order relationship parameter of disinfection ($R^2 = 0.8993$) [27]. It was concluded from Figure 4.3 that up to 2.5 mg/L of CD disinfection was very effective competing with organic matter. The plateau from 2.5 mg/L to 3.5 mg/L of CD shows that rate of oxidation of organic matter is more prominent at these doses as compared to disinfection. After 3.5 mg/L of CD again disinfection process is active. From the figure it was demonstrated that maximum reduction of TCC (<1,000) was related to the highest dose of chlorine i.e. 5 mg/L. Hence, it was concluded that CD was one of the best process control parameters in disinfection process [1] [3] [11] [21] [27] [28].

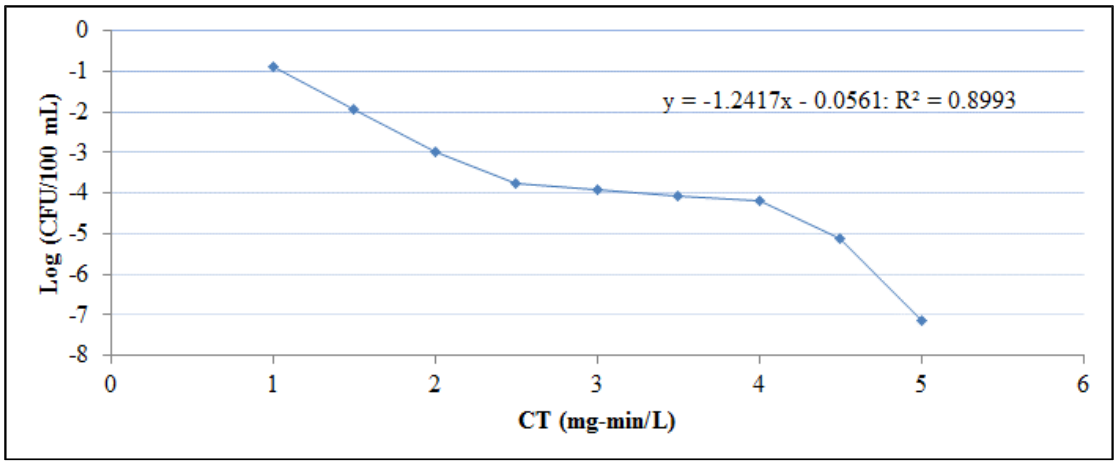


Figure 4.3: Inactivation of TC by CD response curve

The Figure 4.4 represents the inactivation kinetics or relationship between contact time and log reduction of TC. The data show that the contact time is highly correlated with reduction in TCC with a strong correlation (high R^2 value) at each dose of chlorine disinfection [3] [27] [29] [30]. The minimum contact time of disinfection was 5 min and maximum contact time of disinfection was 20 min. This longer chlorine contact duration resulted in less TC survival in treated wastewater. Hence, it was concluded that contact time is also one of the best process control parameters for disinfection.

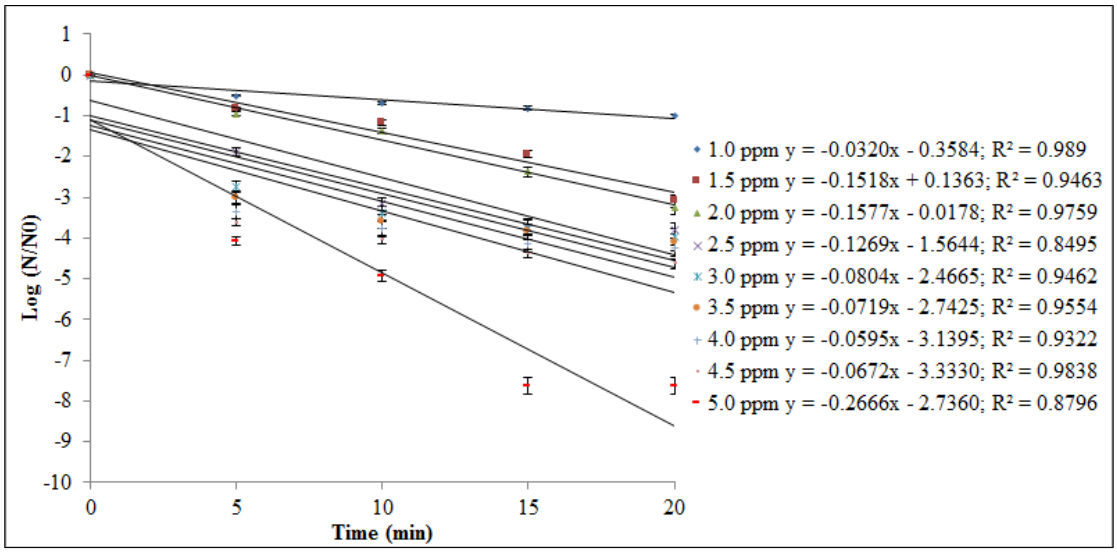


Figure 4.4: Plot of TC inactivation vs contact time

4.6 Relationship between total chlorine and TC

Total chlorine residual was experimentally determined by chlorine residual photometer at different CDs and contact time according to the methods described in Chapter 3. Relationship between total chlorine residue and TC was derived from the results at each dose of chlorine.

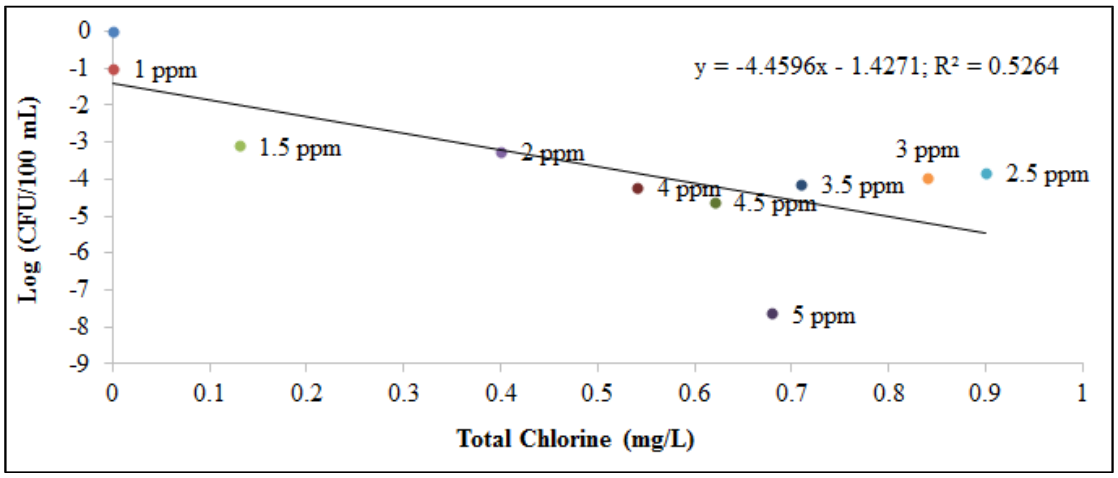


Figure 4.5: Relationship between total chlorine and log TC at different CD

It can be concluded from the Figure 4.5 that R^2 i.e. coefficient of determination shows only 50% variation between microbial contamination and chlorine residual at different CDs, which is relatively weak relationship between the two variables. As the chlorine concentration increases, microbial count decreases. These results were in good agreement with the findings of Martinez et al. and Lechevallier et al. [26] [31].

4.7 Breakpoint Chlorination Curve

The optimum CD was determined and confirmed from the breakpoint curve by measuring total residual chlorine at each stage of experiment. It was found that 4 mg/L with a contact time of 20 minutes was optimum for TC to be brought to the WHO standards for reusing wastewater.

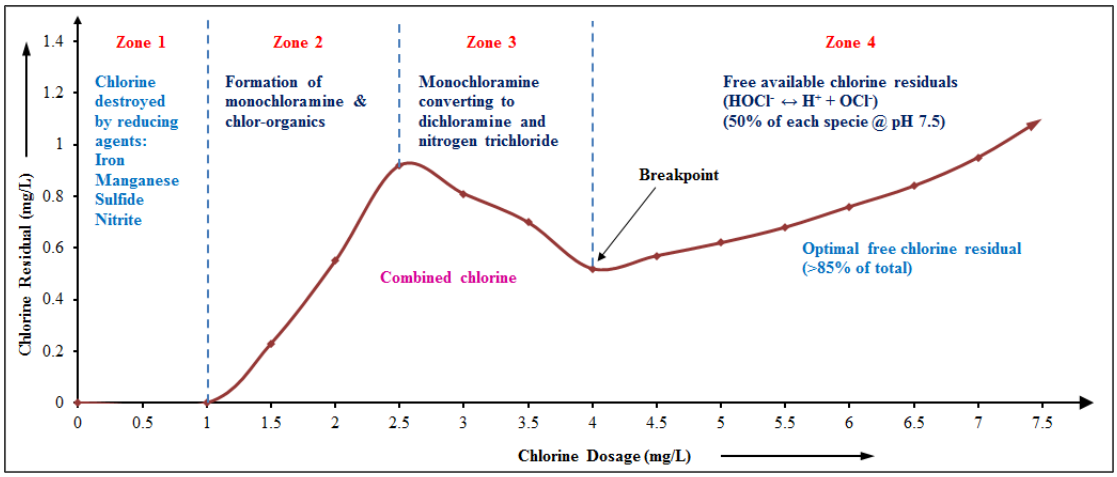


Figure 4.6: Graphical representation of breakpoint chlorination

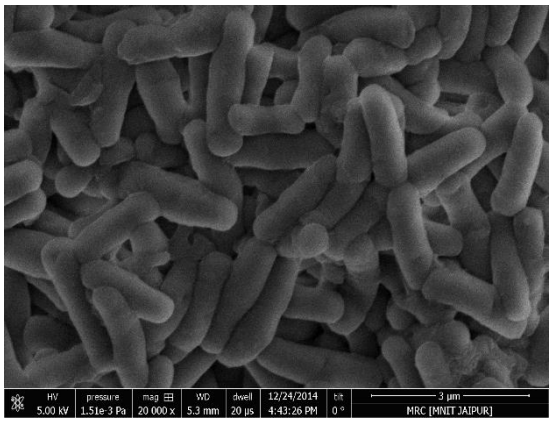
From the Figure 4.6, it was observed that as soon as chlorine (1 mg/L) was added to wastewater, it combined with certain substances such as ammonia, ferrous iron, and hydrogen sulphide, hence no disinfection occurred (zone 1) as organic and inorganic substances exerted a demand that had to be satisfied [11]. The lag phase was due to the instant chlorine demand created by oxidizable compounds, which render chlorine ineffectively for disinfection [2].

When chlorine was further added to the sample (1.5 mg/L, 2 mg/L and 2.5 mg/L), it reacted with ammonia that was present in wastewater sample and resulted into formation of monochloramine (NH_2Cl), dichloramines and trichloramines (combined forms of chlorine) [34]. Due to combined residual form of chlorine, the total chlorine residual first increased to a certain point (2.5 mg/L) and then it is dropped with further addition of chlorine as depicted in zone 2 and zone 3 of figure. In this case, the residual chlorine increased at 2.5 mg/L and after this point the combined residual dropped because the chloramines were converted to trichloramines which are the weakest disinfectants [35]. The point at which chlorine demand was satisfied and all ammonia was oxidised to nitrogen gas (N_2) or trichloramine (NCl_3) and at which the free chlorine residual began to rise is called breakpoint (zone 3). The further addition of chlorine after breakpoint resulted into free chlorine residual formation which continued to disinfect as it behaved like strong disinfectant (zone 4) [36] [37]. After this breakpoint, the free chlorine residual was about 80% of the total chlorine residual. Hence, the breakpoint curve determined that the optimum CD was 4 mg/L at 20 minutes of contact time for complete disinfection and satisfying WHO standards of 1000 CFU/100 mL for reusing wastewater for agriculture purposes [5] [38].

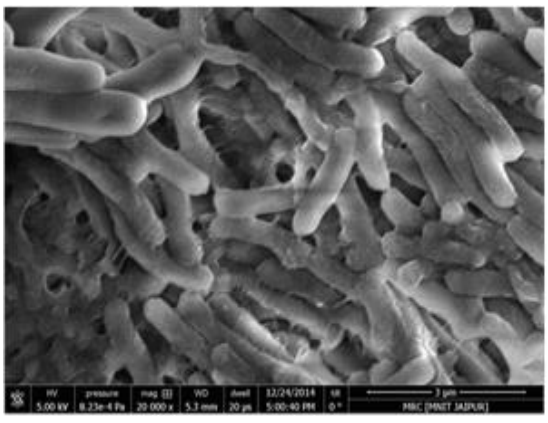
4.8 SEM Analysis

A better understanding of the mechanism by which chlorine kills cells would help in defining effective chlorine treatments and in optimizing strategies for chlorination [39]. SEM analysis helps by giving a characteristic three dimensional appearance and is useful for analysing the surface structure of the samples [40] [41]. Thus, SEM analysis of the samples before and after chlorine disinfection was carried out to observe the microbicidal effect of chlorine as a disinfectant on the bacterial surface.

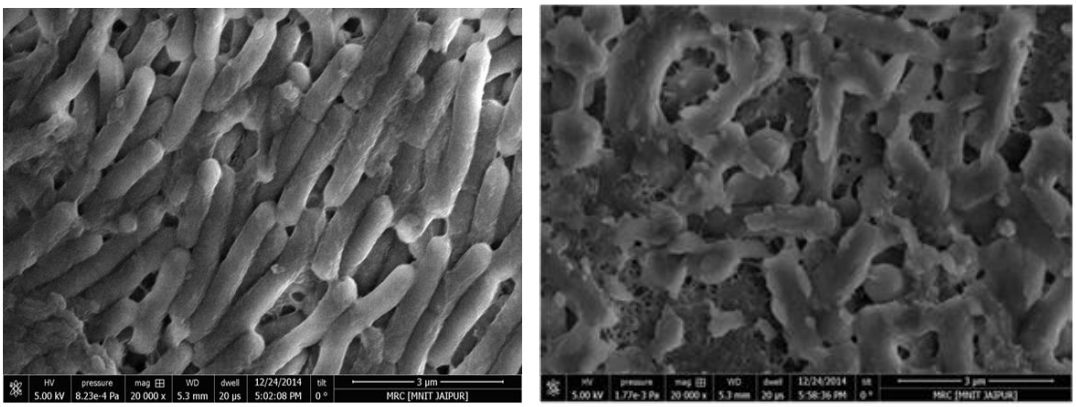
Figure 4.7 (a) represents the image of the gram negative rod shaped bacteria (TC) present in the secondary treated wastewater sample of RBC sewage treatment plant, which was not dosed with chlorine (blank). The size of these rod shaped bacteria varies between 1 to 5 µm in length and 0.25 – 1.0 µm in diameter. The bacterial surfaces were very smooth showing proper rod shaped firm structure of gram negative bacilli with sharp edges. However, from Figure 4.7 (b) it can be observed that when the secondary treated effluent was dosed with 1.5 mg/L chlorine for 20 minutes, slight deformation of cell membrane occurred. It was observed that on increasing the CD, more destructive effect on bacterial surface was seen, which finally resulted in deformation of cell membrane and structure of microbes, as depicted in Figure 4.7 (c). Bacterial cells showed shrunken surfaces in the images due to destructive microbicide effect of chlorine. Figure 4.7 (d) proves that at high doses of chlorine (around 4 mg/L for 20 minutes), bacterial cell lysis occurs which finally results in death or inactivation of microorganisms [37] [42]. Thus, SEM monographs provide support to the results represented in section 4.4 that with increasing CD more reduction is observed in number of microorganisms.



(a)



(b)



(c) (d)

Figure 4.7: TC (a) before chlorination, (b) post chlorination 1.5 mg/L for 20 minutes, (c) post chlorination 2.5 mg/L for 20 minutes, (d) post chlorination 4 mg/L for 20 minutes

Calomiris [43] supports the above mentioned findings who gave a "multiple hit" theory of chlorine inactivation. He asserts that bacterial death probably results from chlorine attacking a variety of bacterial molecules or targets, including enzymes, nucleic acids and membrane lipids. The sequence of events which have been observed in SEM analysis during chlorination includes disruption of the cell wall barrier by reactions of chlorine with target sites on the cell surface and release of vital cellular constituents from the cell which results in termination of cellular functions within the cell followed by death of the microorganism means, it is no longer capable of growing or causing disease [23].

At low chlorine levels, microorganisms that survive the treatment may get injured rather than inactivated. Under suitable conditions injured cells might repair cellular damage and recover. But when the rate of used chlorine is increased to control chlorine resistant pathogenic microorganisms during the disinfection process, the risk of the formation of DBPs also increased [39] [44]. An appreciable nature of sub lethal injury, repair and risk of DBPs is therefore important in devising chlorination strategies with the use of appropriate CD and in developing combination treatments with synergistic actions against the target microorganisms. Effective microbial control by chlorine requires suitable disinfection design criteria to ensure protection of public health and minimize contaminating effects of the chlorination process.

4.9 Optimization of Chlorination Process Using CCD

Microbial resistance to low doses of chlorine was observed in the above study by few coliform species such as *Serratia/Hafnia*, which in turn required much higher doses of chlorine to achieve TCC within WHO standard of 1000 CFU/100 mL. This may consequently increase the formation of disinfection by-products (DBPs) which are considered as human carcinogens. An optimization strategy for chlorination was attempted to achieve counts for rest of the species within 1000 CFU/100 mL except for *Serratia/Hafnia* so that the initial disinfection with chlorine could be terminated at this dose, which may then be followed by another disinfection process (UV light or ozone) against which such resistance was not observed.

Optimization was carried out by using central composite design (CCD) of statistical software Design of Experiments 7.0.0 for Windows [45] [46]. The optimized results were used for experimental verification, which were in good agreement with the predicted results. Optimization can also help in controlling the disinfection process so that the use of excessive chlorine for disinfection can be avoided thus preventing harm to aquatic life by high chlorination dosages and to maintain a specific chlorine residual level adequate to perform the desired disinfection. This would further result in decreased formation of the THMs. The optimized results of chlorination would be useful in designing a hybrid disinfection strategy which includes chlorination followed by UV or Ozone [27] [47]-[49]. This strategy may help in reducing the use of high CD and thereby reducing the by-products of chlorination without sacrificing the economy of the overall disinfection process. CCD of experiments and data analysis were applied to optimize and assess the relationship among two independent variables (1) CD and (2) contact time, which are presented in Table 4.5.

Table 4.5: Independent variables of the CCD design

Variables	***Factor***	***Level (-1)***	***Mean***	***Level (+1)***
A	Dose (mg/L)	1	2.75	4.50
B	Time (min)	5	12.50	20.00

The efficiency of the disinfection process was evaluated by analysing the effect of CD and contact time (independent variables) on the TCC (dependent variable). Each independent variable was varied between -1 and +1 at the determined ranges based on

a set of preliminary experimentation as shown in Table 4.5. These two settings for each factor encompassed the complete range that was found feasible based on the previous studies and experimentally determined values. The use of two levels also reduced the number of experiments that were required to be performed without affecting the efficacy of the selected DOE model. Further, optimum settings of parameters could then be found between the low and the high range of the selected levels. Twenty one experiments were conducted with single replica and the mean value was selected for graphical analysis of the data obtained, according to the plan mentioned in Table 4.6.

Table 4.6: Response values for different experimental conditions

Run	***Factor A: Dose (mg/L)***	***Factor B: Time (min)***	***Response TCC (CFU/100 mL)***
1	4.50	20.00	400
2	2.75	12.50	6000
3	4.50	12.50	600
4	2.75	12.50	600
5	2.75	20.00	2000
6	2.75	5.00	220000
7	1.00	5.00	5.3E+006
8	2.75	12.50	8000
9	4.50	20.00	200
10	4.50	12.50	600
11	2.75	20.00	2000
12	1.00	12.5	2.8E+006
13	1.00	20.00	1.74E+006
14	2.75	12.50	8000
15	1.00	12.50	2.8E+006
16	4.50	5.00	3800
17	4.50	5.00	3800
18	2.75	12.50	6000
19	1.00	5.00	5.3E+006
20	2.75	5.00	220000
21	1.00	20.00	1.74E+006

4.9.1 Validation of the Statistical Model

A regression model equation was developed between the response and input variables which is presented in Equation 4.2.

$$TCC = +51{,}015.15 - 1.607E{+}006*A - 7.130E{+}005*B + 8.354E{+}005*A*B + 1.442E{+}006*A^2 + 2.847E{+}00*B^2 \quad (4.2)$$

It was observed from the Equation 4.2 that when the effect of a factor was negative (as in the above case both the factors had negative value) and factor was changed from low to high level, TCC decreased.

Normal probability plots present a suitable graphical method for judging the normality of the residuals. These plots for TCC are illustrated in Figure 4.8.

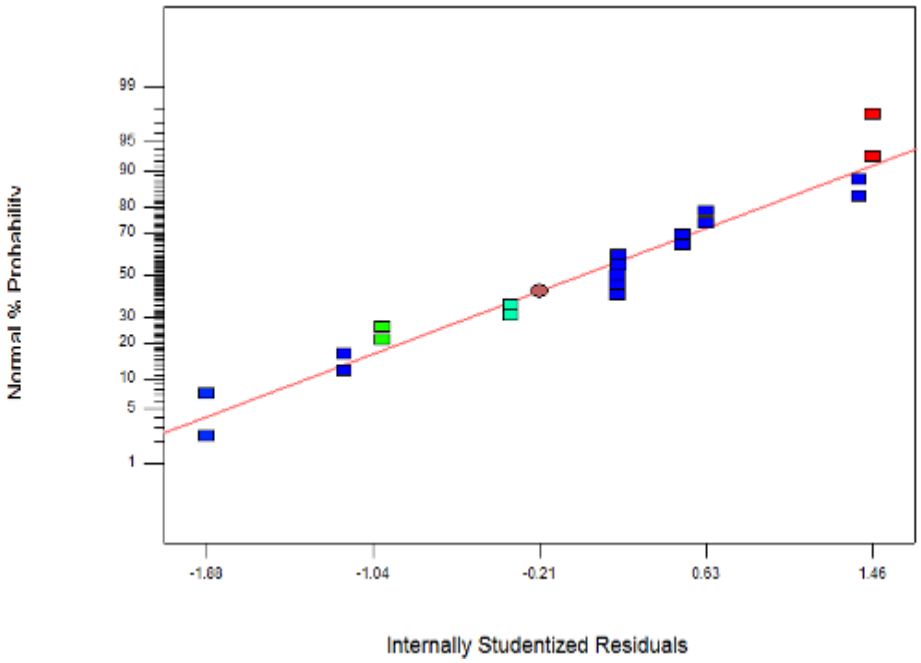


Figure 4.8: Normal plot of residuals

4.9.2 ANOVA

The statistical adequacy of the model was justified through analysis of variance (ANOVA) for regression model. ANOVA values for the quadratic regression model (obtained from CCD) employed in the optimization of chlorination are listed in Table 4.7. On the basis of the experimental values, statistical testing was carried out using F test for ANOVA.

The statistical significance of the second order equation revealed that the regression was statistically significant (p-value<0.0001). The results of the significance of the regression coefficients and ANOVA for the regression model indicate that the response equation proved to be suitable for the CCD experiment [50] [51]. The model's p-values less than 0.0001 indicates that the model terms were significant at 95% probability level [52]. Based on the ANOVA results, the model reported a high R^2 value of 96.50% for the present study. Also, an acceptable agreement with the adjusted determination coefficient was necessary. This indicates that the regression model provides a good explanation of the relationship between the independent variables and the response.

Table 4.7: ANOVA for TCC

Source	***Sum of squares***	***df***	***Mean square***	***F-value***	***p-value Prob>F***	
Model	5.659E+013	5	1.132E+013	82.65	< 0.0001	Significant
A-Dose	3.224E+013	1	3.224E+013	235.45	< 0.0001	
B-Time	4.767E+012	1	4.767E+012	34.81	< 0.0001	
AB	6.324E+012	1	6.324E+012	46.18	< 0.0001	
A^2	1.150E+013	1	1.150E+013	83.94	< 0.0001	
B^2	2.914E+011	1	2.914E+011	2.13	0.1653	
Residual	2.054E+012	15	1.369E+011			
Lack of Fit	2.054+012	3	6.477E+011	1.705E+006	< 0.0001	Significant
Pure Error	4.820E+006	12	4.017E+005			
Cor Total	5.864E+013	20				

4.9.3 Interaction Plots for Various Operating Parameters

To understand the impact of each variable and their interaction, three dimensional plots and interactive plots were made for the estimated responses. These responses were the basis of the model polynomial function for analysis, to investigate the interactive effect of the two factors on the TCC within the experimental ranges, as shown in Figures 4.9 and 4.10.

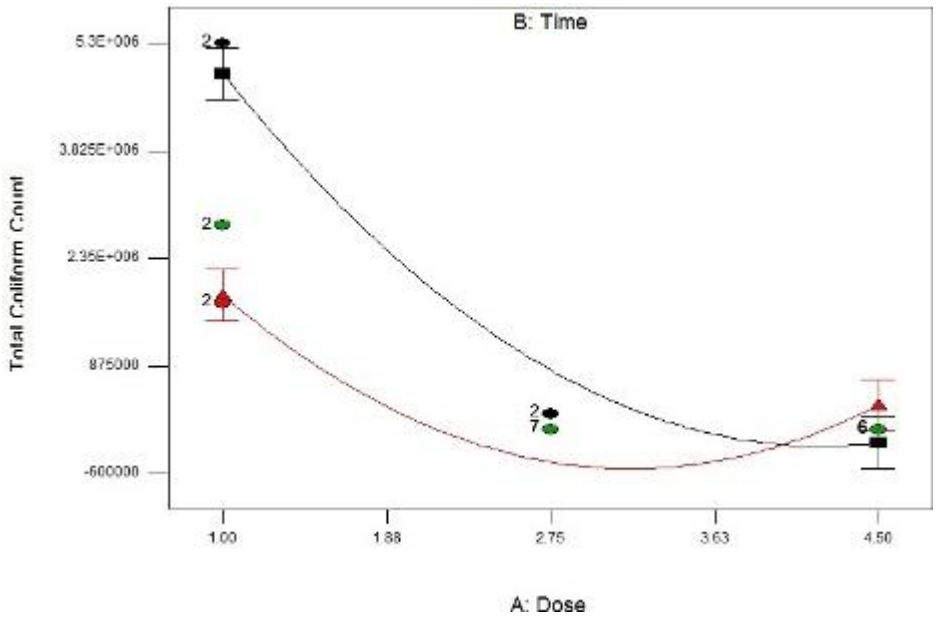


Figure 4.9: Interaction plots for TCC as a function of dose and time

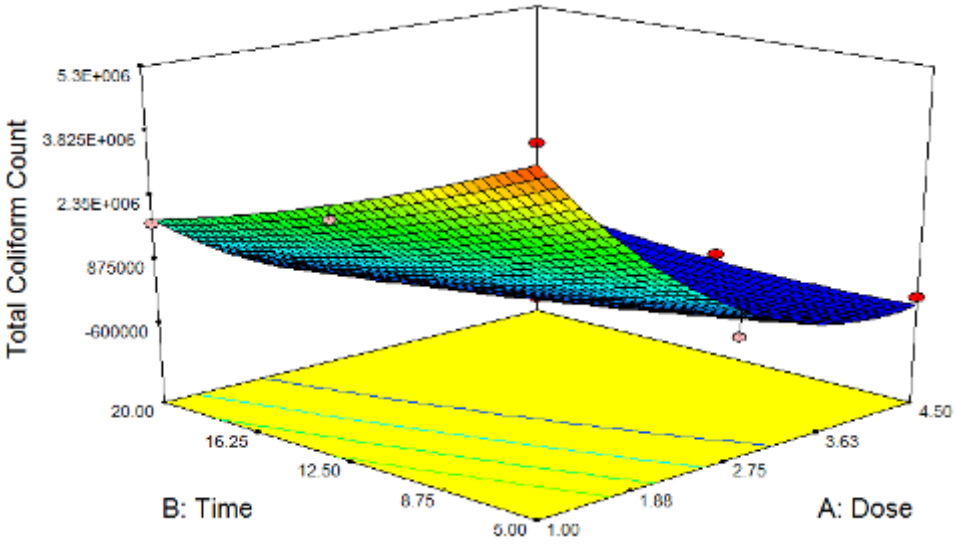


Figure 4.10: 3D Surface plots for TCC as a function of dose and time

4.9.4 Optimization of the Disinfection Process for TCC

Optimization refers to improvement in the performance of a system in order to obtain maximum benefit from it. The main objective of the optimization here was to determine the optimum values of variables. In optimization the target TCC was set as 3,200 CFU/100 mL. Since, the results of preliminary experiments showed that at this stage all species came within the prescribed standards except for *Serratia/Hafnia*. The values for *Serratia/Hafnia* were neglected for creating a basis for hybrid disinfection studies as these were resistant to low doses of chlorine. The variables (i.e. dose and time) were set in a range to achieve this target. The optimization results are shown in Table 4.8. Experiments were conducted at these conditions and comparison was made between the experimental results and the predicted results.

Table 4.8: Optimization and validation values

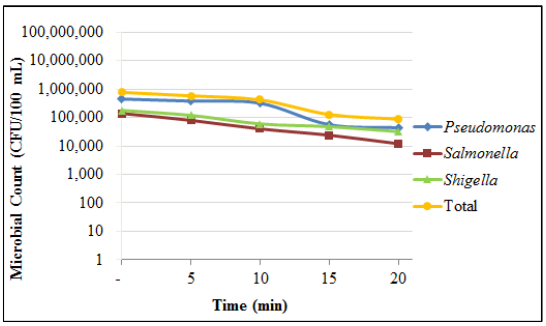
Variables	***Unit***	***Values from optimization***
Dose	(mg/L)	2.50
Time	(min)	16.83
Residual TCC (predicted)	CFU/100 mL	3,204
Residual TCC (experimental)	CFU/100 mL	3,000

The optimized CD for the given target was 2.5 mg/L for contact time of 16.83 min and at this dose the predicted value of TCC was 3,204 CFU/100 mL. The experimental verification of these optimum conditions confirmed a good agreement with the predicted results as the TCC obtained after performing experiments was 3,000 CFU/100 mL. It can be concluded from the results that using optimized CD of 2.5 mg/L for 16.83 min of contact time may be more useful as the first step of disinfection for removing all coliforms species susceptible to chlorine instead of 4 mg/L of CD for 20 min to confirm to the TCC norms. This may then be followed by another disinfection process for taking care of the chlorine resistant species. Thus a reduction in CD of about 47% would be possible and hence a consequent reduction in THM formation. This may further help in reducing the overall cost of the disinfection process by using a suitable disinfectant like UV or ozone in series, which is effective against the chlorine resistant species.

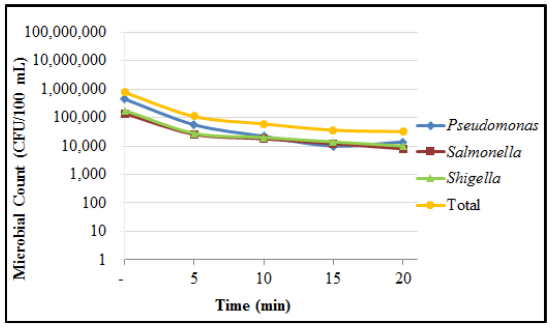
4.10 Effect of Chlorine Disinfection on Pathogen Removal in Batch Mode

Experiments were carried out to understand the efficiency of chlorine disinfection on different pathogens under batch regime. In this study, the primary aim was to comprehend the effect of contact time and applied dose of chlorine on pathogen versus coliform removal efficiency of secondary treated effluent. The experiments were carried out till the complete removal of microbes was achieved.

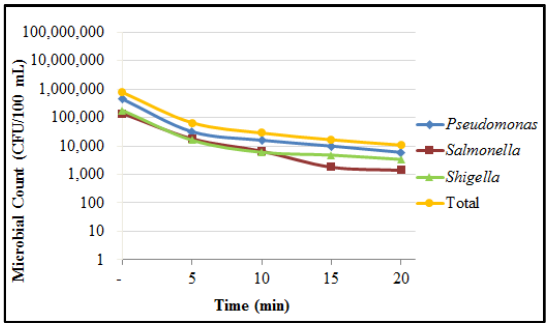
The set of experiments was carried out for 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, and 5 mg/L of CD and for contact time of 5, 10, 15, and 20 minutes. The samples were collected at regular time interval of 5 minutes and were used for the enumeration of pathogenic bacteria. The graphical representation of results is shown in Figure 4.11.



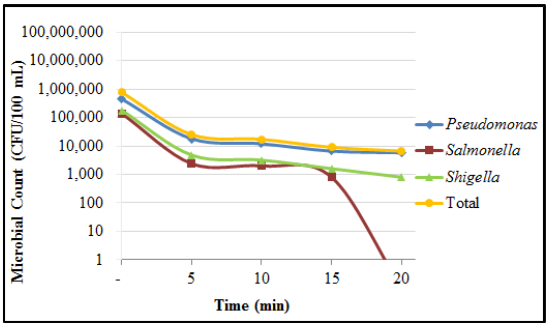
(a) 1 mg/L



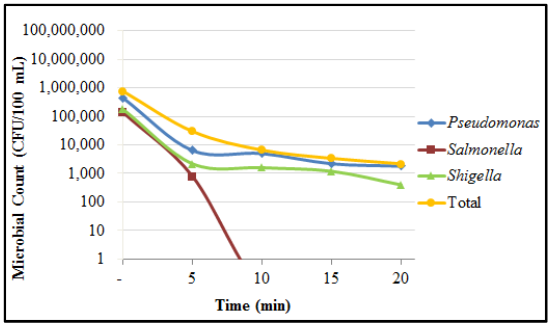
(b) 1.5 mg/L



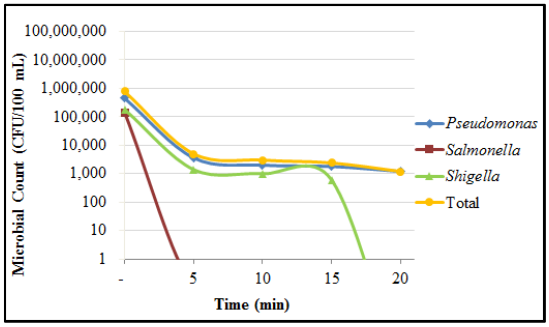
(c) 2 mg/L



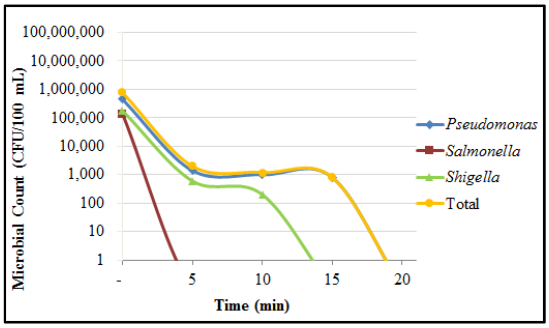
(d) 2.5 mg/L



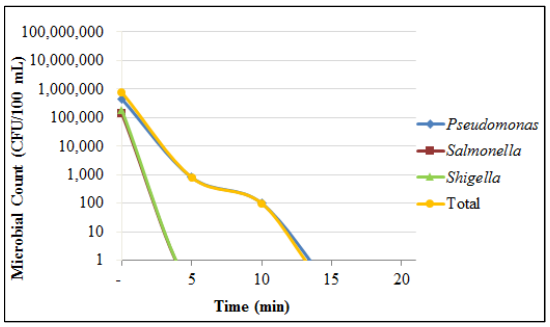
(e) 3 mg/L



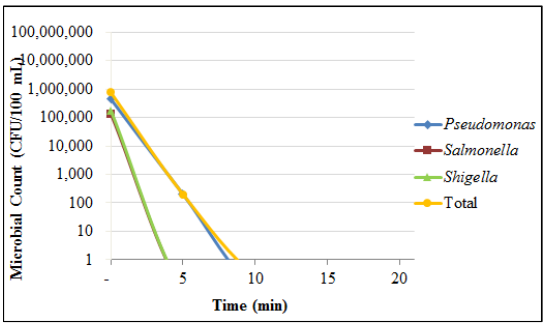
(f) 3.5 mg/L



(g) 4 mg/L



(h) 4.5 mg/L



(i) 5 mg/L

Figure 4.11: Pathogen removal profile for CD of (a) 1 mg/L, (b) 1.5 mg/L, (c) 2 mg/L, (d) 2.5 mg/L, (e) 3 mg/L, (f) 3.5 mg/L, (g) 4 mg/L, (h) 4.5 mg/L, (i) 5 mg/L

It is evident from the Figure 4.11 (a) that only one log reduction was observed in the population of pathogens after 1 mg/L of CD at 20 min of CT and same trend was

observed at 1.5 mg/L of CD as shown in Figure 4.11 (b). It was observed that on further increasing CD up to 2 mg/L same trend followed, as presented in Figure 4.11 (c).

The dose of 2.5 mg/L was again very effective as at this dose tremendous decrement in the counts of *Salmonella* was observed up to 15 min and their count reduced to zero on further increasing the CT. In the same way reduction in the counts of *Shigella* was also observed and its counts reduced to below 1000 CFU/100 mL. At 3 mg/L the same trend in reduction continued with 2 log reduction in TCC as represented in Figure 4.11 (e).

In Figure 4.11 (f) it was observed that at 3.5 mg/L of CD and at 20 min of CT, maximum number of microbial count was observed in the case of *Pseudomonas* species. Again, *Pseudomonas* did not respond much at this dose so addition of further CD was required. Even after 20 minutes of CT, the counts for pathogen could not be brought below 1,000 at this dose of chlorine. However, the total count was still above the standards only due to *Pseudomonas* as, *Salmonella* and *Shigella* counts dipped to zero.

The graphical representation of results in Figure 4.11 (g) shows that at 4 mg/L of CD the most sensitive species (i.e. *Salmonella*) reached zero at 5 minutes of CT and count for *Shigella* also finally reached to zero at 15 min of CT. *Pseudomonas* dominated the total counts after 5 minutes of disinfection at this dose, with its curve almost coinciding with that of total pathogen count. *Pseudomonas* seemed to be the sturdiest among all species against chlorination but its count also reduced at 15 minutes of CT and reached zero at 20 minutes. As the TCC reached zero at 20 minutes of CT therefore this would be the recommended dose for disinfection.

On further increasing the CD up to 4.5 mg/L, it was observed that only *Pseudomonas* survived till 10 min after which complete removal of pathogens was achieved as represented in Figure 4.11 (h). At 5 mg/L of CD, very low count of *Pseudomonas* was observed at 5 min of CT which were also reduced to zero if further CT was provided as represented in Figure 4.11 (i).

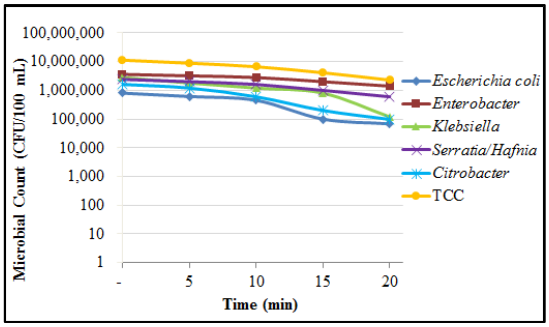
Thus, it was concluded that at 3.5 mg/L of CD all the considered pathogens reached zero except for *Pseudomonas. Pseudomonas* had the lowest removal percentage confirming its high resistance to the chemical action of the disinfectant at all the tested concentrations and exposure times [53]. The resistant nature of *Pseudomonas* is due to its cell envelope, which has higher content of phospholipids and polysaccharide and a lower concentration of fatty acids. The capsular EPS on cell

membrane appeared to reduce membrane permeation by disinfectants as suggested by deformation of key functional groups in EPS and cell membrane (the C-O-C stretching of carbohydrate and C=O stretching of ester group) [54] [55]. The combined results supported that capsular EPS, acting either as a disinfectant consumer (for chlorine inactivation) or limiting access to reactive sites on cell membrane, provides a protective role for bacterial cells against regulatory residual disinfectants by reducing membrane permeation. Delcour [52] reported that another reason for high resistance of *Pseudomonas is* because of the high Mg^{2+} content in outer membrane, which aids in producing strong LPS-LPS links and furthermore, because of their small size, the porins may not permit general diffusion through them.

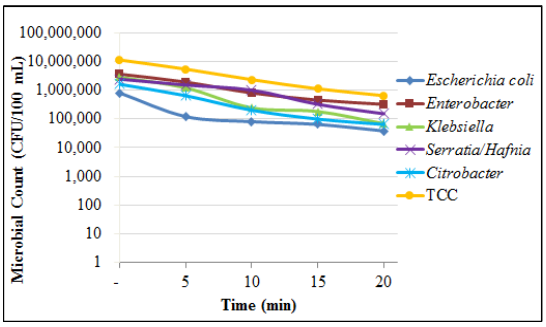
Henceforth, dose of 4 mg/L was effective against all the pathogens. It was also concluded that the pattern of pathogen removal across the treatment technologies closely matched with that of TC removal. These results of the present study were also supported by the finding of Poswal et al. [21]. Further, it was confirmed that the role of coliforms as indicator was duly justified as all pathogens almost vanished till the WHO norm for reuse was met.

4.11 Disinfection with Chlorine in Continuous Mode

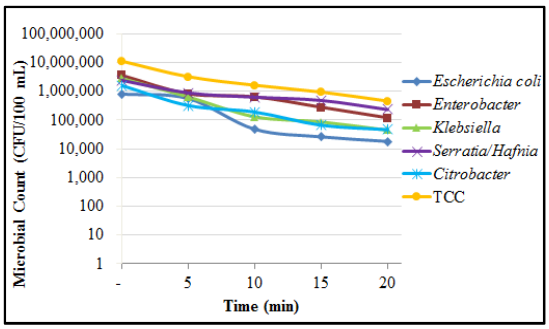
In the next set of experiments, an attempt was made to determine the effect of chlorine disinfection on TC in continuous mode. In a continuous process, the flow of sample and CD is continuous with constant flow. Chlorination of secondary treated effluent was carried out according to the methods explained in Chapter 3. Range of CD chosen for continuous process was from 2 mg/L to 6.5 mg/L and total contact time provided for each CD was 20 min. The effects of different CDs of 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, and 6.5 mg/L were examined for contact time of 5, 10, 15, and 20 min on the five dominant microbial species and TC for the continuous mode process are represented in the Figure 4.12. Initially, chlorination was also carried out at 1 and 1.5 mg/L of CD but at these initial CDs, only a negligible reduction in microbial counts was observed hence, in further experiments the initial dose adopted was 2 mg/L.



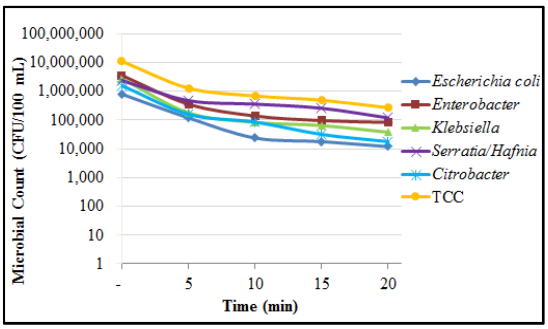
(a) 2 mg/L



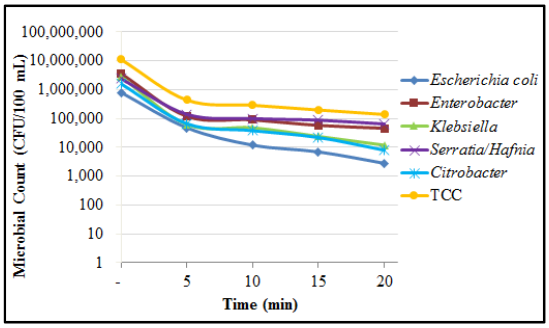
(b) 2.5 mg/L



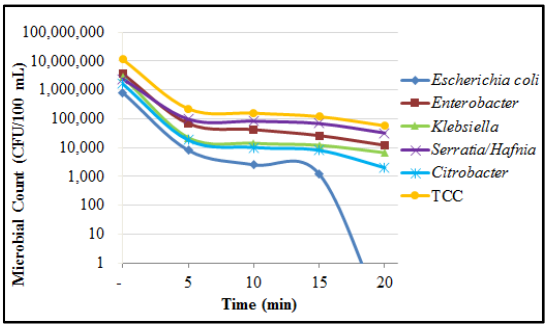
(c) 3 mg/L



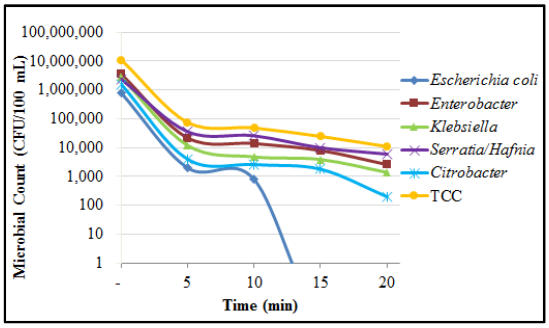
(d) 3.5 mg/L



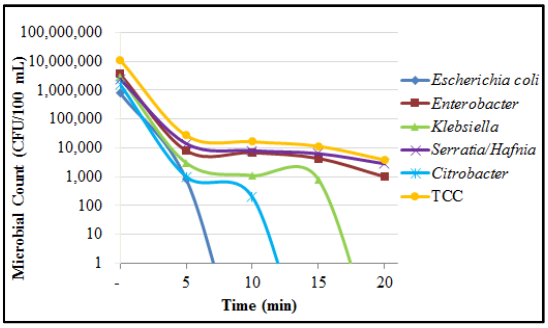
(e) 4 mg/L



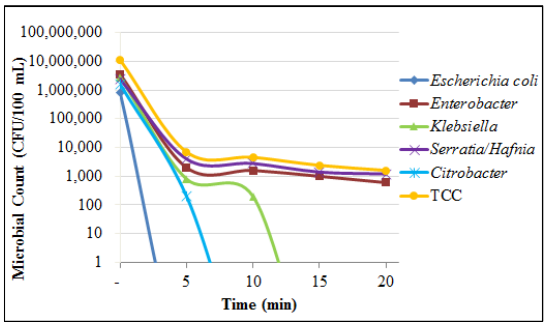
(f) 4.5 mg/L



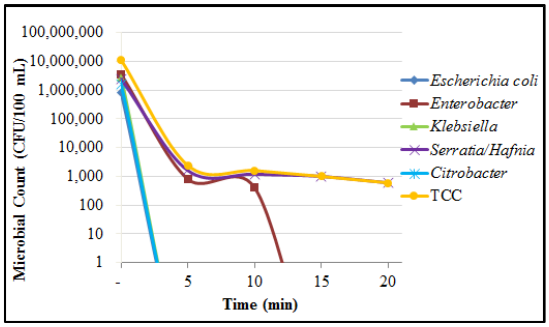
(g) 5 mg/L



(h) 5.5 mg/L



(i) 6 mg/L



(j) 6.5 mg/L

Figure 4.12: TCC removal profile for CD of (a) 2 mg/L, (b) 2.5 mg/L, (c) 3 mg/L, (d) 3.5 mg/L, (e) 4 mg/L, (f) 4.5 mg/L, (g) 5 mg/L, (h) 5.5 mg/L, (i) 6 mg/L, (j) 6.5 mg/L

It is evident from the Figure 4.32 (a) that at 2 mg/L, negligible decrement was observed in the microbial counts after 20 min of CT. Typically, zero log reduction was observed for most of the species including TC. This might possibly be due to the high initial chlorine demand of oxidizable substances present in the wastewater sample as discussed in section 4.4.1 of this chapter [19] [20]. At 2.5 mg/L of CD, only one log reduction was observed for TC after 20 minutes as shown in Figure 4.12 (b). Maximum reduction was observed in the counts of *E.coli*, *Klebsiella* and *Citrobacter*. This might possibly be due to the combined forms of chlorine as they were expected to require larger contact time for disinfection as explained in section 4.4 (USEPA). At 3 mg/L of CD, as represented in Figure 4.12 (c) very less decrement was observed in the population of TC. At 20 min of CT, the same trend was observed with only one log reduction as was observed at 2.5 mg/L.

It was observed that in Figure 4.12 (d) at 3.5 mg/L, and 20 min of CT was very effective as it showed the reduction of *Enterobacter* species to some extent. It could be predicted that *Serratia/Hafnia* were the sturdiest among the above species against chlorination as observed in the batch process too [21].

It can be seen from Figure 4.12 (e) that at 4 mg/L CD and 5 min of CT, minimal number of microbial count was found in *E.coli.* Thereafter, a continuous decrement in the value of microbial count was found in *Klebsiella* and *Citrobacter*. Decrement was also observed in the counts of *Enterobacter* and *Serratia/Hafnia* at 20 min of contact time. But only one log reduction was observed in TCC, as the counts of the two resistant species were still high. At 4.5 mg/L and 20 min of CT the most sensitive species was

E.coli and maximum microbial count was again observed in *Enterobacter* and *Serratia/Hafnia* as represented in Figure 4.12 (f).

At 5 mg/L of CD, as represented in Figure 4.12 (g), a similar trend followed as was with 4.5 mg/L of CD but a slight difference was observed in the TCC. *E.coli* was found to be the most sensitive species followed by *Citrobacter* and *Klebsiella*. At this stage, at 20 min of CT, TCC had reduced up to three logs due to reduction in the counts of all the five species. At 5.5 mg/L CD and up to 15 min of CT *E.coli*, *Citrobacter* and *Klebsiella* also reduced to minimum count and reached zero as shown in Figure 4.12 (h). A decrement of microbial count was also observed for *Enterobacter* and *Serratia/Hafnia* at 20 min of CT with three log reduction in TCC.

It is represented in Figure 4.12 (i) that at 6 mg/L and 5 minutes of CT, the count for *E.coli* was zero. After 10 and 15 min of CT, same trend was observed for *Klebsiella* and *Citrobacter*. At the same CT, the two resistant species i.e. *Enterobacter* and *Serratia/Hafnia* were competing with each other and finally at 20 min of contact time there was reduction in counts for *Enterobacter* as well. *Serratia/Hafnia* had not responded much to this dose at 15 and 20 min of contact period and the reduction in counts was same as it was at 10 min.

It is observed from Figure 4.12 (j) that at 6.5 mg/L and 5 min of CT, minimal microbial count was found in the *E.coli*, *Citrobacter* and *Klebsiella*. After 10 min of CT, count for *Enterobacter* was also reduced. And at 15 min of CT, the counts of all the above considered microbes reached zero except for *Serratia/Hafnia*, which seemed to be the sturdiest among the above species against this dose of chlorination. However, at 20 minutes of CT, a further reduction in count of *Serratia/Hafnia* was observed and the count also reached within the standard norms. The four log reduction was finally achieved at 20 min of CT at this CD, which is necessary in the case of wastewater reuse. It was concluded that 6.5 mg/L of CD was sufficient to bring the counts of *Serratia/Hafnia* within norms and the TCC at this specific dose was 600 CFU/100 mL, which was also within the norms. But it was only *Enterobacter* and *Serratia/Hafnia* due to which such high doses of chlorine up to 6 to 6.5 mg/L were required, as these microbes were resistant to low doses of chlorine. It has also been reported in the literature that microbial resistance to low doses of chlorine results in its much higher dosing to achieve TCC within WHO standard of 1000 CFU/100 mL than those needed without the presence of these resistant species.

The TCC obtained before and after disinfection at the different predetermined CDs are presented in Table 4.9 where reduction in microbial counts in terms of CFU/100 mL is presented in terms of both % removal and log reduction and Table A.2 (Appendix A) represents data in terms of MPN/100 mL. Exposure of treated effluent to the optimum CD (6.5 mg/L for 20 minute) was capable of reducing TCC from 112 x 10^5 CFU/100 mL to 600 CFU/100 mL. Almost 99.99 % removal of TC was obtained at the optimum dose.

Table 4.9: Removal of microbial organisms (TC) at different CD in terms of CFU/100 mL

S. No.	***CD***	***TCC before disinfection***	***TCC after disinfection***	***% Removal***	***Log reduction***
1	2.0	112 x 10^5	18 x 10^5	83.22	0
2	2.5	112 x 10^5	64 x 10^4	94.28	1
3	3.0	112 x 10^5	46 x 10^4	95.89	1
4	3.5	112 x 10^5	28 x 10^4	97.75	1
5	4.0	112 x 10^5	14 x 10^4	98.75	1
6	4.5	112 x 10^5	57 x 10^3	99.49	2
7	5.0	112 x 10^5	11 x 10^3	99.90	3
8	5.5	112 x 10^5	38 x 10^2	99.96	3
9	6.0	112 x 10^5	16 x 10^2	99.98	3
10	6.5	112 x 10^5	6 x 10^2	99.99	4

4.12 Breakpoint Chlorination Curve for Continuous Mode

As soon as chlorine was added to the reactor, the readily oxidizable substances, such as iron (Fe), manganese (Mn), hydrogen sulphide (H_2S) and organic matter reacted with chlorine and reduced most of it to chloride ion, as represented by zone 1 in Figure 4.13.

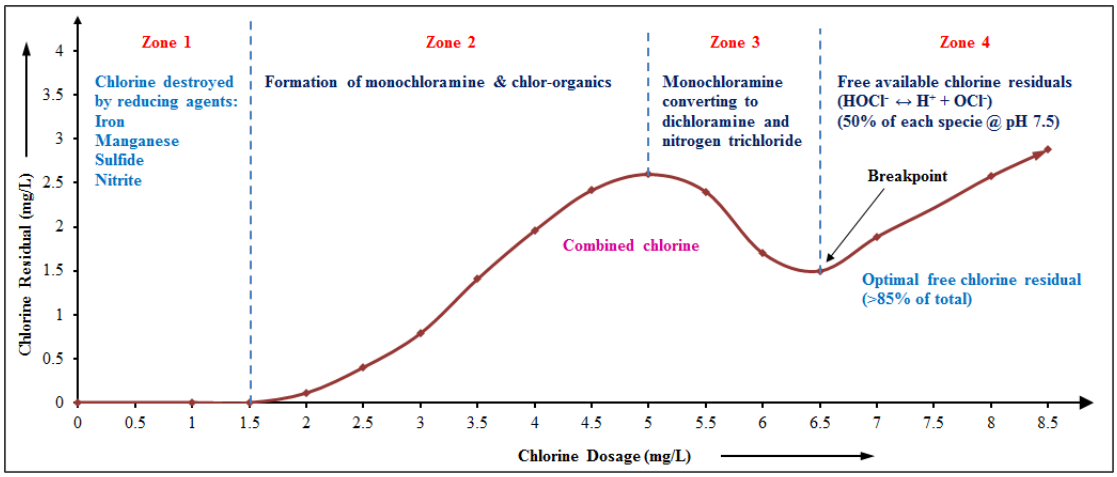


Figure 4.13: Graphical representation of breakpoint chlorination in continuous process

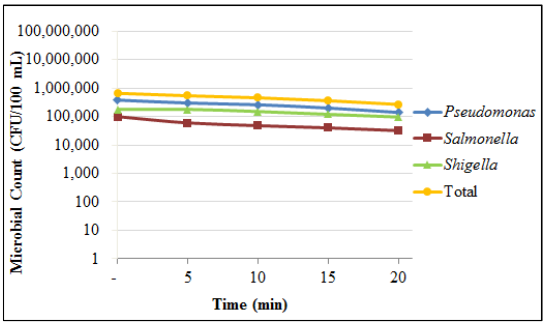
After meeting this immediate demand, the chlorine continued to react with the ammonia and formed chloramines [11] [34], from CDs of 2 to 5 mg/L (zone 2). Between 5 mg/L and the breakpoint (6.5 mg/L), chorine was reduced to chloride ions and some chloramines were oxidized to form nitrogen trichloride (zone 3). Continued addition of chlorine past the breakpoint resulted in a proportional increase in the free available chlorine (non-reacted hypochlorite), which is represented by zone 4 in the graph [36] [37]. Hence, the optimum CD for chlorination in continuous process was 6.5 mg/L at 20 minutes of CT for complete disinfection and satisfying WHO standards of 1000 CFU/100 mL [5].

The results of batch and continuous studies showed that the continuous process requires slightly increased concentration of CD (6.5 mg/L) to achieve the disinfection standard. The possible reason for this may be that more chlorine escaped in continuous process compared to the batch process (4 mg/L) due to which high CD is required.

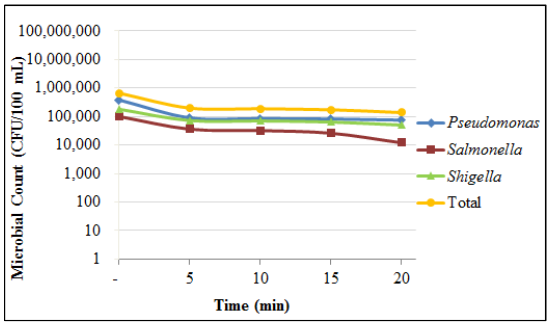
4.13 Effect of Chlorine Disinfection on Pathogen Removal in Continuous Mode

Experiments were carried out to understand the effect and efficiency of chlorine disinfection on different pathogenic bacteria under the continuous process. In this study, the primary aim was to comprehend the effect of contact time and applied dose of chlorine on pathogen versus coliform removal efficiencies of secondary treated effluent. The experiments were continued until the complete removal of microbes was achieved.

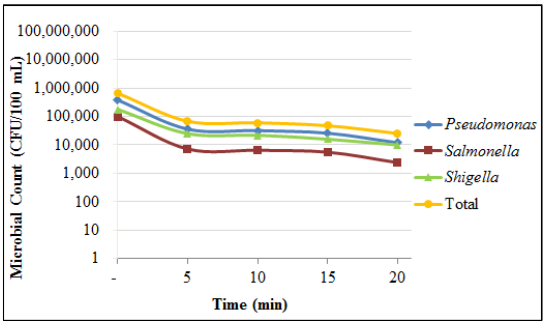
This set of experiments was carried out for the same doses as it was for TC removal i.e. from 2 mg/L to 6.5 mg/L of CD and for total CT of 20 minutes. Samples were collected at regular time interval of 5 minutes and were used for enumeration of pathogenic bacteria. The graphical representation of results is presented in figures given below.



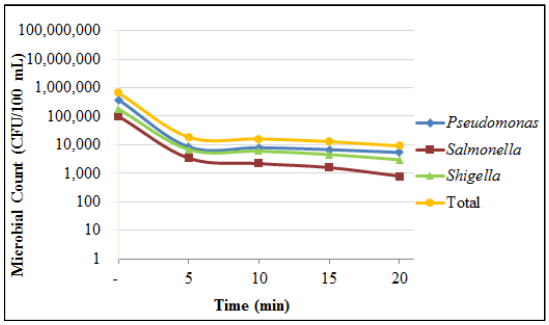
(a) 2 mg/L



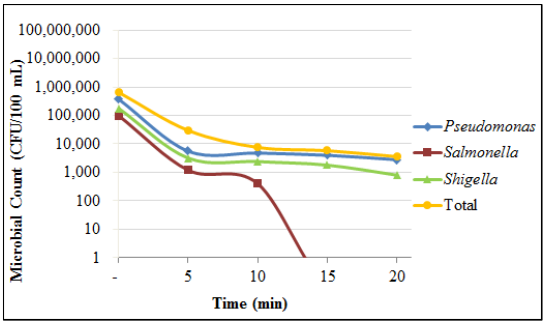
(b) 2.5 mg/L



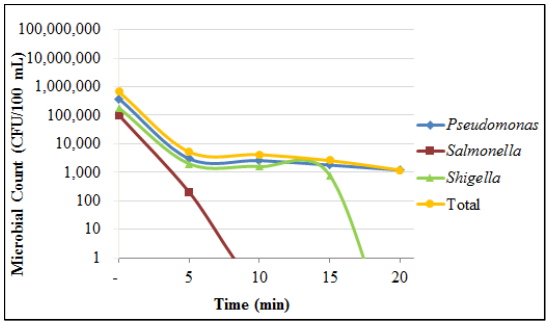
(c) 3 mg/L



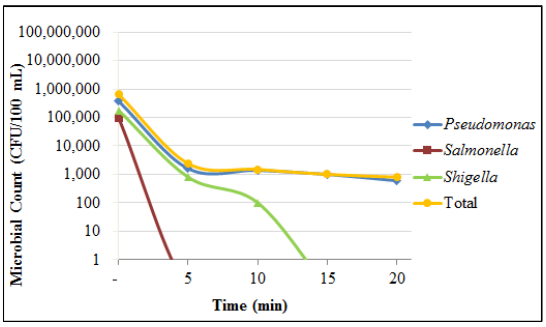
(d) 3.5 mg/L



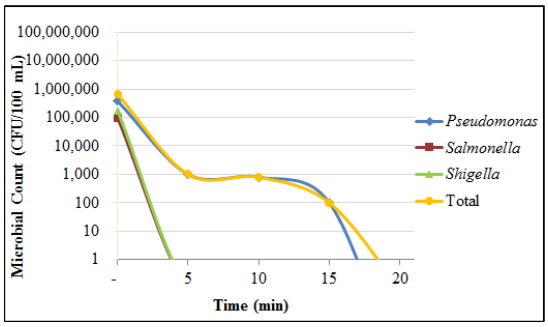
(e) 4 mg/L



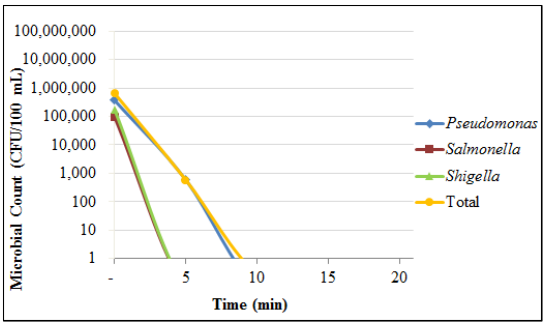
(f) 4.5 mg/L



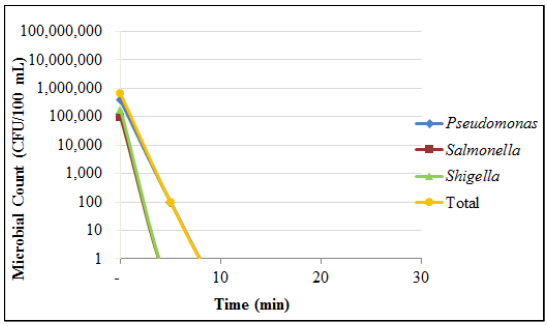
(g) 5 mg/L



(h) 5.5 mg/L



(i) 6 mg/L



(j) 6.5 mg/L

Figure 4.14: Pathogen removal profile for CD of (a) 2 mg/L, (b) 2.5 mg/L, (c) 3 mg/L, (d) 3.5 mg/L, (e) 4 mg/L, (f) 4.5 mg/L, (g) 5 mg/L, (h) 5.5 mg/L, (i) 6 mg/L, (j) 6.5 mg/L

It is evident from the Figure 4.14 (a) that at 2 mg/L of CD, only one log reduction was observed in the population of *Salmonella* and *Shigella* after 20 min of CT. It was observed that no additional reduction was observed in TCC at 20 min of CT, which may be due to insufficient CD because of high chlorine demand of certain organic and inorganic substances present initially. At 2.5 mg/L as shown in Figure 4.14 (b) decrement was observed up to one log in the population of pathogens. At 3 mg/L of CD and 5 min of CT, two log reduction was observed in the counts of *Salmonella* but pathogen counts could not be brought below 1,000 at this dose of chlorine even after 20 min of CT. This may be due to the fact that combined forms of chlorine are less effective than free forms as represented in Figure 4.14 (c). The two pathogenic species *Pseudomonas* and *Shigella* showed resistance at this dose of chlorine.

It is seen from Figure 4.14 (d) that at 3.5 mg/L and 5 minutes of contact time of CD, a continuous decrement of up to two logs in the value of microbial count was found in all the three species. The next 10 to 15 minutes were not so effective for *Pseudomonas* and *Shigella*. However, there was a continuous decrement in the counts

of *Salmonella* as it was sensitive to chlorine. At 20 min of CT counts for *Shigella* and *Pseudomonas* were competing with each other. 4 mg/L of CD was very effective with continuous reduction in the microbial counts. Counts for *Salmonella* reached zero at contact time of 15 min. *Shigella* also reached below norms at 20 min of contact. Again the counts for *Pseudomonas* were still high at this dose so further addition of CD was required, represented in Figure 4.14 (e).

On increasing the CD to 4.5 mg/L, there was further decrement in the microbial counts. The most sensitive species i.e. *Salmonella* reached zero at 10 min of CT. At 20 minutes, the counts for *Shigella* finally reached zero. Even after 20 minutes of CT, the pathogen counts for *Pseudomonas* could not be brought below 1,000 at this dose. Hence, the total count was still above the 1,000 mark at this time only due to *Pseudomonas* resistant nature as shown in Figure 4.14 (f).

From the Figure 4.14 (g) it was concluded that at 5 mg/L, *Salmonella* and *Shigella* reached zero at 15 minutes of CT. *Pseudomonas* seemed to be the sturdiest among the above species against chlorination but counts for it also reduced below 1000 CFU/100 mL at 15 minutes and finally reduced to zero at 20 minutes of contact time.

It was observed that at 4.5 mg/L of CD all the considered pathogen reached zero except for *Pseudomonas*, which offered the highest resistance to the chlorine. The resistant nature of *Pseudomonas* is already discussed in section 4.9 of this chapter [53]-[55]. At 5.5 mg/L of CD, TCC reached zero. It may be concluded that, 5.5 mg/L of CD was effective against pathogenic microbes as represented in Figure 4.14 (h). The total counts reduced to zero and hence this would be the recommended dose for disinfection for pathogens. Experiments were also carried out using 6 mg/L and 6.5 mg/L of CD, where it was observed that at these doses 5 min of CT was sufficient for complete removal of pathogens as noticed from Figure 4.14 (i) and (j). It was observed that the CD required for inactivation and removal of pathogens was generally less than that required for removal of TC from secondary treated effluent of STP in continuous process.

4.14 Determination of THMs

Disinfection is a very important step in water treatment process to reduce pathogens and prevent the regrowth of bacteria in the distribution system [56]. However, the use of chlorine as a disinfectant can accelerate the formation of DBPs, when chlorine

species like OCl^-/HOCl react with the dominant fraction of aquatic NOM comprised of humic and fulvic substances [57]-[60]. Thus, the major disadvantages of chlorination process is the formation of DBPs that includes THMs and HAAs [61]-[64]. The formation process of these DBPs has been explained with a flow chart in Figure 4.15. These DBPs have diverse negative effects on environment, human health which include carcinogenicity and birth defects [65]-[67].

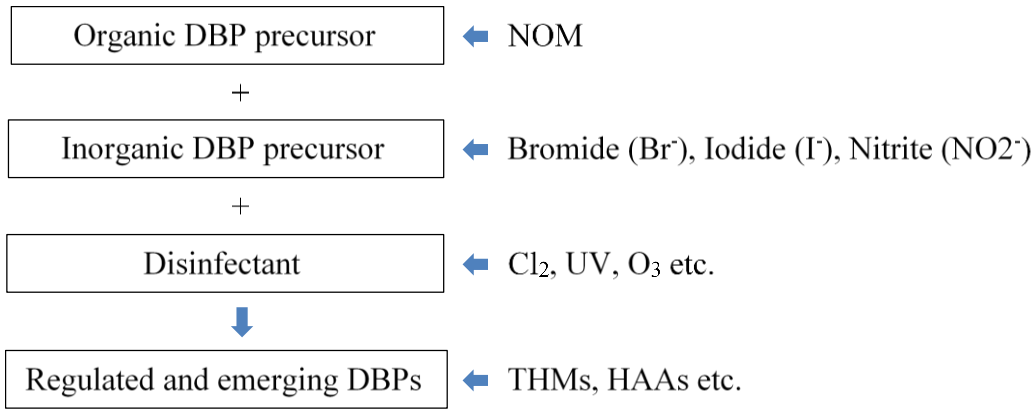


Figure 4.15: Formation process of DBPs

This research investigates the formation of only one class of the halogenated organics, i.e. the trihalomethanes to assess the fate of DBPs. THMs including $CHCl_3$, $CHCl_2Br$, $CHClBr_2$ and $CHBr_3$ were analysed based on methods described in Chapter 3 with GC-MS/MS [68]. Wastewater samples were examined for THMs before and after chlorine treatment. The two CDs at which THMs were analysed were 1.5 mg/L and 2.5 mg/L.

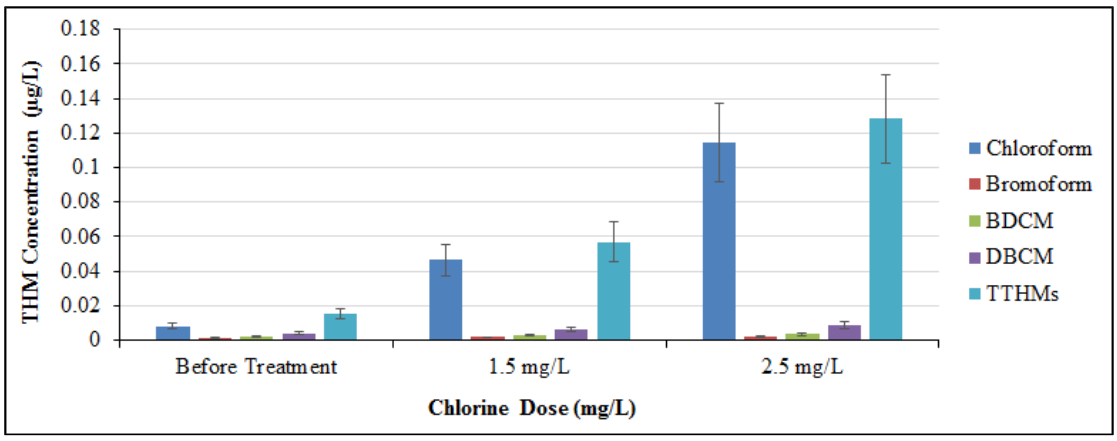


Figure 4.16: Changes in concentration of THM species with CD (RBC)

From Figure 4.16 it was concluded that THMs with fairly good concentration of bromide compounds were present in secondary treated effluent of RBC prior to chlorination. Davis [69] reported that the amount of salt in water reflects the value of bromide. It seems that THMs were also formed in small amount by chemical reactions between certain chemicals that include chlorine and bromide salts, which were discharged with the wastewater from laboratories of MNIT. The additional chlorination to the wastewater in the disinfection process then adds to the amount of THMs as it has been reported that increasing CD and exposure time will subsequently increase THMs [58] [66]. In agreement with the above statement it was concluded that in the present study as the concentration of chlorine increases from 1.5 to 2.5 mg/L, the production of THMs also increased. This has been validated statistically by applying two tailed t-test at 95% confidence interval. The p value is 0.0089 for samples treated with 1.5 mg/L for 20 min. and for samples treated with 2.5 mg/L for 20 min it is 0.0310. Formation of these by-products continues as long as precursors and disinfectants are present.

It was observed that $CHCl_3$ was the major THM which accounted for more than 50% in secondary treated effluent (before chlorination). It further increased to 81% at 1.5 mg/L CD for 20 min of CT and at 2.5 mg/L of CD for 20 min of CT, its concentration increased to more than 89% among all four THMs. A similar conclusion was also drawn by Siddiqui et al. [68], who analysed water samples at 58 locations and found that $CHCl_3$ was the major THM, which accounted for 91% of the total THMs.

The presence of bromide changes the relative concentrations of the different by-products [17] Increment in the concentration of $CHBr_3$ was observed by 42% at 1.5 mg/L of CD and by 58% at 2.5 mg/L of CD when compared to the concentration in secondary treated water. After $CHCl_3$, the next largest fractions of THMs were $CHCl_2Br$ and $CHClBr_2$. The $CHCl_2Br$ increased by 47% at 1.5 mg/L of CD and at 2.5 mg/L of CD it was increased by 84% when compared to its concentration in secondary treated water. The $CHClBr_2$ also increased by 63% at 1.5 mg/L of CD and the concentration of which increased to 126 % at 2.5 mg/L of CD when compared to its initial concentration in secondary treated water. This is possibly because when the ratio of CD to bromide ions increases, the formation of brominated THMs is favoured. TTHMs increased by 8 times after 2.5 mg/L CD when compared to their concentration in secondary treated water.

The presence of bromide ions in chlorinated water results in an increase in the formation of brominated THMs. During chlorination, bromide ions are oxidised to

hypobromous acid (HOBr), which reacts more readily with organic precursors than chlorine, forming brominated THMs [17] [70]. Bromination reactions similar to the chlorination reactions produce chlorinated organic DBPs. The combined action of chlorine and hypobromous acid leads to the formation of mixed chloro-bromo THMs [17] [70]. But it was observed that the $CHCl_3$ concentration and TTHMs concentration did not exceed the maximum permissible value i.e. 100 µg/L [65] [67]. Even at 2.5 mg/L of CD the TTHM concentration is very low i.e. 0.1284 µg/L. These findings are supported by some previously published studies, which also indicates that $CHCl_3$ was the most abundant THM species followed by $CHCl_2Br$ and $CHClBr_2$ [58] [59] [64].

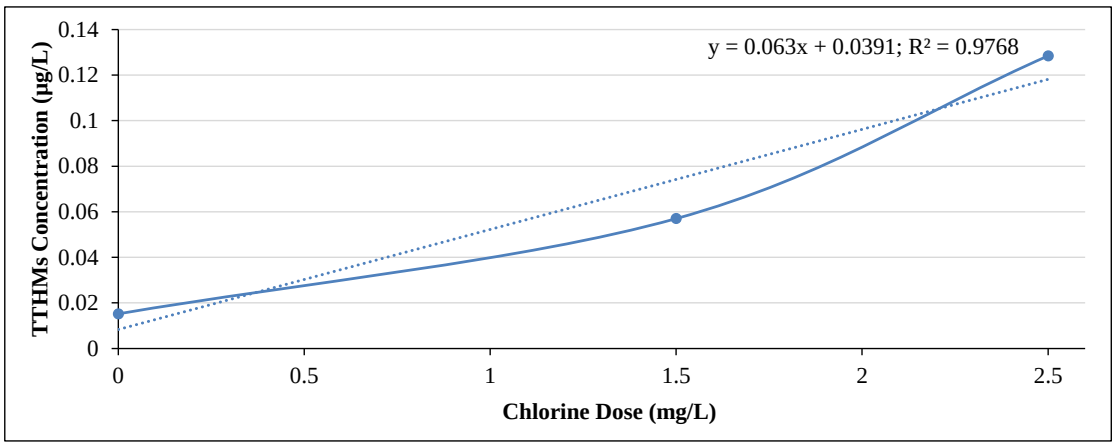


Figure 4.17: Relationship between CD and TTHM concentration

Figure 4.17 exemplifies that added CDs have reviewed strong relationship (R^2 values 97.68%) to THM formation. Adding chlorine to water leads to the formation of HOCl and OCl^-. The formation of these two species depends on the pH of water. It has been reported that in acidic conditions, HOCl dominates whereas in the alkaline solution the OCl^-dominant [3] [11] [17]. This information was useful in arriving at a conclusion in the present study that as the pH of the secondary treated effluent was in range of 7.5 to 7.8, the HOCl fraction might dominate resulting in the formation of THMs.

As discussed in section 4.4, high CD up to 17.5 mg/L was required in disinfection process of secondary treated effluent from STP, which is based on ASP to achieve the WHO standards for TC. Hence, use of such high CD will result into formation of excessive DBPs which in turn will have negative impact to the surrounding environment. To get a clue for this, an attempt was made to analyse THMs. It was

observed that on increasing CD up to a value of 7.5 mg/L, which was higher than what was sufficient for disinfection of secondary treated effluent of RBC, increase in TTHMs was observed by 13.5 times compared to initial concentration. It has been observed that there is a moderately steep increase in THM production as the CD is increased, until sufficient chlorine has been added to meet the full chlorine demand of the water [64] [65]. So, it was concluded that if the CD was increased up to 17.5 mg/L there will be tremendous increase in DBPs which can harm the surrounding environment.

The chromatograms represented below reflect a substantial increase in concentration of THMs, following different treatment processes.

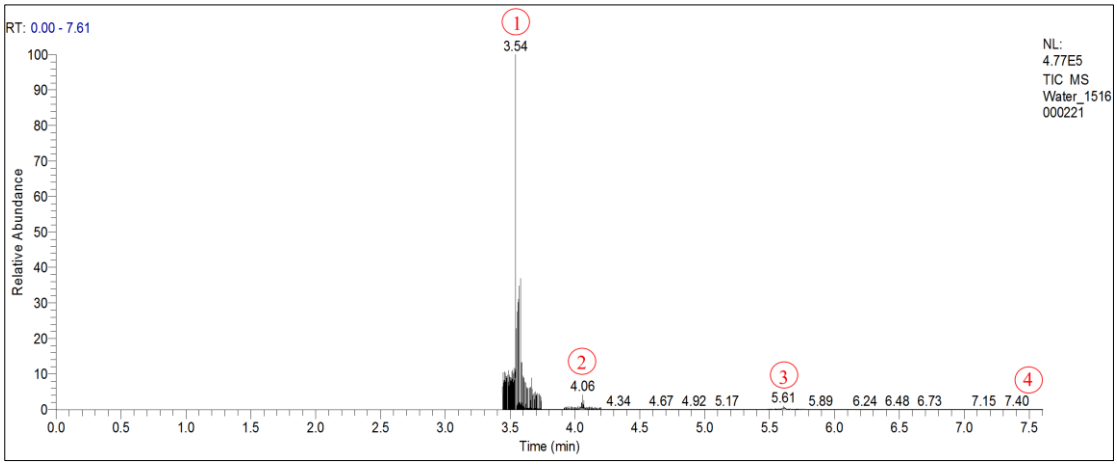


Figure 4.18: GC-MS/MS chromatogram (representing peaks of 1-4 of four analytes) obtained from MTBE extracts prepared from a representative wastewater sample before chlorination.

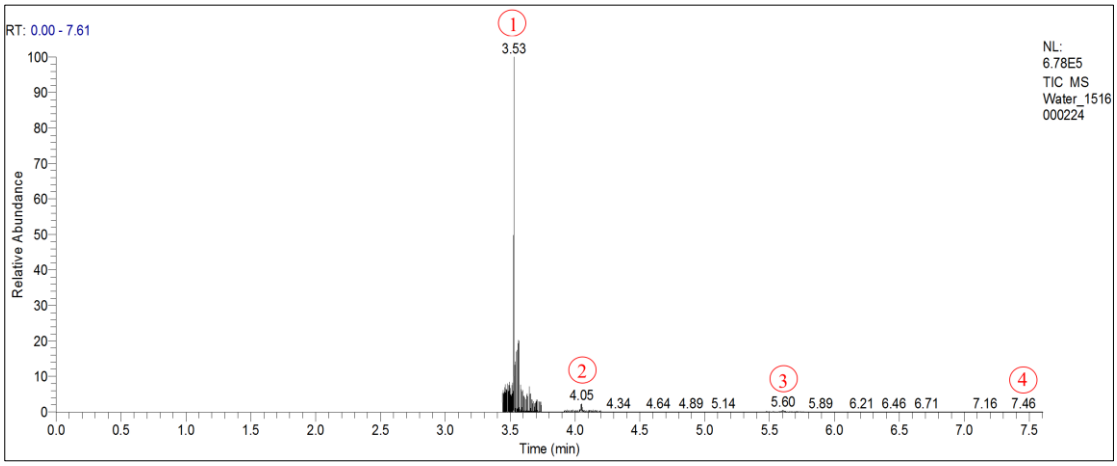


Figure 4.19: GC-MS/MS chromatogram (representing peaks of 1-4 of four analytes) obtained from MTBE extracts prepared from a representative wastewater sample after chlorination at CD of 1.5 mg/L.

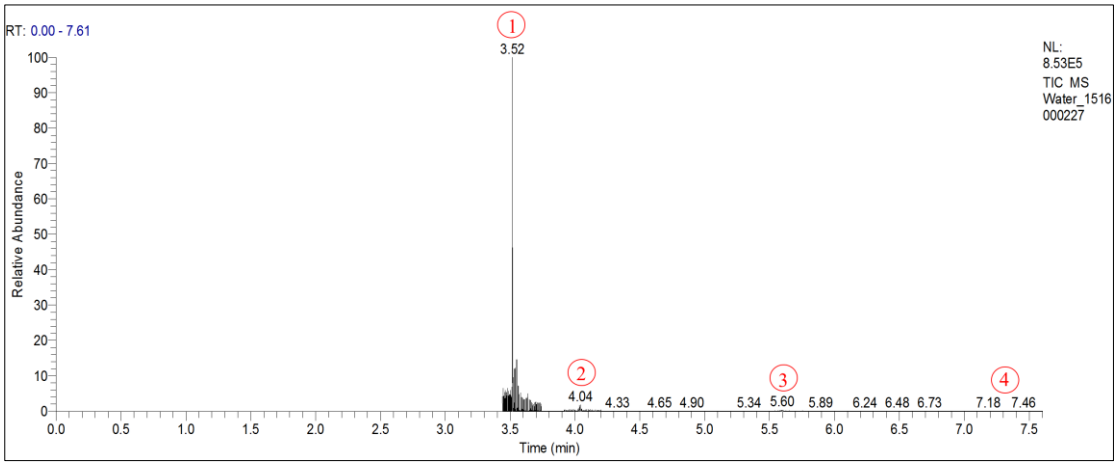


Figure 4.20: GC-MS/MS chromatogram (representing peaks of 1-4 of four analytes) obtained from MTBE extracts prepared from a representative wastewater sample after chlorination at CD of 2.5 mg/L.

In Figures 4.18, 4.19, and 4.20, the peaks of four analytes have been presented at their corresponding RT. As in the present research the analysis has been carried out by GC-MS/MS the peaks of only selected analytes were obtained on the chromatographs. Peaks of certain analytes such as bromoform are not clearly visible due to their less area when compared to area of $CHCl_3$ which has highest concentration (largest peak) among THMs. Table E.3 (Appendix E) presents the retention time, area and concentration of the four analytes in three samples. From these data, it was observed that the higher concentration of an analyte with increasing dose is confirmed by higher area of the corresponding analyte peak in chromatograph.

It was observed that compounds such as $CHCl_3$ and $CHCl_2Br$, which are reported human carcinogens and can seriously impact downstream public water supplies, should be minimized through their reduced formation by decreasing the applied doses. Thus the use of alternative disinfectants must be explored to effectively reduce the formation of THMs in treated effluent. According to WHO [5] [71] and USEPA [3] ozone and UV disinfection are two viable alternative to chlorine, which are now frequently used in the field.

Chapter Summary

This chapter addressed the research objectives 1, 3, 7 and 8. It showed the results of the chlorine disinfection in batch and continuous modes. The results indicate the dominancy of *Enterobacter* and *Serratia/Hafnia species* in the secondary treated effluent of RBC, which results in high chlorine doses for disinfection. The appropriate chlorine dose and contact time required for achieving the WHO standards for reusing wastewater in agriculture purposes is 4.5 mg/L for 20 minutes for batch process. For continuous process it is 6.5 mg/L for 20 minutes of contact time which shows that in continuous process chlorine demand will be higher than in the batch mode as some of the chlorine escapes from the continuous reactor. Similar trends for pathogen removal were observed in the experiments confirming coliforms were suitable indicators for pathogens.

From the results of batch process it was observed that at 2.5 mg/L CD and 20 minutes of contact time almost all the species were inactivated except for *Serratia/Hafnia* which seemed to be the most resistant one. In order to achieve the WHO standards, an extra addition of CD up to 4.5 mg/L was required, which might result into formation of more carcinogenic DBPs. To prove this, an attempt was also made to analyse THMs, one of the major group of DBPs. It was concluded from the study that at 2.5 mg/L of CD the increment observed in TTHMs was 8 times than the initial concentration in wastewater. This proves that if CD is further increased than the concentration of these THMs may very high and will increase carcinogenicity in the disinfected water. On the other hand chlorinated wastewater is often dechlorinated to reduce the toxic effect of free and combined chlorine residual which in turn increases the cost of the process. Optimization of the disinfection scenario may allow plants to control the formation of regulated and emerging DBPs. However, new and improved methods are needed to disinfect pathogens in treated wastewater, especially the highly resistant ones. The toxicity and high cost problems identified with chlorination have promoted evaluation and consideration of various alternative disinfection methods as ozonation and UV treatment.

Next chapter deals with the results obtained from the disinfection of the secondary treated effluent with ozone.

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Chapter 5

Chapter 5

Ozone Disinfection of Secondary Treated Effluent

Since 1970s, the use of chlorine has been reported to be associated with adverse environmental consequences [1] [2]. Chlorination of secondary treated wastewater leads to the formation of certain carcinogenic DBPs; hence alternative disinfectants have been considered widely for disinfection including ozone and UV radiations [3] [4]. The recent research is mainly focused on the efficient and eco-friendly disinfection technology for the removal of TC and pathogenic organisms [5] [6]. This part of the study is on ozone disinfection of secondary treated effluent, which is expected to provide a basis for hybrid disinfection strategy.

Ozone is an attractive disinfection alternative as it is a strong germicide and simultaneously oxidizes organic matter thereby improving the wastewater quality [3] [7]-[13]. It is an exceptionally good disinfectant that has faster disinfection kinetics and is more potent to eliminate most microorganisms when compared with other widely used chemical disinfectants [8] [11]. It has been reported that ozone disinfection is very effective for removal of TC and resistant microbes including pathogens [9] [12]. As opposed to chlorine, ozone does not leave any trace of residual product upon its oxidative reaction [3] [14]. The Ct values associated with the ozone are orders of magnitude lower than those associated with any other oxidant [6] [15]. It has been reported that low ozone concentration and short contact times are sufficient for disinfection purposes [3] [9] [11] [16] [17].

The study was conducted to evaluate the effectiveness of ozone treatment on the disinfection of secondary treated effluent from the STP installed at MNIT campus. It investigates the COD variation during the disinfection process and finds an optimum dose of ozone disinfection for secondary treated effluent of RBC in order to achieve the WHO standards for irrigation and agriculture purpose [18]. During these experiments, the effect of ozone on physicochemical and biological characteristics of the effluent in semi-continuous process was recorded. This part of the study also includes results of SEM analysis to demonstrate the bactericidal effect of ozone doses to get a clue to its inactivation mechanism. In another part of the study, the results of optimization of ozone doses using statistical analysis are included in order to avoid its excess dosing.

At the end, the effect of ozone on THM formation was studied with the help of GC-MS/MS.

5.1 Characteristics of Secondary Treated Effluent and their Effect on Ozone Disinfection

The physicochemical characteristics of the secondary treated effluent of RBC prior to disinfection are presented in Table 5.1. Effects of wastewater characteristics on ozone disinfection are also presented in the Table 5.1.

Table 5.1: Characteristics of secondary treated effluent of RBC and their effect on ozone disinfection

S. No.	***Quality parameters***	***Secondary treated effluent***	***Effect on ozone disinfection* [8] [11]**
1	pH	7.7 ± 1.20	It has some effect on the efficiency of ozone. pH change may affect ozone decomposition rate as at low pH hydroxide ions are less in water.
2	BOD	15.83 ± 1.50 mg/L	The degree of interference depends on their functional groups and chemical structures.
3	COD	105.35 ± 1.73 mg/L	The degree of interference due to organic matter.
4	Turbidity	41.10 ± 1.50 NTU	Organic matter reacts directly with ozone or reacts with hydroxyl radicals and decreases ozone stability.
5	TSS	15 ± 5 mg/L	The organic matter scavenges ozone and hydroxyl radicals which shields the micro pollutants and microorganisms from oxidation. Hence reduces efficacy.

5.2 Ozone Disinfection of Secondary Treated Effluent

The secondary treated wastewater was treated with different transferred ozone doses (TOD). The TOD has been considered as the most important and effective parameter

in disinfection of wastewater by ozone treatment [19]. The objective was to determine the TOD required to meet the WHO standards for TCs [11]. At the same time TOD was also determined for complete removal of pathogens from the effluent.

Table 5.2: Effect of ozone on physiochemical characteristics of secondary treated effluent of RBC

S. No.	***Quality parameters***	***Secondary treated sample***	***Ozonated sample***	***% Reduction***
1	pH	7.7 ± 1.10	7.2 ± 1.2	-
2	BOD	15.83 ± 1.50 mg/L	12.26 ± 2.4 mg/L	22.55
3	COD	105.35 ± 1.73 mg/L	26.22 ± 5.11 mg/L	75.23
4	Turbidity	41.10 ± 1.50 NTU	20.54 ± 1.8 mg/L	50.02
6	TSS	15 ± 5 mg/L	8 ± 2.1 mg/L	45.33

Table 5.2 presents the average values of different physicochemical parameters before and after ozone treatment and reduction in their values in terms of percentage. It was observed that exposure of secondary treated effluent to the optimum ozone dose was capable of reducing some of the physicochemical parameters, which hinders disinfection process. Significant reduction in COD, turbidity and TSS was observed which proves that ozone is a good oxidising agent. It is necessary to mention that at low ozone doses a slight increment in BOD value was observed as ozone can oxidise recalcitrant compounds and thereby increase effluent biodegradability but on increasing ozone doses, further continuous decrement in BOD was observed [3]. Ozone partially oxidises organic materials which are further broken down into smaller biologically degradable molecules that can be removed by filtration [7]. These results are in good agreement with the previous studies which reports a considerable reduction in COD, BOD, turbidity and TSS [3] [7] [11] [12] [19].

5.2.1 Ozone Disinfection Profile for TC Reduction

Experiments were carried out at different ozone doses ranging between 15-42 mg/L, according to the protocol explained in Chapter 3. The effects of subsequent doses were observed on the five dominant microbial species (*Escherichia coli, Enterobacter, Klebsiella, Serratia/Hafnia,* and *Citrobacter*) and on overall TC.

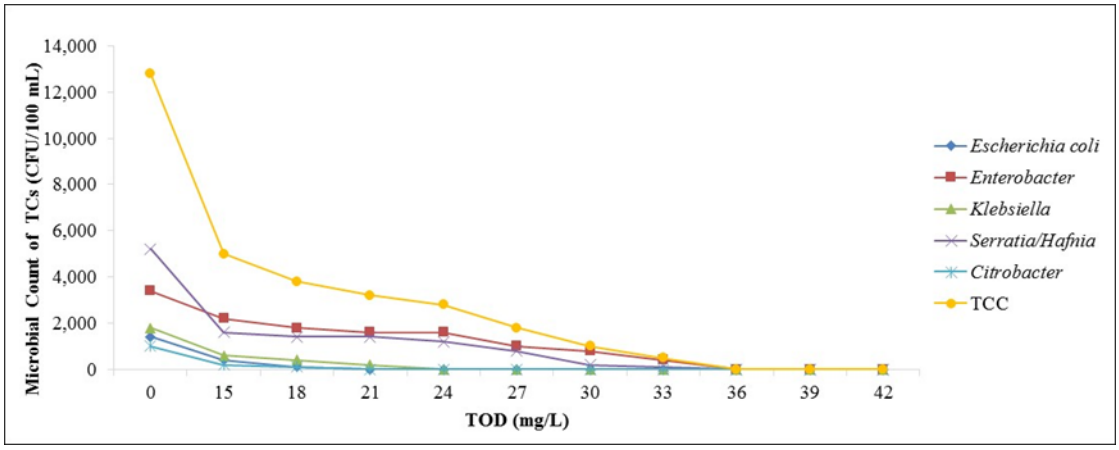


Figure 5.1: (a) Inactivation of coliforms at different TOD (the initial values of TC are represented in thousands)

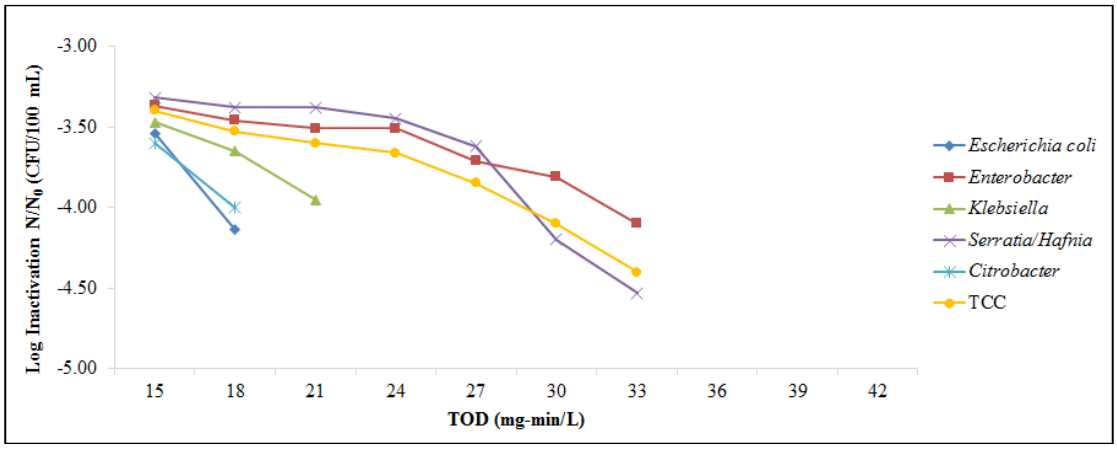


Figure 5.1: (b) Log inactivation of coliforms at different TOD

Figure 5.1 (a) represents the changes in the inactivation of microbial species as a function of ozone dose and Figure 5.1 (b) presents the changes in the inactivation rate of microbial species expressed in terms of log (N/No) as a function of ozone dose in order to explain log reduction. Lower concentrations of ozone were ineffective when organic matter was present as it interferes with the action of ozone on the bacterial cells [20]. Hence, it was observed from the above figure that only the population of *E.coli* and *Citrobacter* were reduced at very initial dose of ozone i.e. 15 mg/L as these were the most sensitive species to ozone [11]. Considerable reduction in the counts of *Klebsiella* was also observed at this dose. Due to excellent oxidative nature of ozone, even at low doses, the reduction of up to 2 logs was observed in most of the species except for *Enterobacter* and *Serratia/Hafnia*. A dose of 18 mg/L was not much effective for overall reduction in counts. *E.coli*, which was another sensitive coliform

species to ozone, was eliminated at 21 mg/L, followed by removal of *Citrobacter*. At 24 mg/L ozone dose *E.coli*, *Klebsiella* and *Citrobacter* were almost eliminated. However, the other two gram negative strains i.e. *Enterobacter* and *Serratia/Hafnia* were resistant to such low ozone doses. The counts for *Serratia/Hafnia* at 27 mg/L of ozone dose were able to meet the WHO standard [18]. But the counts for the most resistant species i.e. *Enterobacter* reduced below 1000 CFU/100 mL norm at 30 mg/L of ozone dose, which also brought the TCC within the limits.

It has been already discussed in Chapter 4 that being gram negative *Serratia/Hafnia* have the lipopolysaccharide (LPS) content in the outer membrane, which makes them difficult to obliterate, thus possibly resulting in the consumption of a high amount of any oxidant either chlorine or ozone [21-25]. Another possible reason for the resistant nature may be due to its variable cell wall permeability and due to the secretion of beta-lactamases as reported by Delcour [26]. It is also discussed in Chapter 4 that *Serratia*/*Hafnia* were resistant to many antimicrobial agents due to certain characteristics such as their ability to survive in aerobic and anaerobic conditions (unique membrane) [23] [25].

The TOD (Ct 30 mg/L) required to meet the WHO norms was 62% times lesser than the total chlorine dose of 80 mg/L (4 mg/L for 20 min) as described in Chapter 4. Further the addition of ozone dose up to 36 mg/L totally eliminated all resistant strains. *Enterobacter* was also one of the resistant species to ozone. The resistant nature of *Enterobacter* to ozonation was in agreement with the observation of Martinez et al. and Gayet et al. [25] [27], who reported that *Enterobacter* exhibited phenotypes of multiresistance and is encapsulated.

The results of the study confirmed that ozone is one of the fastest and efficient known bactericide. The ozone disinfection was very effective for TC and chlorine resistant microorganisms such as *Enterobacter* and *Serratia/Hafnia*. The overall killing rate of ozone was found to be more than 99% for the concentration of microorganisms up to 10^5 CFU/100 mL in secondary treated effluent, which is in agreement with the previous studies [16] [19] [28] [29]. Hence ozone emerges as a solution and a good alternative to chlorine for offering disinfection treatment of the effluent. Ozone results into true destruction and not a displacement of the microbes and pollutants [7]. Another very important property of ozonation is that when a good disinfection is achieved, defined as 1000 CFU/100 mL, inactivation of viruses, protozoa and other micro pollutants is also achieved [11] [16] [30], but it was not monitored in the present study.

5.2.2 COD Variation of Sample with Different Doses of Ozone

The effect of ozone on COD depends on the effluent matrix, the initial value of COD and whether the organics are easily oxidizable or recalcitrant among other factors. From Figure 5.2 it can be observed that a systematic decrease in COD was observed with the increasing ozone dose.

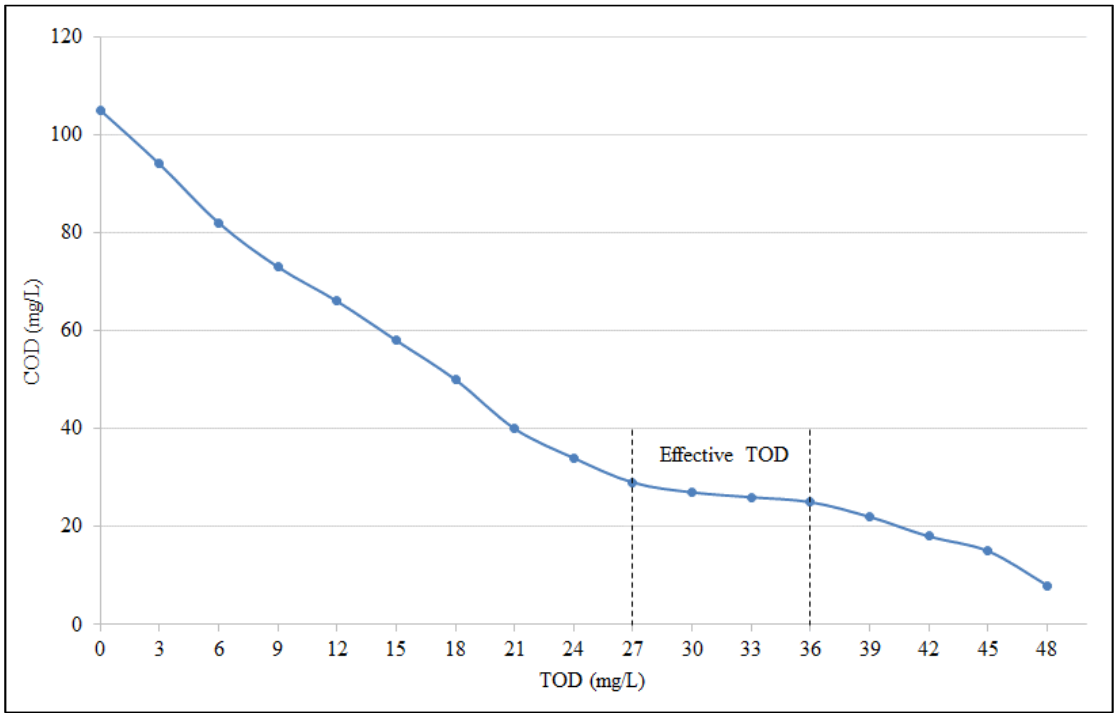


Figure 5.2: COD variation of secondary treated sample with different doses of ozone

Initially, there was simultaneous reduction of TC and organic matter with both the processes competing for applied ozone for doses between 10-20 mg/L. The COD of the samples continued to decrease at a high rate between doses of 20-27 mg/L, during which the disinfection process is almost constant. Above a dose of 27 mg/L, the rate of COD removal decreased significantly and the doses between 30-33 mg/L (effective transferred ozone dose) probably produced disinfection of resistant coliform species with partial reduction of the remaining organic matter. Above 40 mg/L of ozone dose, the TCC became zero and virtually very less reduction in COD was found, which shows that the consumption of ozone had been decreased at this stage. Thus ozone has proven effective as a strong oxidising agent. Paraskeva et al. [12] also reported increased COD reduction at higher ozone doses. For doses in the typical disinfection range (i.e. 4 to 10

mg/L) the average COD reduction reported was 20 to 30%, at 20 mg/L ozone dose, the reduction was 30 to 50% and while at higher doses (10-30 mg/L), 30-75% reduction was observed.

Table 5.3 represents the effect of ozone doses on removal of TC in terms of CFU/100 mL and Table B.1 (Appendix B) represents data in terms of MPN/100 mL. The results of inactivation of TC by the pour plate method at different TOD are shown in Figure B.1 (Appendix B) and colilert results are represented in Figure B.2 (Appendix B), indicating removal of microbes in terms of MPN/100 mL.

Table 5.3: Effect of different ozone doses on TC in terms of CFU/100 mL on secondary treated effluent

S. No.	***TOD (mg/L)***	***TCC in sec. treated effluent***	***TCC in ozonated effluent***	***Standard deviation***	***% Reduction***	***Log reduction***
1	15	128×10^5	50×10^3	80.10	99.96	2
2	18	128×10^5	38×10^3	75.23	99.97	2
3	21	128×10^5	32×10^3	60.54	99.97	2
4	24	128×10^5	28×10^2	36.65	99.97	2
5	27	128×10^5	18×10^2	32.39	99.98	3
6	30	128×10^5	10×10^2	24.02	99.99	4
7	33	128×10^5	50×10^1	22.17	99.99	5
8	36	128×10^5	<30	18.06	99.99	5
9	39	128×10^5	0	12.38	99.99	6
10	42	128×10^5	0	1.31	100	6

The reaction of ozone with organic and inorganic compounds creates an ozone demand, which should be satisfied during ozonation before developing a measurable residual and before any ozone is available to satisfy primary disinfection requirements [11]. It has been reported that the rate of reaction between disinfection of indicator microorganisms and oxidants is fast [47]. Disinfection results indicate that the ozonated effluent quality fulfils the established guidelines of WHO of 1000 CFU/100 mL for TC at TOD of 30 mg/L so that wastewater can be reused without restrictions for irrigation and agriculture purpose. A 4 log reduction in TC was observed at this corresponding ozone dose. It may be because organic matter was getting oxidised at this state predominantly as evidenced by COD removal in Figure 4 as well. At initial doses, organic matter would require some time to get oxidised and continues to exert oxygen demand till the formation of some end products, which do not undergo oxidation. While at TOD 39 mg/L the sample became sterile, which was supported by the previous studies that higher the ozone dose, the higher will be the bacterial removal [2] [8]. Greater removal of up to 5 to 6 log reductions has been reported with higher doses of ozone [41] [48].

5.3 SEM Analysis

SEM images have characteristic three dimensional appearances, which help in understanding the decontamination mechanism of the treated microorganisms. Microscopic observation and SEM analysis showed that microorganisms were rapidly destroyed by ozone and finally the cell membrane was ruptured followed by cell death [31] [32].

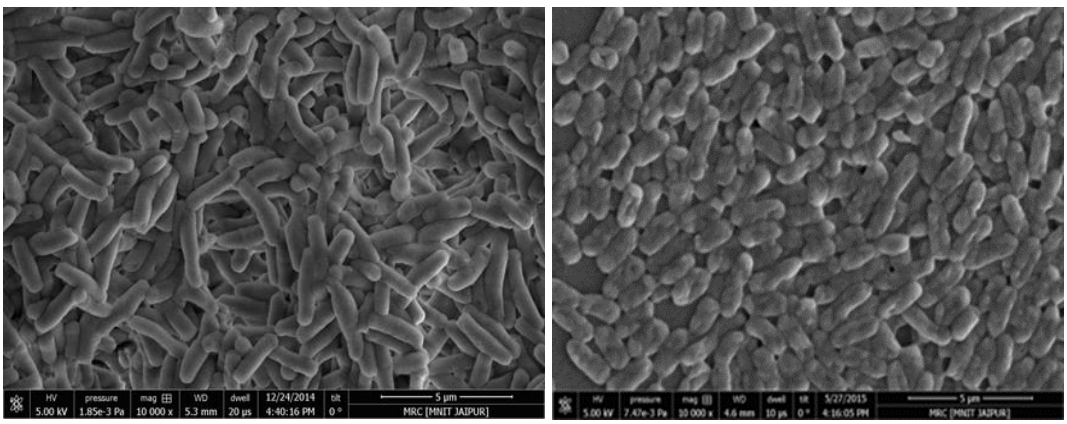
Four samples were prepared for SEM analysis to study the effect of ozone dose on cell physiology of microorganisms. First sample was of secondary treated effluent before ozonation (blank), second sample was treated with an ozone dose of 15 mg/L, third sample was treated with 30 mg/L and fourth sample was treated with higher ozone dose of 36 mg/L as represented in Figure 5.3 (a), 5.3 (b), 5.3 (c), and 5.3 (d).

Figures 5.3 (a) shows SEM images of samples before disinfection. It was observed that initially the bacterial surface was very smooth, showing sharp edges and appeared very turgid with gram negative bacteria of size 2 µm (approx.). Figure 5.3 (b) represents SEM image of the sample treated with low ozone dose of 15 mg/L. Figure

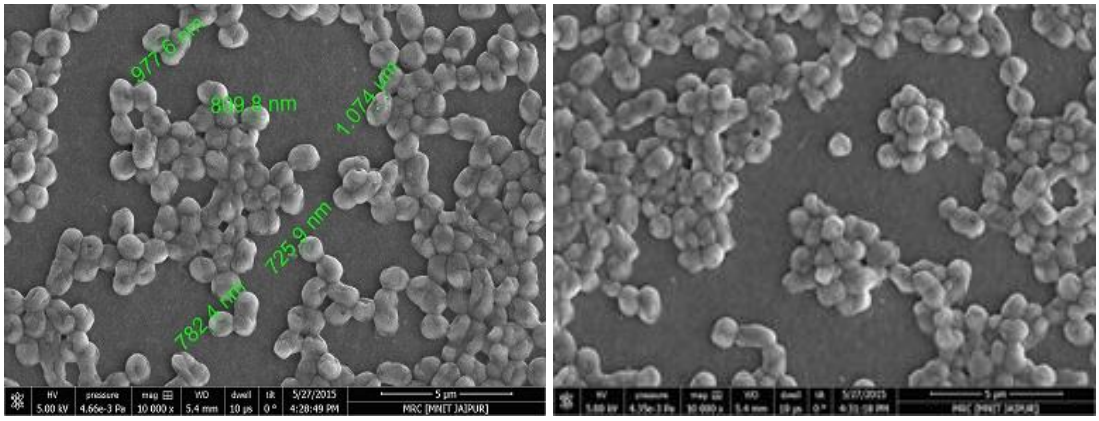
5.3 (c) represents SEM image of the sample, treated with ozone dose of 30 mg/L, which was found sufficient for meeting disinfection requirements as per WHO norms in experiments.

The oxidizing mechanism of ozone may involve direct reactions of molecular ozone on the microorganisms and also free radical-mediated destruction by hydroxyl (OH) radicals, which are formed on decomposition of molecular ozone in the indirect pathway [33]. It was confirmed that due to oxidation process, ozone changed the ultra-structure of bacteria. It resulted into deformation of cell membrane and structure by damaging its surface. Bacterial cells treated with ozone showed collapsed, shrunken pattern in images and size of bacteria was also reduced to nm range. These findings are also supported by Thanomsub et al. [34]. This occurs because of its high oxidation potential, ozone oxidizes cell components of the bacterial cell wall. This is a consequence of cell wall penetration. The mechanism of bacterial inactivation by ozone occurs by general inactivation of the whole cell. Ozone by oxidative mechanism breaks cell membrane, cell wall, chromosomes, carbon nitrogen bonds between sugar and bases, DNA hydrogen bonds, as well as sugar phosphate bonds leading to depolymerisation and leakage of cellular constituents and irreversible enzyme inhibition within microorganisms [9] [11].

When the cellular membrane was damaged during this process, the cell wall ruptured, resulting in cellular lysis [9] [11] [35]. It was observed that low doses of ozone resulted in destruction of cells as observed from Figure 5.3 (b) and (c) whereas further higher doses lead to cell lysis and the size of the cells reduced to half, as observed from Figure 5.3 (d). Hence, SEM images showed partial to complete destruction of the cell membrane, leading to its lysis as a result of the oxidation process.



(a) (b)



(c) (d)

Figure 5.3: TC (a) before ozonation, (b) ozonation 15 mg/L, (c) after ozonation 30 mg/L, (d) after ozonation 36 mg/L

5.4 Optimization of Ozonation Process Using One Factorial Design

In the present study for statistical data analysis, the DOE software (version 7.0.0.0) was used. One factorial design of RSM was applied to assess the relationship among ozone dose (independent variable), TC and COD (dependent variables or response). The independent variable was varied over two levels between high and low based on a set of preliminary experiments. Optimum settings of parameters can be found between this low and high range. Table 5.4 presents the levels used for the process parameter in one factor design. The two levels for a variable, reduced the number of experiments required to be performed.

Table 5.4: Independent variables of the one factorial design

Variables	***Factor***	***Level (-1)***	***Mean***	***Level (+1)***
A	Dose (mg/L)	15	28	42

Twelve runs of the single factorial design were carried out and the design matrix for the factor and response is presented in Table 5.5.

Table 5.5: Response values for different experimental conditions

Run	***Factor A: Dose (mg/L)***	***Response TCC (CFU/100 mL)***	***Response COD (mg/L)***
1	42	0	14.11
2	28.50	1400	30.12
3	35.25	200	23.00
4	28.50	1400	28.18
5	28.50	1200	29.06
6	15.00	4900	78.75
7	28.50	1200	28.79
8	28.50	1400	30.12
9	15.00	4900	78.18
10	21.75	3000	48.13
11	42.00	0	14.11
12	28.50	1400	28.05

5.4.1 Validation of the Model

The regression model equation was developed between the response and the input variable and is presented in terms of coded factors by Equation 5.1 and 5.2.

$$TCC = + 1330.77 - 2488.89 * A – 1117.95 * A^2 \quad (5.1)$$

$$COD = + 28.72 - 32.51 * A + 17.16 * A^2 \quad (5.2)$$

From these equations it is clear that increased ozone dose results in a consequent decrease in both the TCC and the COD, which compete together at varying rates for its consumption. Comparison of actual and predicted values for TCC and COD is presented in Figure 5.4 (a) and 5.4 (b).

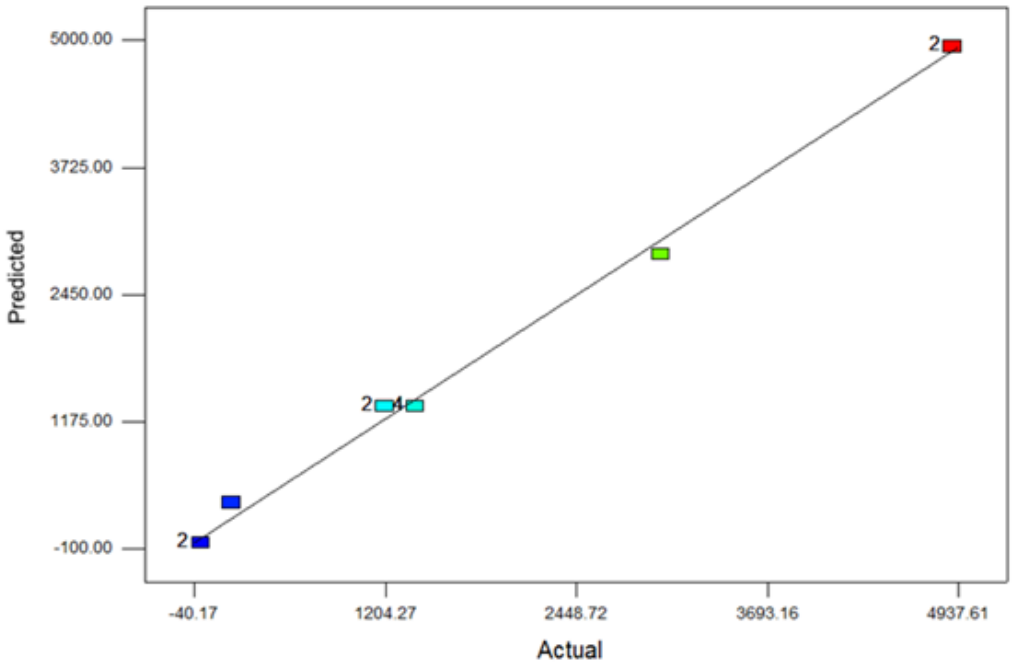


Figure 5.4: (a) Parity plot for the actual and predicted value of TCC (CFU/100 mL)

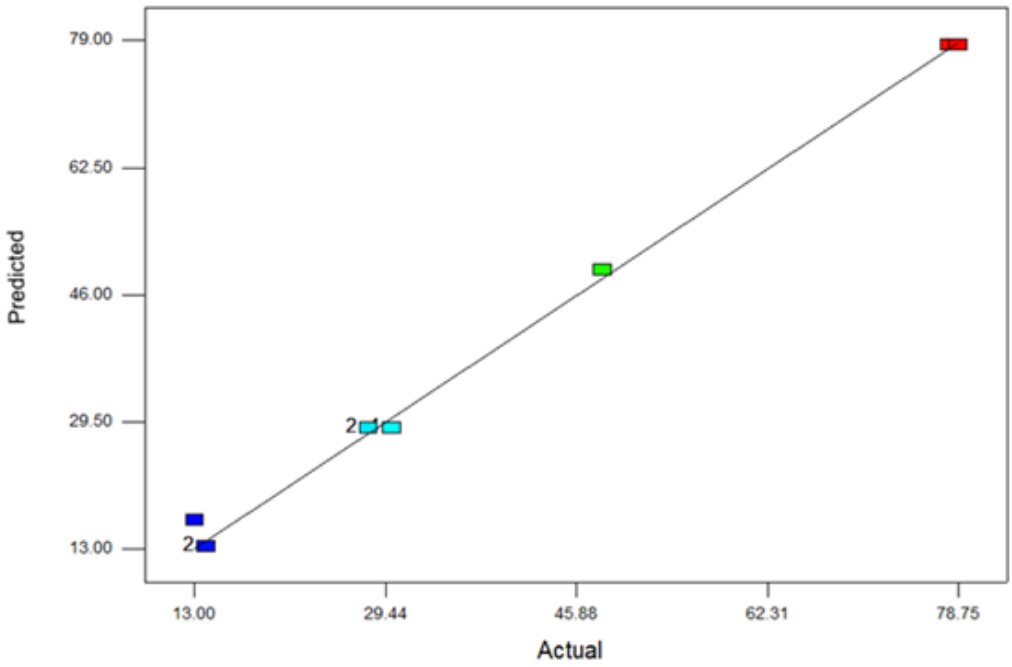


Figure 5.4: (b) Parity plot for the actual and predicted value of COD (mg/L)

5.4.2 ANOVA

The statistical adequacy of the model was justified through ANOVA. The ANOVA values for the quadratic regression model for TCC and COD in case of ozone disinfection are shown in Tables 5.6 and 5.7. The p-value of lower than 0.0001 indicates that the model is statistically significant at 95% confidence interval [36]. The value of

regression coefficient R^2 is higher than 99.65% for TCC and 99.56% for COD, confirming a good fit of the model.

Table 5.6: ANOVA for TCC

Source	***Sum of squares***	***df***	***Mean square***	***F-value***	***p-value Prob>F***	
Model	3.092E+007	2	1.546E+007	1288.01	< 0.0001	Significant
A-Dose	2.788E+007	1	2.788E+007	2322.23	< 0.0001	
A^2	3.046E+006	1	3.046E+006	253.79	< 0.0001	
Residual	1.080E+005	9	12003.80			
Lack of Fit	54700.85	2	27350.43	3.59	0.0845	Not Significant
Pure Error	53333.33	7	7619.05			
Cor Total	3.103E+007	11				

Note: R^2 = 99.65% and R^2 (adj) = 99.57%; df = degree of freedom

Table 5.7: ANOVA for COD

Source	***Sum of squares***	***df***	***Mean square***	***F-value***	***p-value Prob>F***	
Model	5475.11	2	2737.56	1024.28	< 0.0001	Significant
A-Dose	4757.35	1	4757.35	1780.00	< 0.0001	
A^2	717.76	1	717.76	268.56	< 0.0001	
Residual	24.05	9	2.67			
Lack of Fit	18.44	2	9.22	11.49	0.0061	Not Significant
Pure Error	5.61	7	0.80			
Cor Total	5499.16	11				

Note: R^2 = 99.56% and R^2 (adj) = 99.47%; df = degree of freedom

5.4.3 Interaction Plots for Various Operating Parameters

Figures 5.5 (a) and 5.5 (b) show the interaction effects of factor and responses. On increasing the value of a factor (i.e. TOD) there was a negative effect on the response and the values of both TCC and COD decreased simultaneously.

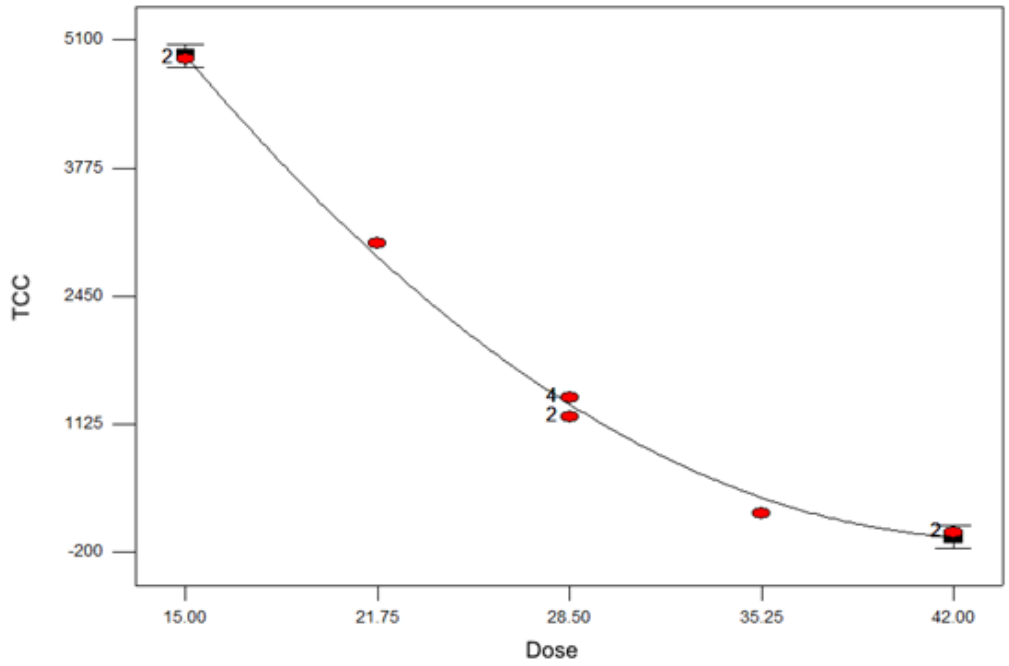


Figure 5.5: (a) Interaction plots for TCC as a function of ozone dose

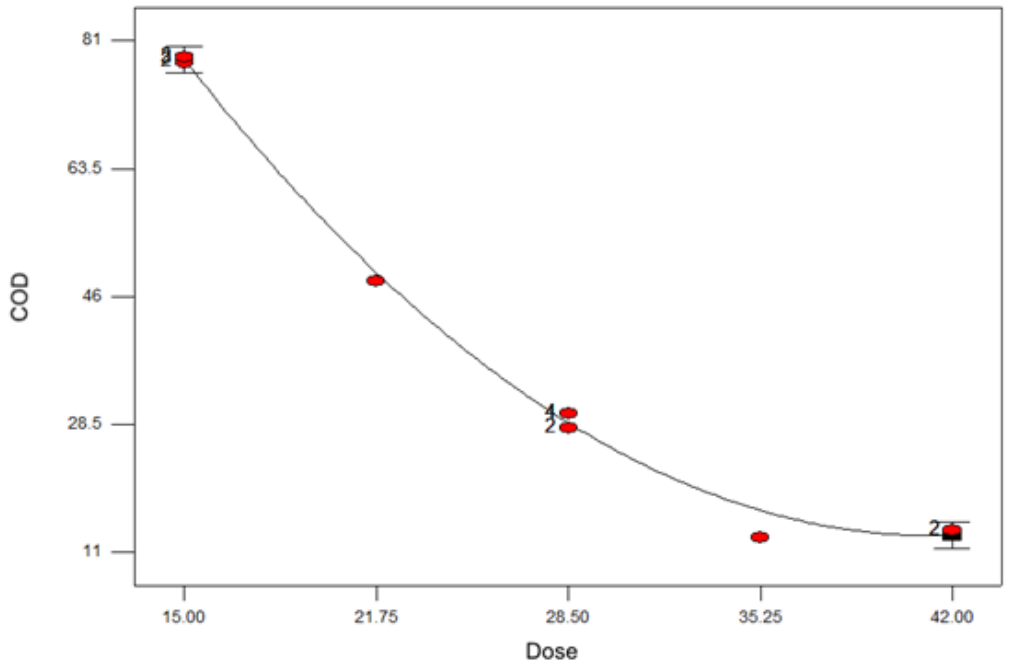


Figure 5.5: (b) Interaction plots for COD as a function of ozone dose

5.4.4 Optimization of the Ozone Disinfection Process

The main objective of the study was to determine the optimum value of ozone dose for TC to be brought within WHO norms [18] and to obtain the corresponding value of COD at this ozone dose. In the present study the target TCC was defined as 1000 CFU/100 mL for ozone dose in a range of 15 to 42 mg/L and for the minimum COD.

Table 5.8 represents the optimised results. It was found that the ozone dose of 30 mg/L was sufficient to achieve the target and the value of COD at this dose was 21mg/L.

From a similar study carried out on same effluent with chlorine disinfection, it was observed that the optimised CD of 4 mg/L for 20 min; or 80 mg/L of Ct produced equivalent effect. If we compare Ct, it is found that ozone (30 mg/L) has an edge over chlorine (80 mg/L) and was 62% times lesser. The residual COD with ozone was also lower at 21 mg/L when compared to chlorine residual COD of 58.88 mg/L, confirming higher potential of ozone for simultaneous disinfection and oxidation of organic matter. The experimental verification for these optimised sets of conditions also confirmed good agreement with the predicted results. Thus data derived from the experimental studies may be very useful in arriving at cost economics of the two types of disinfection processes.

Table 5.8: Optimization and validation values

Variables	***Unit***	***Values from optimization***
TOD	mg/L	30.00
Residual TCC (predicted)	CFU/100 mL	1,000
Residual TCC (experimental)	CFU/100 mL	800
Residual COD (predicted)	mg/L	21.00
Residual COD (experimental)	mg/L	22.00

5.5 Ozone Disinfection Profile for Pathogen Removal

Ozone is a powerful oxidising agent which can destroy any type of pathogenic and non-pathogenic bacteria. Hence, the experiments were carried out at different doses of ozone ranging from 15-42 mg/L to understand the efficacy of ozone disinfection on different pathogenic bacteria under semi-continuous regime. The primary aim of this study was to test the effect of TOD on coliform versus pathogen removal efficiency of secondary treated effluent. The experiments were continued till the complete elimination of pathogens was achieved. The results are represented in Figure 5.6 (a) and 5.6 (b).

As it is shown in Figure 5.6 (a), *Pseudomonas*, *Salmonella* and *Shigella*, were chosen as target microorganisms in order to evaluate the disinfection efficiency of ozone on pathogens. It is important to mention that a relatively low ozone dose of 24 mg/L totally inactivates *Salmonella* from the secondary treated effluent sample, which

was in agreement with previously reported data, that *Salmonella* was very sensitive to ozone [11]. A higher efficiency of ozonation was also observed for *Pseudomonas* [37]. A significant reduction of *Pseudomonas* was obtained, with subsequent ozone doses, whereas, it was found to be resistant to low doses of chlorine. A TOD of 27 mg/L was enough for total inactivation of *Pseudomonas*. This ozone dose, required for inactivation of *Pseudomonas*, was comparable to the TOD reported by Bataller et al. [12]. As compared to all other three microorganisms, a slightly higher resistance was offered by *Shigella*, which required an ozone dose of 30 mg/L for its complete inactivation. Hence, from the present study it is concluded that the ozone dose of 27 mg/L is required to bring the counts of pathogens to satisfy WHO regulation (1000 CFU/100 mL), but for their complete inactivation from wastewater the ozone dose required would be 30 mg/L. Figure 5.6 (b) represents the reduction in pathogens on a logarithmic scale. Hence, it was concluded from above results that ozone reduced the content of pathogenic microorganisms resistant to chlorine which is supported by the findings of Selma et al. [38].

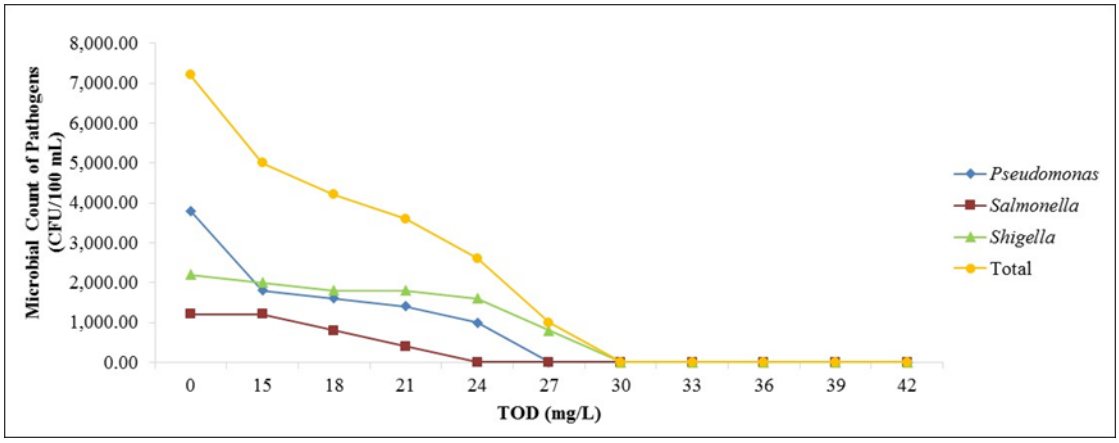


Figure 5.6: (a) Inactivation of Pathogens at different TOD (the initial values of pathogens are represented in hundreds)

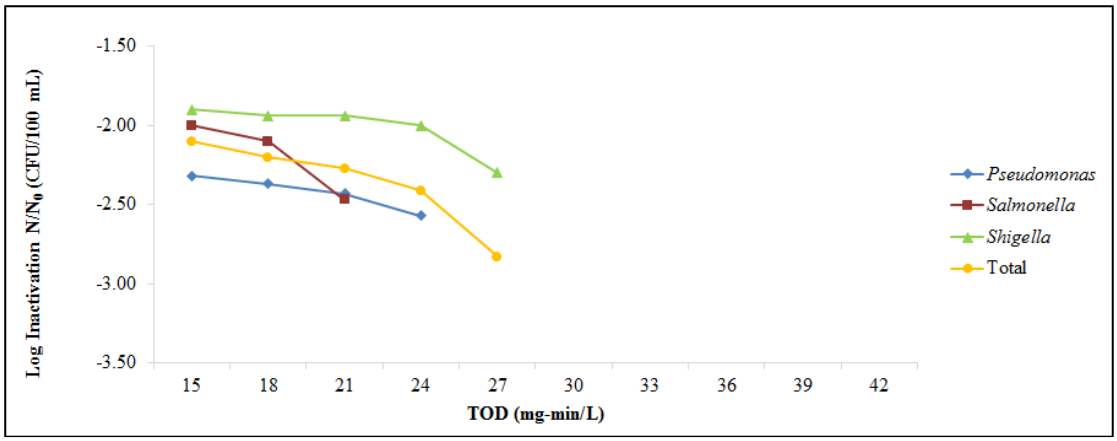


Figure 5.6: (b) Log Inactivation of Pathogens at different TOD

5.6 Determination of THMs

Ozone reacts with natural products present in the water to form numerous DBPs such as aldehydes, ketones, mono and dicarboxylic acids etc. [39]. But these by-products are less toxic as compared to chlorinated DBPs [11] [40].Ozone has been widely used to control THMs and other DBPs as it reported to produce fewer chlorinated DBPs and provide excess disinfection efficacy [39]. Hence, in the present study instead of analysing less effective DBPs which are normally produced by ozonation, the effect of ozone on four THMs was studied and Triple Quadrupole GC-MS/MS was employed for detection and identification of THMs at CEG laboratory. A single optimised dose of ozone i.e. 30 mg/L was chosen to study the effect on four THMs. The results are shown in Figure 5.7.

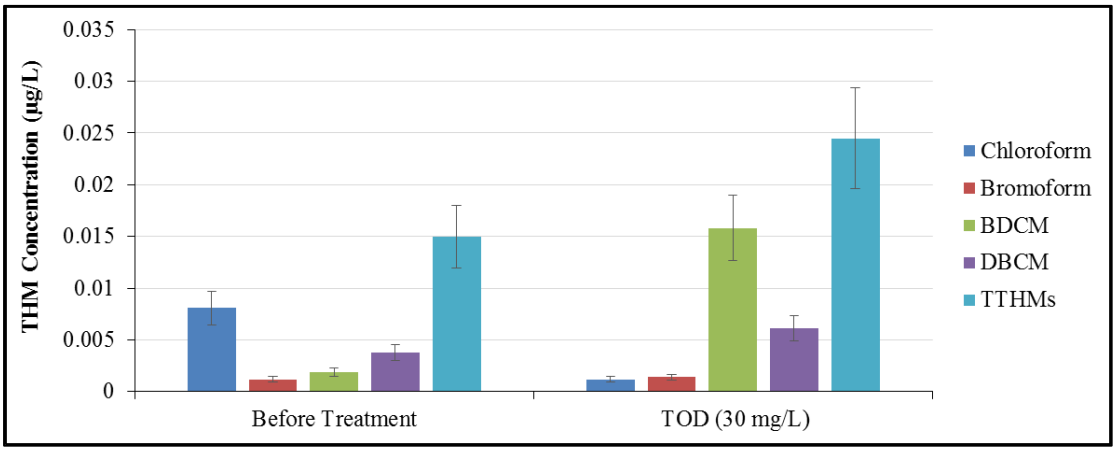


Figure 5.7: Changes in concentration of THM species with ozone dose (RBC)

From the Figure 5.7 it can be observed that after ozone treatment, the reduction in $CHCl_3$ (Equation 5.3) was observed to be 85% when compared to its concentration in secondary treated effluent, probably through the following reaction:

$$6\ CHCl_3 + 6\ O_3 \rightarrow 6\ CO_2 + 3\ H_2O + 9\ Cl_2O\ [40] \quad (5.3)$$

On the other hand, ozone can oxidise any bromide ion (Br^-) if present in the water to bromated (BrO_3^-) [41] [42]. The oxidation reaction with Br leads to the production of hypobromous acid (HBrO) and hypobromite (BrO^-). The HBrO and BrO^- can further react with organic matter present in water to form brominated organic compounds, similar to those formed by chlorine [11] [44]. If Br^- ion is present in the raw water halogenated DBPs may be formed [11] [42]. It was reported by Nan [45] that ozonation enhanced the yields of all detected chlorine DBPs except $CHCl_3$. Similarly, it was also observed in the present study that after ozone treatment there was increment in the concentration of three brominated species. The $CHBr_3$ was increased by 16%, increment in $CHCl_2Br$ was 7.3 times and $CHClBr_2$ increased by 60% when compared to their initial concentration in secondary treated wastewater. THMs speciation gradually shifts from chlorinated species to mixed bromochloro species with increasing ozone concentration, while only a slight increase in $CHBr_3$ was detected [45]. Validated statistically by applying two tailed t-test at 95% confidence interval where the p value is 0.0053.

When compared to chlorination, it was concluded that after ozonation, the increment in the three bromo products is 66%. This is supported by a finding that reactions between organic compounds with HBrO/BrO^- are faster than those with HOCl/ClO^- [39] [47]. Hence, it was concluded, that the secondary treated effluent of RBC naturally had some concentration of Br^- in it due to which these brominated products were formed. In absence of Br in water sample, ozonation would have minimized the formation of halogenated DBPs [42].The increment in TTHMs (when compared to secondary effluent) due to ozone disinfection was 61% which was very less as compared to the chlorination process. But, it was observed that reduction in TTHMs was by 80% as compared to chlorination. However, $CHCl_3$ concentration was 94 times more in chlorination process than that of formed by ozonation. Hence, it was concluded that one of the most important benefits of using ozone as a disinfectant alternative is that it reduces the formation of one of the most prevalent human carcinogenic DBPs, namely, $CHCl_3$. Overall chlorination resulted into formation of

four times more TTHMs as compared to ozonation, these results are supported by the findings of Hu [39].

In general, brominated DBPs are now being recognised as toxicologically important because there is an indication that brominated DBPs may be more carcinogenic than their chlorinated analogs [8] [11]. The future regulations are likely to focus more on individual concentrations of DBPs, as the ever increasing evidence of epidemiological studies indicates that different individual species may have different health effects [4] [46]. The chromatograms represented below reflect a substantial change in concentration of THMs, after treatment of secondary treated sewage effluent.

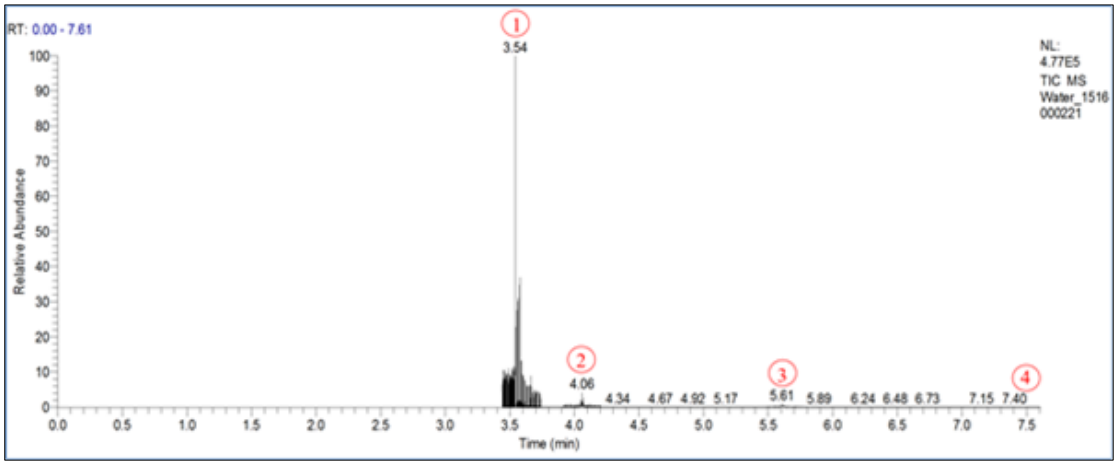


Figure 5.8: GC-MS/MS chromatogram obtained from MTBE extracts prepared from a representative wastewater sample before chlorination.

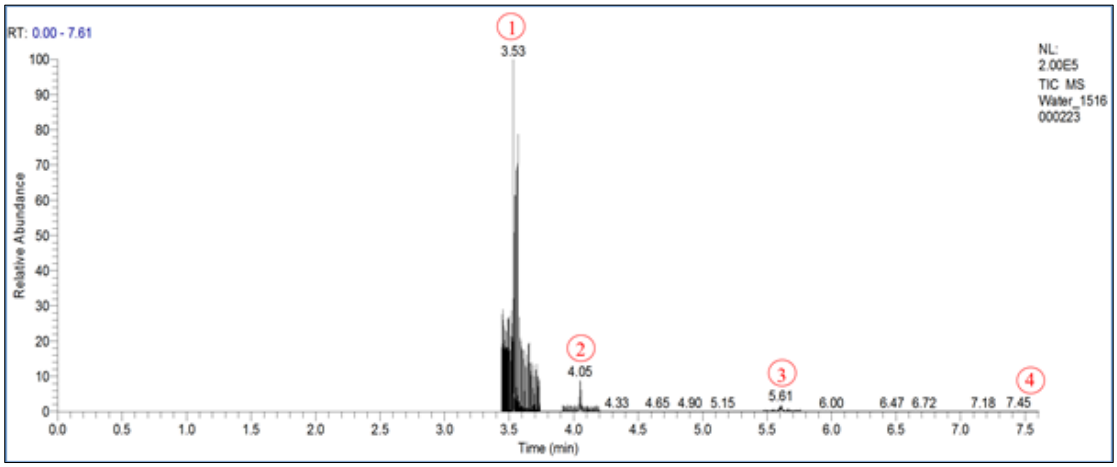


Figure 5.9: GC-MS/MS chromatogram (representing peaks of 1-4 of four analytes) obtained from MTBE extracts prepared from a representative wastewater sample after ozone treatment at 30 mg/L.

In Figures 5.8 and 5.9, the peaks of four analytes have been represented at their corresponding RT. The Table B.2 (Appendix B) represents the RT, area and measured concentration of the four analytes in two samples. From the Table F.2 it may be concluded that the higher concentration of an analyte with an increasing dose is confirmed by higher area of the corresponding analyte peak in chromatograph. Since, the three bromo products have high concentration after ozone treatment, their area is also high when compared to raw sample whereas, the concentration of $CHCl_3$ decreased after ozone treatment, so its area reduced when compared to raw effluent.

Chapter Summary

This chapter focuses on Objectives 4, 7 and 8 of the present research as listed in Chapter 1. It has explained results of effect of ozone disinfection on inactivation of TC and pathogens. The experimental ozone dose obtained for achieving 99% inactivation (1000 CFU/100 mL) of TC is 30 mg/L. From the results it was concluded that *E.coli* was very sensitive to ozone, hence lays doubt on the pertinence of such microbes as indicators for ozone treatment. By contrast, the higher resistance of *Enterobacter* confirms that they can be good candidates for resistant microorganism indicator. A similar trend was followed by TC and pathogens for achieving the WHO standards of reusing wastewater at a similar TOD of 27 mg/L. For the complete inactivation of pathogens from the effluent sample, a TOD of 30 mg/L was required. It was also observed that, ozonized effluent shows a considerable reduction in physicochemical characteristics such as COD, TSS, and turbidity.

The optimum TOD suggested by one factorial design to guarantee an adequate disinfection was 30 mg/L, at which the corresponding minimum COD value was 21mg/L. When effect of ozone disinfection on THMs was studied it was observed that there was a tremendous decrease in $CHCl_3$ concentration after ozone oxidation whereas the concentration of brominated species increased marginally.

Thus, ozone is a highly effective disinfectant requiring lower Ct for TC (30 mg/L) than chlorine. It has a further benefit that no residuals are left after treatment; hence it does not leave any toxic potential on aquatic organisms on disposal of treated sewage. However, due to its relatively higher cost compared to chlorine, we decided to try a hybrid process, to optimize its use. The hybrid disinfection strategy will include chlorination followed by ozone which help to avoid excessive use of chlorine, with a hope to achieve the desire disinfection efficiency with lesser production of THMs using nominal ozone doses.

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Chapter 6

Chapter 6

UV Disinfection of Secondary Treated Effluent

The increased awareness of the many limitations of chemical disinfectants, specifically those of Cl_2 and O_3, has resulted in the selection of UV as an alternative method for disinfection with many attractive features and benefits [1]-[3]. The use of UV radiations as an alternative to other disinfectants has gained widespread use due to increasing demand for an effective, low cost and environmental friendly way with leaving no residuals to disinfect wastewater so that it can be reused for several purposes [3]-[5]. As discussed previously in Chapter 2, the UV disinfection uses high intensity UV rays at a germicidal wavelength of 253.7 nm to destroy microorganisms [6]. The use of UV for disinfection is a fast, efficient, safe and cost effective process [4] [7] [8].

The present study was carried out to investigate the effectiveness of UV radiations on dominant microbial species, TC and on selected pathogens for disinfection of secondary treated effluent. Disinfection was carried out in a column designed for UV exposure as discussed in Chapter 3. The images of SEM analysis indicates the effect of UV dose on cell structure, explaining their inactivation mechanism. It has been reported in the previous studies that, after UV disinfection most bacteria are able to repair damage in their nucleic acids [9] [10]. Hence, an attempt was made to study the effect of subsequent exposure to visible light on reactivation of UV disinfected effluent. At last, the effect of UV radiations on THM formation was also studied by GC-MS/MS.

6.1 Characteristics of Secondary Treated Effluent

The physicochemical characteristics of the secondary treated effluent of from the STP prior to disinfection and their effect on UV disinfection are presented in Table 6.1.

Table 6.1: Characteristics of secondary treated effluent and their effect on UV disinfection

S. No.	***Quality parameters***	***Secondary treated effluent***	***Effect on UV disinfection* [2] [3] [11]**
1	pH	7.7 ± 1.20	At neutral pH inactivation rate is very slow.
2	BOD	15.83 ± 1.50 mg/L	Minor effect, if any. If a large portion of the BOD is humic and/or unsaturated (or conjugated) compounds, then UV transmittance may be diminished.
3	COD	105.35 ± 1.73 mg/L	High degree of interference due to organic matter.
4	Turbidity	41.10 ± 1.50 NTU	Organic matter will absorb UV radiation or shield microbes from UV radiation, resulting in reduced microbial disinfection.
5	TSS	15 ± 5 mg/L	Shielding of embedded bacteria and efficacy of UV radiation to reach targeted microbes.

6.2 UV Disinfection of Secondary Treated Effluent

Three fixed doses of UV radiations were considered to carry out disinfection of secondary treated effluent in order to meet the WHO standards for TC for reusing the wastewater for irrigation purposes [12]. At the same time, the UV dose was also determined for complete removal of pathogens from the effluent.

Table 6.2: Effect of UV on physiochemical characteristics of secondary treated effluent

S. No.	***Quality parameters***	***Secondary treated sample***	***UV disinfected sample***	***% Reduction***
1	pH	7.7 ± 1.10	7.7 ± 1.50	-
2	BOD	15.83 ± 1.50 mg/L	15.14 ± 2.26 mg/L	4.30
3	COD	105.35 ± 1.73 mg/L	92.56 ± 5.10 mg/L	12.14
4	Turbidity	41.10 ± 1.50 NTU	36.00 ± 1.20 NTU	12.40
6	TSS	25.00 ± 5 mg/L	22.18 ± 2.1 mg/L	11.28

From Table 6.2 it was observed that UV disinfection does not showed any changes in the physicochemical characteristics of treated effluent as it showed negligible reduction. Thus, it is concluded that in this process, the UV radiations have higher specificity for disinfection than the chemical processes, where organic matter interferes significantly. A much lower reduction in (BOD and COD) was noticed with UV treatment when compared to chlorination and ozonation. These observations, are supported by Rowe et al. [13] and Zion et al. [14] who reported that UV disinfection does not remove dissolved organic and inorganic particles in the water as it directly attacks on the nucleic acid of microbes.

6.2.1 UV Disinfection Profile for TC Removal

Experiments were carried out at specific UV doses, according to the protocol explained in Chapter 3. The effect of these doses was observed on the five dominant microbial species (*Escherichia coli, Enterobacter, Klebsiella, Serratia/Hafnia,* and *Citrobacter*) and on TC that are considered as the most conservative indicators for disinfection [15] [16].

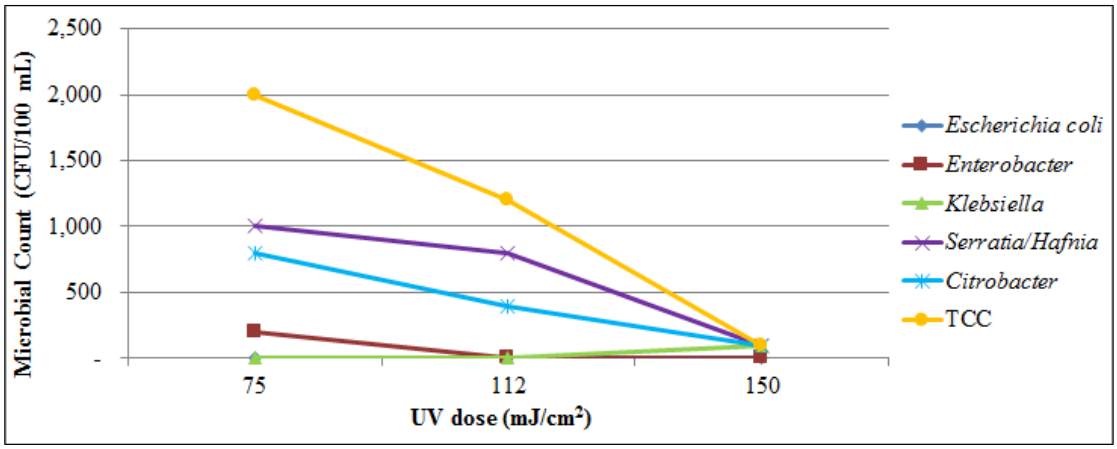


Figure 6.1: Reduction of five dominant coliform species and TC in secondary treated effluent by UV light

Figure 6.1 presents the reduction in the counts of five dominant coliform species and TC in secondary treated effluent with UV irradiations. At a UV dose of 75 mJ/cm^2, among the five dominant bacterial species, *E.coli* was the most sensitive to UV irradiations followed by *Klebsiella* and *Enterobacter.* The counts for *Citrobacter* were comparatively high but were within the norms. The population of *Serratia/Hafnia* were

also relatively high and it was concluded that the dose of 75 mJ/cm^2 was not sufficient to achieve the overall TCC within WHO norms. UV dose was further increased up to 112 mJ/cm^2, where it was observed that *Enterobacter* was eliminated and the, counts of *Serratia/Hafnia* and *Citrobacter* reduced further. But still the TCC was not within the WHO prescribed norms. Hence, a further higher dose of 150 mJ/cm^2 was opted. This increased UV dose, reduced the counts of nearly all species to zero and counts for *Serratia/Hafnia* were near to zero. Results of the present study were also supported by the findings published in literature by Abou et al. who reported that a UV dose of 164 mWs/cm^2 reduced TCC by 3 logs [17]. Yu et al., reported that disinfection efficiency increased relatively slowly with the dose ranging from 75 mJ/cm^2 to 200 mJ/cm^2 [18].

It was observed that UV radiations were very effective in reducing the counts of most of the species including chlorine resistant ones, namely *Enterobacter* and *Serratia/Hafnia*, since UV radiations directly damage DNA and RNA that carry genetic information instead of attacking outer membrane. It exemplifies that, its microbicidal efficiency is not affected by the high lipid content present in the outer membrane of chlorine resistant species as detailed in Chapter 4 [3].

It was further observed from the above results that different microbial species have a wide range of sensitivity to UV radiations. The different sensitivities to UV radiation of bacterial strains are caused by cellular DNA repair systems [19] [20]. In the present study *Serratia/Hafnia* was found to offer slightly high resistance among all five dominant species. Some specific proteins must be turned on to help in repairing the damaged DNA, which may be the reason for some cells of *Serratia/Hafnia* to survive. Zion et al. supported this finding that strains of *Serratia/Hafnia* are more UV resistant due to their active repair systems [14]. In the present study the order of reduction among all the tested microbial species is exactly similar to that reported by Martiny et al. which was *E.coli, Klebsiella, Enterobacter, Citrobacter* and *Serratia* being the most resistant one [21].

The results demonstrated that the disinfection efficiency by UV exposure increased with increasing dose of UV exposure and the required UV dose for microbial log removal in any water sample is influenced by wastewater characteristics [18] [22] [23].

Table 6.3: Effect of different UV doses on TCs in terms of CFU/100 mL in secondary treated effluent

S. No.	***UV dose (mJ/cm^2)***	***TCC in sec. treated effluent***	***TCC in UV treated effluent***	***Standard deviation***	***% Reduction***	***Log reduction***
1	75	110×10^5	20×10^2	37.61	99.98	4
2	112	110×10^5	12×10^2	30.18	99.98	4
3	150	110×10^5	10×10^1	16.32	99.99	5

From Table 6.3 and C.1 (Appendix C) it is observed that at initial UV dose of 75 mJ/cm^2, a 4 log reduction was seen in TCC, but still the counts for TC were not within the standards. The above results are marginally in good agreement with the data published by Nozaic who stated that UV doses between 40 and 70 mJ/cm^2 generally gave 2 to 3 log reductions in indicator organisms [24]. Second phase was carried out at UV dose of 112 mJ/cm^2, where counts reduced further and 4 log reduction was achieved but still the counts were not within the standards. In the last phase a higher UV dose i.e. 150 mJ/cm^2 was adopted to satisfy WHO norms where 5 log reduction was observed in the TCC. These data has been supported by the findings of Sommer et al. who reported that for 6 log reduction, a UV irradiation of up to 300 mJ/cm^2 was required [6]. From the results of the present study it was concluded that at UV dose of 150 mJ/cm^2, up to 99.999% reduction occurred in bacterial counts, which suggests good effectiveness of UV radiation in disinfection process [2]. The pour plate results of inactivation of TCC at different UV doses are represented in Figure C.1 (Appendix C) and colilert results are presented in Figure C.2 (Appendix C), showing removal of microbes in terms of MPN/100 mL.

6.2.2 UV Disinfection Profile for Pathogen Removal

The UV radiations have a greater effectiveness on a wide range of pathogens. Pathogens are more susceptible to UV radiations as compared to chlorine [24]. In the present study the effect of three subsequent UV doses was observed at the same time for few of the common pathogenic species from the same sample, which was tested for TC. The Figure 6.2 represents results for pathogenic counts before and after UV disinfection.

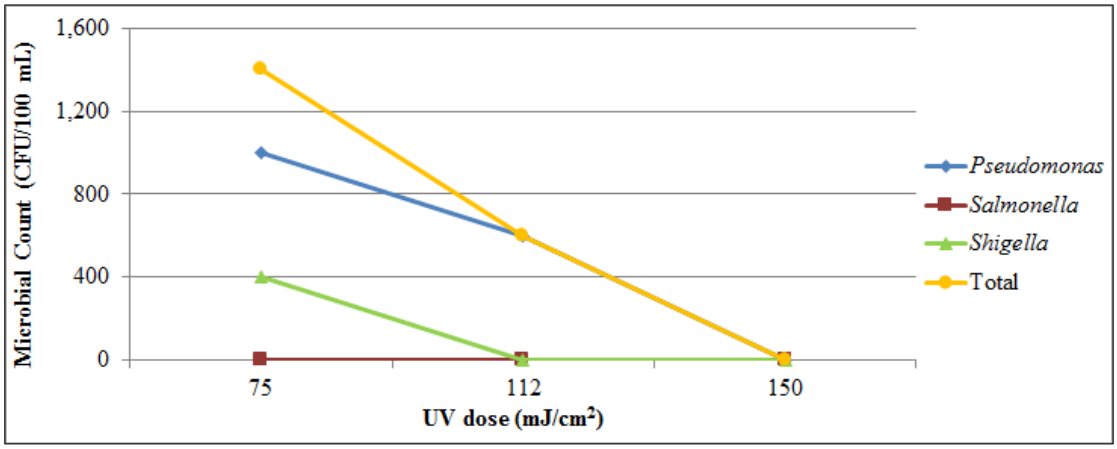


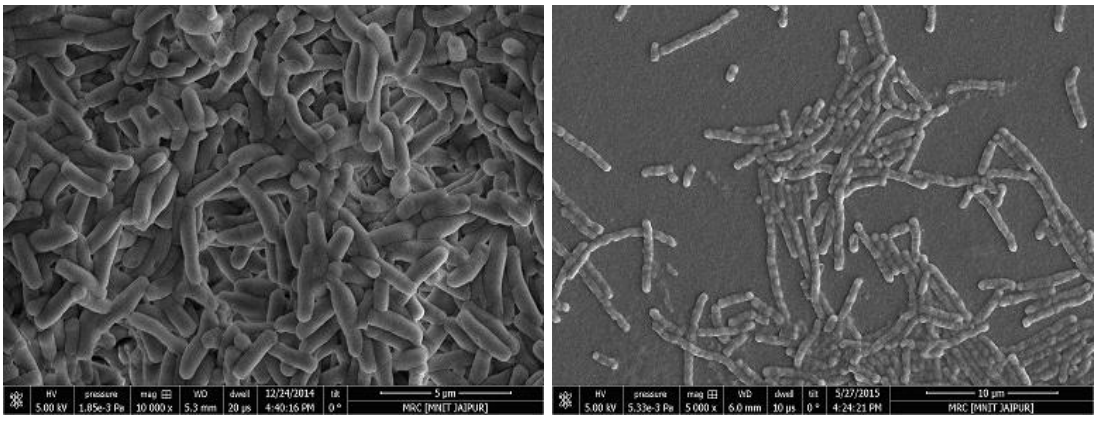
Figure 6.2: Effect of UV light on reduction of pathogens in secondary treated effluent

The results demonstrate that *Salmonella* followed by *Shigella* were sensitive species against UV disinfection. It was also observed that *Pseudomonas* was comparatively more resistant to UV radiations, as its counts were high at UV dose of 75 mJ/cm^2 with minimum counts for *Salmonella*. UV dose of 112 mJ/cm^2 was effective for removal of *Shigella* which was completely eliminated but counts for *Pseudomonas* were still high. At a higher dose of UV radiation i.e. 150 mJ/cm^2, *Pseudomonas* also reduced to zero. Results were supported by the literature findings where it is reported that a dose of 50 mWs/cm^2 was required for 1 to 2 log inactivation of *Pseudomonas* in the secondary treated wastewater, and a much higher UV dose was required to achieve 99.999% reduction [4] [7] [25].

Hence, it was concluded that a UV dose of 150 mJ/cm^2 was sufficient to achieve total removal of pathogens. It was also inferred that disinfection mechanism of UV radiations would require a contact time of the order of few seconds to accomplish pathogen inactivation [2]. The required UV doses for a given pathogen log removal are influenced by wastewater quality [8].

6.3 SEM Analysis

The SEM was used to identify the morphological changes in bacterial cells exposed to UV radiation [26]. The rod shaped morphology of gram negative bacilli (TC) appeared normal, ellipsoid, 1-2 µm along on the anterior-posterior axis with smooth continuous outer membrane in secondary treated effluent before UV disinfection as shown in Figure 6.3 (a).



(a) (b)

Figure 6.3: SEM image of TC (a) before UV disinfection; (b) after UV disinfection 75 mJ/cm^2

The secondary treated effluent when exposed to UV light, it was observed that, the outer membrane of cells was no longer smooth and regular. They became little shrunken, twisted and small holes appeared on the surface as shown in Figure 6.3 (b). With increasing dose, irregularities on the external surface appeared more prominent, particularly at both ends of the rod shaped structure. When compared to chlorination, which kills the bacteria by damaging the cell membrane, UV treatment does not damage the membrane too much but damages bacterial DNA to block replication. Nucleic acids are the major targets in bacteria during UV disinfection [4] [27].

An interesting thing was noticed from SEM photographs that after exposure to UV irradiations microbes formed long filamentous chains by attaching themselves with each other as evident from Figure 6.3 (b), which probably indicates their efforts for survival as a response to UV disinfection. This observation has also been supported by Connor et al. [20], Ganesan [28], Young [29] and Edwards et al. [30] who reported that in adverse conditions *E.coli* or gram negative bacilli, elongate without a significant change in their diameter and are more likely to grow in chains as this response would increase the surface area available for attachment. Hence, rods and filamentous cells have an advantage in environment with sizeable shear, which help them in their survival [29]. Each individual microbe joins together for many different activities including complex communications and decision making [31]. Cell signalling occurs within the attached cells which helps them in their survival during drastic conditions. It has been reported that this chain formation is a special phenomenon of multi cellularity as these

gram negative bacilli like *Serratia marcescens* fall within the Phylum Proteobacteria, which do not form endospores for their survival and form chain like structure [29]. This signal transduction chain can help them to send messages back and forth which is required for their survival [31]. Hence, SEM images were helpful to examine the pattern of damage to the cell membrane caused by UV radiations.

The mechanism of microorganism destruction is that it causes molecular rearrangement in DNA and RNA, which in turn blocks replication [3] [4]. The most common products resulting from damage by UV radiation are thymine dimers, which are formed when two adjacent thymine molecules become fused. The formation of these dimers and other photoproducts prevents the DNA from being able to replicate, effectively killing the cell [20].

6.4 Reactivation of Microorganisms

Exposure to low doses of UV radiations does not lead to the full destruction of bacterial cells as bacteria can be induced into a viable but nonculturable state at low UV doses in which the bacteria are dormant and no longer form colonies, but can later re-vegetate and start dividing again [32]. If the intensity of UV radiation provided for disinfection is not sufficient, bacteria can repair some of their DNA damages through light dependent (photo reactivation) or light independent (dark repair) mechanisms and become more resistant to these treatments. Such kind of reactivation process is a natural defence mechanism of microorganisms for their survival [33].

In this set of experiments, after being exposed to UV disinfection, tertiary treated effluent was passed through visible light column (as described in Chapter 3) to study the effect of visible light in the repair mechanism of cells. The results are shown in Figure 6.4 and 6.5.

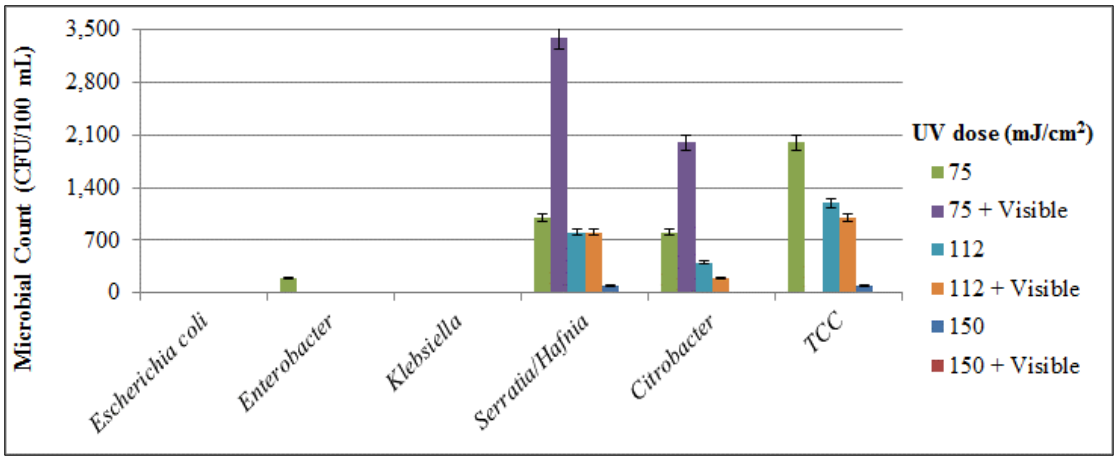


Figure 6.4: Effect of visible light on reactivation of TC

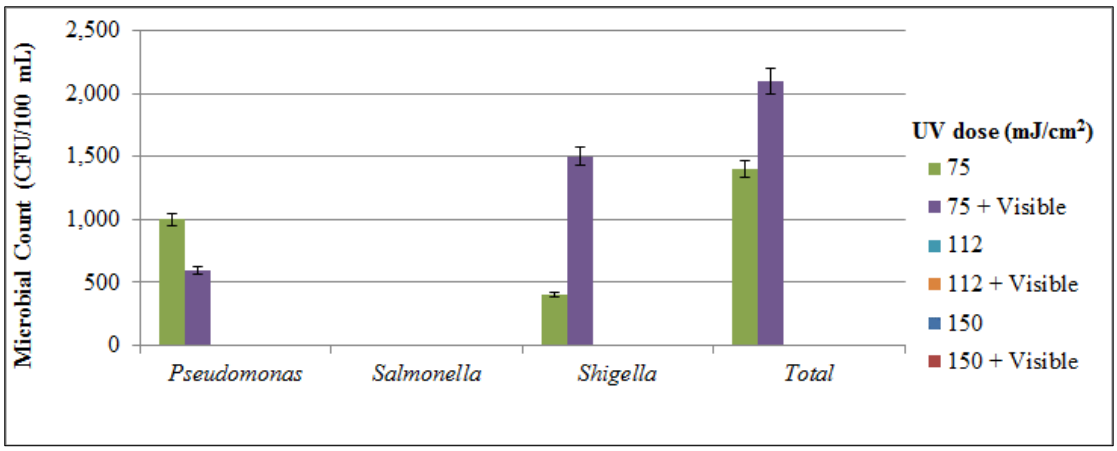


Figure 6.5: Effect of visible light on reactivation of pathogens

From Figure 6.4 and 6.5 it can be observed that when the UV treated effluent at 75 mJ/cm^2 was exposed to the visible light, then this exposure has shown to be a growth enhancing factor for reappearance of certain microbes like *Serratia/Hafnia* and *Citrobacter* among coliforms [9], while among pathogens, the reactivation was observed in *Shigella* [3] [25]. The results of the present research are supported by the findings of Brahmi et al. [8], Zenoff et al. [9], Carson et al. [10], and Zhang et al. [34]. One of the possible reasons behind this phenomenon could be that during UV exposure period at low dose, the members of these species went to dormant or inactive state for some time due to mutation in their genetic material [34]. As soon as they were exposed to visible radiations due to photo reactivation and DNA repair mechanism, microbes were again activated and reproduced as a result of which their counts increased [35]. There might be a possibility that such mechanism occurred because of adaptation of microbes for this situation as in RBC the biofilm attached on shaft keeps on rotating

and shaft is overall covered with just few openings or window. Due to this during rotations, sometimes the attached microbes have to face dark period and sometimes they were exposed to sunlight. Similar situations were available during UV disinfection and visible light exposure and as they were familiar and could manage to survive at low UV doses of 75 mJ/cm^2. It was seen that, an increment in counts by 2.4 times was observed in *Serratia/Hafnia, Citrobacter* increased by 1.5 times, and *Shigella* increased by 2.7 times.

Photoreactivation repair is carried out by an enzyme known as photolyase, which reverses the UV induced damage [25] [36]. This enzyme is first activated by visible light which then repairs damaged DNA. Photolyase binds to the dimer, which is formed due to UV mutation and splits into two normal pyrimidine bases [20] [33]. This enzyme has been found in bacteria. It is considered to be responsible for a significant amount of repair system and allow further replication of them. Though it was clear that dormant bacteria can resuscitate under certain conditions but from the present study it was noticed that not all of the species can do this. On the other hand if damage is too extensive then it simply cannot be tolerated by the microorganisms and will almost self-destruct themselves leading to cytotoxic condition [6]. As in the present study also, no reactivation was observed at high UV dose of 112 and 150 mJ/cm^2.

The outcome of the present study is adequately supported by previous studies, which shows that all UV treated bacteria were not dead. It has been validated in literature by measuring the expression of bacterial genes using reverse transcriptase with quantitative polymerase chain reaction. The results of polymerase chain reaction (PCR) test showed that no significant change in expression of a ribosomal gene occurred. This indicates that most of the UV treated bacteria retained the ability to synthesise proteins and thus were not dead [34] [37].

6.5 Determination of THMs

In the present study THMs refer only to chlorinated and brominated species, i.e. $CHCl_3$, $CHCl_2Br$, $CHClBr_2$ and $CHBr_3$. The concentration of THMs was analysed before and after UV disinfection.

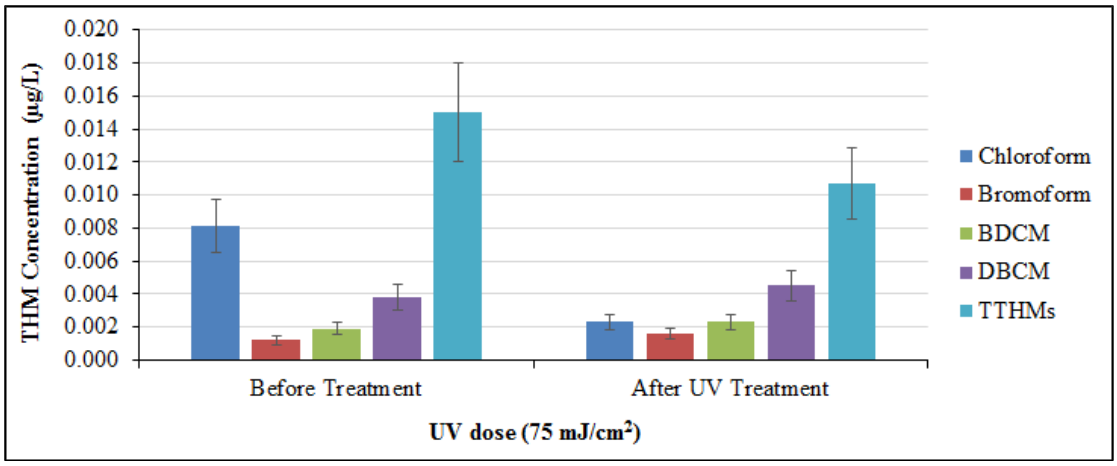


Figure 6.6: Changes in concentration of THM species with UV radiation (RBC)

Figure 6.6 shows that with increasing UV dose the concentration of $CHCl_3$ decreased while there is slight increment in the bromine fraction of halogens among formed THMs. The concentration of $CHCl_3$ reduced by 71% when compared to initial concentration. On the other hand $CHCl_2Br$ increased by 21%, $CHClBr_2$ increased by 18% and $CHBr_3$ increased by 33% when compared to their initial values in secondary treated effluent. One of the possible reason behind this is that UV removes bromine atoms from larger molecules that participate in THM production. UV irradiation is known to photo rearrange organic matter and this can potentially increase the DBP formation. It was also clear from the present study that though there was very less increment in the concentration of brominated species but when we focus on TTHM concentration than it was observed that TTHMs reduced by 28% after UV treatment when compared to the concentration of TTHMs in secondary treated effluent. Validated statistically by applying two tailed t-test at 95% confidence interval where the p value is 0.0124.

These results of the present study are also supported by another study from literature, which states that UV radiation accelerates DBP formation as UV radiations cleave the bonds between larger organic compounds and bromine liberating, bromide and thereby increasing the total amount of TTHMs [39] [40]-[42]. In another study it was reported that UV does not significantly change the chemistry of water as a result of which formation of DBPs with UV is minimal [40].

When concentration of $CHCl_3$ formed in UV disinfection was compared to that of chlorination it was noticed that it was reduced by 97% and reduction in TTHMs was 91%. These results were in agreement with the results of Spiliotopoulou et al. who

concluded that UV radiations do not accelerate formation of DBPs [39]. It further indicated that if UV exposure is serially given after chlorination, some $CHCl_3$ formed during chlorine reactions may get scavenged in the post UV treatment and hence a hybrid disinfection study may be very useful.

A comparison of chromatograms for samples before (Figure 6.7) and after UV radiations (Figure 6.8) is presented below and differences in concentration of THMs are represented by four peaks.

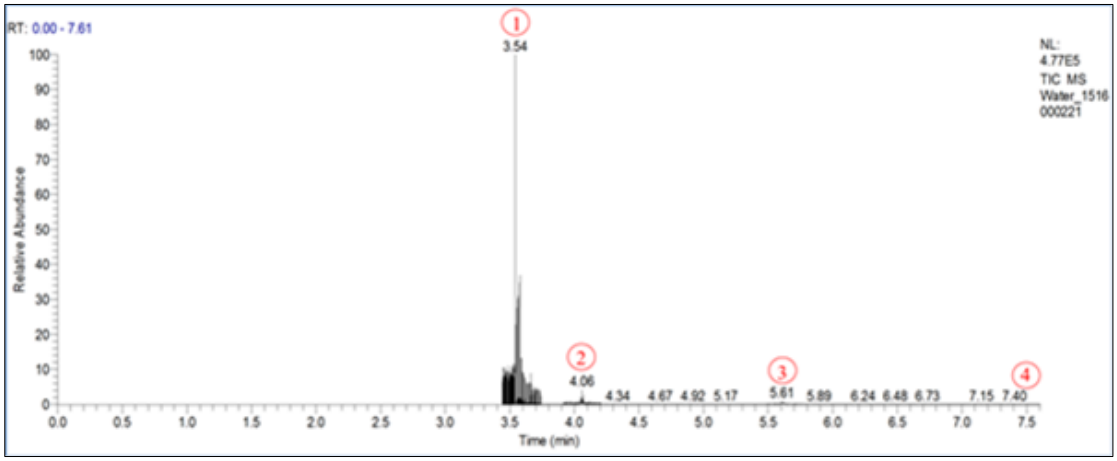


Figure 6.7: GC-MS/MS chromatogram obtained from MTBE extracts prepared from a representative wastewater sample before UV disinfection

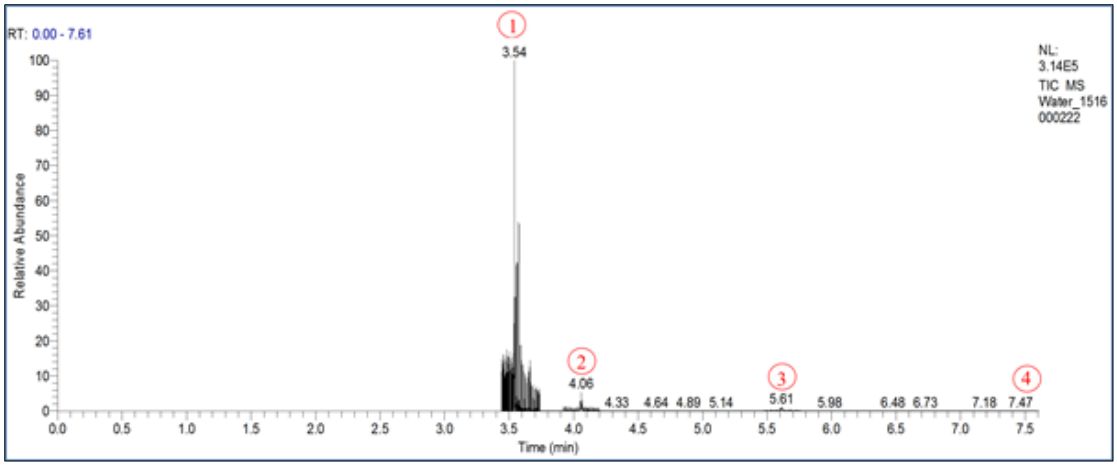


Figure 6.8: GC-MS/MS chromatogram obtained from MTBE extracts prepared from a representative wastewater sample after UV treatment

Table C.2 (Appendix C) presents the RT, area and measured concentration of the four analytes in two samples. It can be concluded from Table G.2 that R.T for the four analytes is same in both the samples, which is a sign of confirmation of an analyte [43]. The area of an analyte peak is directly correlated to its concentration, which means that higher the area of an analyte peak, higher is its concentration.

Chapter Summary

This chapter fulfils the research objective 1, 5, 7 and 8 as listed in Chapter 1. It reports the effect of UV radiations as a disinfectant on inactivation of TCs and pathogens in secondary treated effluent of RBC. It was interpreted from the experimental results that a UV dose of 150 mJ/cm^2 was sufficient for the removal of TCs to meet the WHO standard and for complete removal of pathogenic organisms as a standalone measure.

At UV dose of 75 mJ/cm^2, the reactivation was observed in *Serratia/Hafnia* and *Citrobacter*, among TC while among pathogens, the reactivation was observed in *Pseudomonas* and *Shigella*. On the other hand at UV dose of 150 mJ/cm^2, the reactivation was not seen in microorganisms, since at this dose, complete inactivation of microorganisms occurred. Hence, this dose was sufficient for disinfection. Specific four THMs levels measured after UV treatment were compared with a sample without UV exposure and it was concluded that formation of THMs with UV radiation is minimal. UV disinfection reduced TTHMs was 91%. Though UV treatment accelerates, but does not increase THM formation.

From the results of the present study it was concluded that due to potential health and environmental risk of UV disinfection, a combined disinfection technology should be explored.

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Chapter 7

Chapter 7

Hybrid Disinfection for Secondary Treated Effluent

Chlorination is one of the most widely used methods to disinfect wastewater, despite innumerable objections raised primarily due to the formation of resultant byproducts [1]. In order to reduce the population of coliforms and pathogens in the finished water, a large dose of chlorine needs to be added in the treatment process (as already explained in Chapter 4), a negative effect of which is the formation of DBPs in high concentration many of which have been reported to be carcinogenic especially $CHCl_3$ [2]-[5].

An attempt to reduce the dosage of chlorine can be very useful in lowering the concentration of the DBPs. Other modes of disinfection such as ozonation and UV disinfection also have some drawbacks as discussed in previous chapters. Hence, to overcome these drawbacks different disinfection methods can be optimized in different combinations in order to achieve better disinfection efficacies. There are reports available on hybrid or sequential disinfection involving synergistic benefits for using two or more disinfectants in drinking water treatment, which indicate that the overall inactivation of microorganisms is greater than the sum of the inactivation achieved for each disinfectant individually [6] [7]. Previous studies for drinking water disinfection have evaluated the combination of disinfectants such as UV/Cl_2 and O_3/Cl_2, which primarily addressed the issue of maintaining disinfection capability during travel of water in the distribution system as both UV and O_3 do not leave any residual substances [8]-[11]. For sequential disinfection of wastewater effluents. Hence, little literature could be procured [12] [13].

As discussed in Chapter 1, the novelty of the present research lies in the fact that the species wise analysis for susceptibility to chlorine helped us minimize its dose, as the resistant species would be tackled by the relatively more potent though costlier disinfectants (UV or O_3). It further stresses that the final step of UV or O_3 does not leave any residual and hence this treated sewage would be much less problematic to aquatic life than the Cl_2 treated sewage that has its combined forms, which are toxic to the fish and other aquatic life. A further perceived benefit of the sequential disinfection was the scavenging of some THMs that may add to the overall benefits of the hybrid strategy [14]-[16].

This chapter discusses the results of application of multiple disinfectants in the form of sequential or hybrid disinfection technique to meet the varied requirements for inactivation of microbes, reduction of DBPs and for developing a cost effective process, which can be used for large field scale applications. The results of the first attempt on hybrid disinfection strategy 'A', where a combination of Cl_2 as primary disinfectant and O_3 as secondary disinfectant was included for achieving the WHO [17] standards of 1000 TCC/100 mL for irrigation and agriculture are presented in this chapter. Outcomes of the second attempt of hybrid disinfection strategy 'B' are also discussed, which involved a combination of chlorine as primary disinfectant and UV as secondary disinfectant.

During these experiments, the effect of hybrid disinfection on physicochemical and biological characterization of effluent was recorded. This part of research also includes the results of SEM analysis to demonstrate the physiological effects of hybrid doses on bacterial cells to understand the inactivation mechanism. Another major focus of the study was to analyze effect of hybrid disinfection strategy on THMs by GC-MS/MS.

7.1 Hybrid Disinfection Strategy 'A' using Cl_2 and O_3 for Secondary Treated Effluent

Secondary treated effluent was disinfected using hybrid disinfection technology in which two sequential disinfectants i.e. Cl_2 and O_3 were used. In this strategy optimized CD for removal of susceptible coliform bacteria (2.5 mg/L for 16.83 min or Ct 42.07 mg-min/L) was chosen as primary disinfectant dose followed by different TOD ranging from 6 mg/L to 12 mg/L. Experiments were carried out, in order to obtain minimum ozone dose in hybrid disinfection strategy for achieving the WHO standards [17] and removal of pathogens in the process was also monitored.

The characteristics of secondary treated effluent of RBC and their effect on chlorine, ozone and UV disinfection are already discussed in Chapters 4, 5 and 6. Effect of two hybrid disinfection strategies 'A' and 'B' on physiochemical characteristics of secondary treated effluent of RBC are presented in Table 7.1.

Table 7.1: Effect of hybrid disinfection dose on physiochemical characteristics of secondary treated effluent

S. No.	***Quality parameters***	***Secondary treated sample***	***Hybrid dose 'A' (Cl_2/O_3)***	***% Reduction***	***Hybrid dose 'B' (Cl_2/UV)***	***% Reduction***
1	pH	7.7 ± 1.10	7.6 ± 1.00	-	7.7 ± 1.20	-
2	BOD	15.83 ± 1.50 mg/L	10.41 ± 2.60 mg/L	34.23	12.11 ± 2.64 mg/L	23.49
3	COD	105.35 ± 1.73 mg/L	40.00 ± 3.12 mg/L	61.90	55.33 ± 2.10 mg/L	47.47
4	Turbidity	41.10 ± 1.50 NTU	26.22 ± 1.62 NTU	36.20	30.00 ± 2.04 NTU	27.98
6	TSS	15.00 ± 5.00 mg/L	10.12 ± 2.01 mg/L	32.53	11.01 ± 2.5 mg/L	26.66

From the Table 7.1, it can be observed that hybrid disinfection strategy 'A' with a combination of Cl_2 followed by O_3 was more effective as an oxidant in removing dissolved organics as compared to hybrid disinfection strategy 'B' with a combination of Cl_2 followed by UV radiation. One of the possible reasons behind this could be that Cl_2 and O_3 both are excellent oxidants which work in series producing better organic matter removal. On the other side in strategy 'B', Cl_2 is followed by UV, which has lesser oxidation power compared to the other two disinfectants though it has specificity for deactivating microorganisms. The effect of two hybrid disinfection doses was further studied on five dominant coliform species, TC removal and on removal of three pathogen species.

7.1.1 Hybrid Disinfection for TC Removal

From Figures 7.1 and 7.2, it can be observed that optimized CD (2.5 mg/L for 16.83 min or in terms of Ct 42 mg-min/L) completely removed the chlorine sensitive species such as *E.coli* and *Klebsiella*. The counts for *Citrobacter* were also very low at this stage. Counts for *Enterobacter* were within the WHO standard limit for reuse, but counts for the resistant species i.e. *Serratia/Hafnia* were high resulting in exceedance of standards.

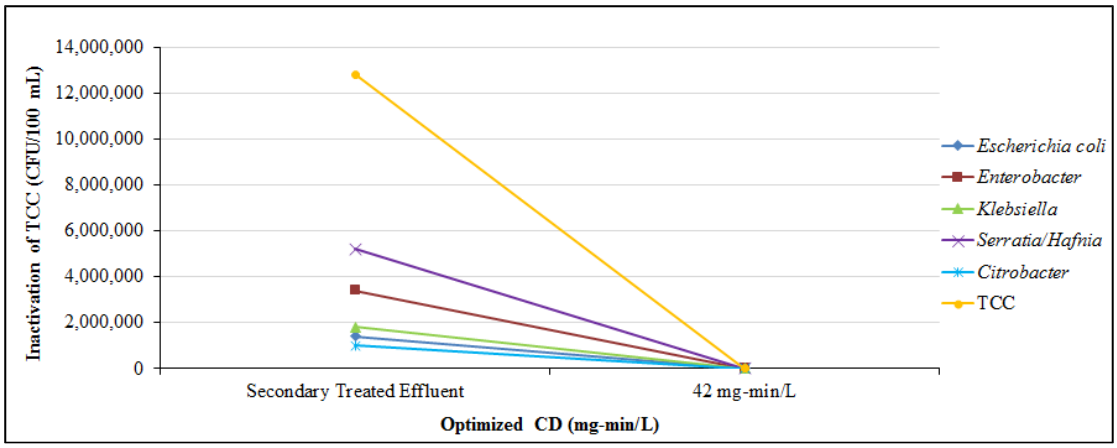


Figure 7.1: Inactivation of TC at optimized CD (42 mg-min/L)

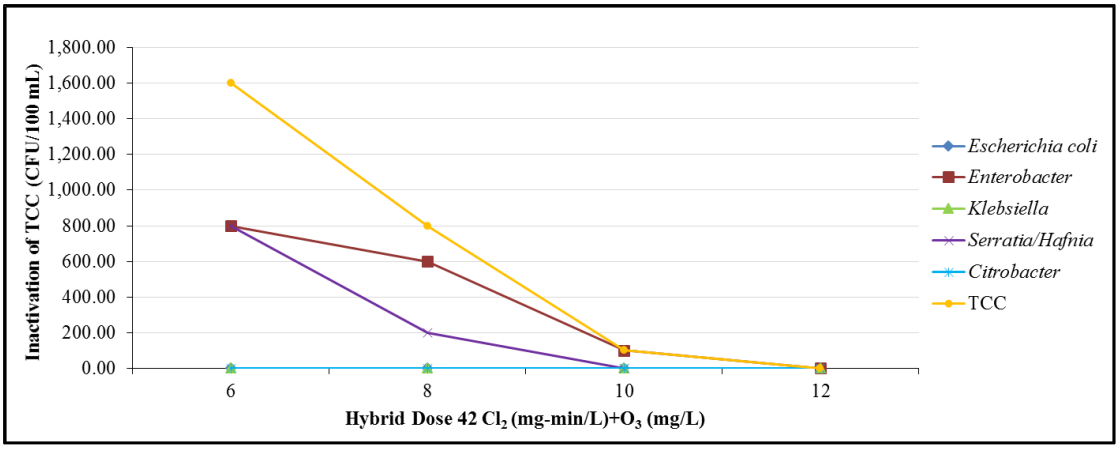


Figure 7.2: Inactivation of TC by hybrid dose Cl_2/O_3

The chlorinated tertiary treated sample was sequentially disinfected with ozone in series to remove the chlorine resistant species and to achieve the disinfection standards. A subsequent low dose of ozone with 6 mg/L further reduced *Citrobacter* to zero. The counts for the two chlorine resistant species (*Enterobacter* and *Serratia/Hafnia*) were also reduced substantially and their individual counts were within the norms though the WHO norms for TCC were not attained. Hence, in the next set of experiments, the optimized CD was kept constant and ozone dose was further increased to 8 mg/L where the TCC reached below 1000 CFU/100 mL. On further increasing the ozone dose up to 10 mg/L, the counts for the most chlorine resistant species *Serratia/Hafnia* also reached zero with very low counts of *Enterobacter*. At 12 mg/L complete inactivation of TC was achieved. But the main focus of present study was to obtain a hybrid disinfection dose to meet the WHO [17] standard, so that the treated water can be reused for irrigation and agriculture practices. Hence, it was concluded that 42 mg-min/L (CD) +

8 mg/L (TOD) as hybrid disinfection dose was sufficient to achieve the required WHO standard for TCC for reusing disinfected wastewater for agriculture purposes.

7.1.2 Hybrid Disinfection for Pathogen Removal

In order to evaluate the disinfection efficiency of hybrid dose on pathogens, species such as *Pseudomonas*, *Salmonella* and *Shigella* were chosen as target microorganisms as discussed in previous chapters. Experiments were carried out, in order to obtain minimum ozone dose in hybrid disinfection strategy for achieving complete removal of pathogens.

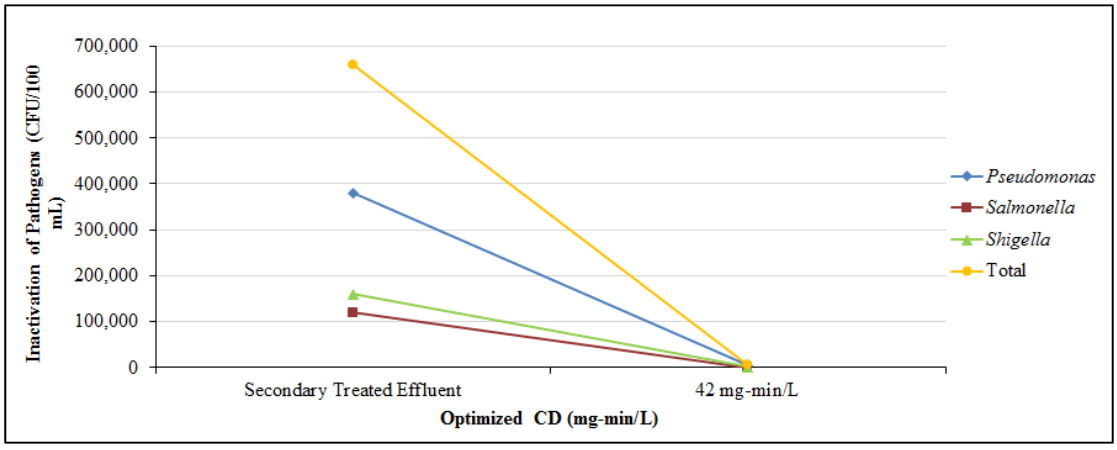


Figure 7.3: Inactivation of Pathogens by optimized CD (42 mg-min/L)

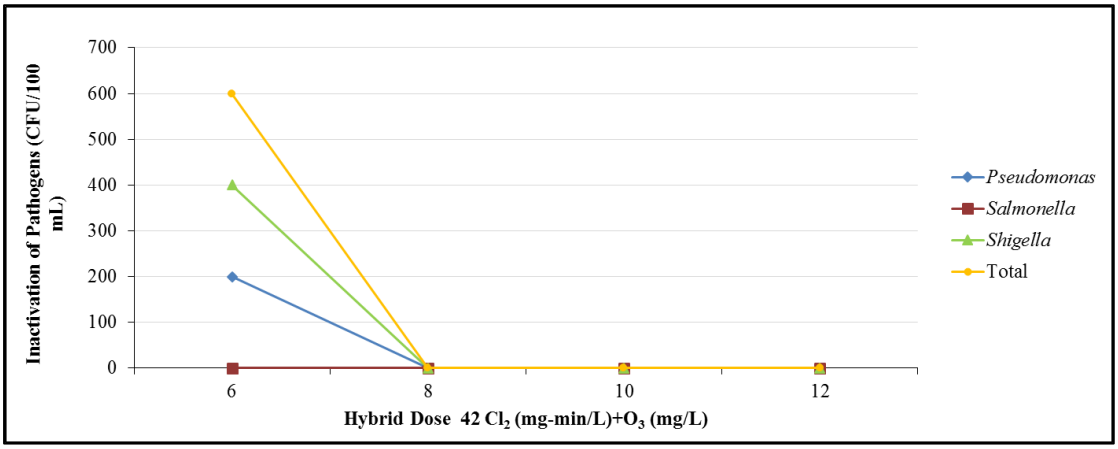


Figure 7.4: Inactivation of Pathogens by hybrid dose Cl_2/O_3

In case of pathogens, at optimized CD as shown in Figures 7.3 and 7.4 out of the three pathogen species considered, counts for *Salmonella* were minimum. The individual counts for *Pseudomonas* and *Shigella* were high due to which the overall

count was also high. As the effect of first set of hybrid disinfection dose (42 mg-min/L CD + 6 mg/L TOD) tremendous reduction in the counts of microbes was observed, where counts for the most ozone sensitive species *Salmonella* reached zero. Total counts for *Pseudomonas* and *Shigella* were within the standards but the goal was to achieve complete removal of the pathogens from treated water, hence ozone dose was increased up to 8 mg/L (42 mg-min/L CD + 8 mg/L TOD) with constant optimized CD. At this stage the counts for the two resistant species *Pseudomonas* and *Shigella* also reached zero. This hybrid dose of 42 mg-min/L (CD) + 8 mg/L (TOD) was able to completely remove pathogens from treated water. These results further indicate that the hybrid dose required to satisfy WHO regulations also provides total inactivation of pathogens. These results were supported by the findings of Souza and Daniel, who reported synergistic effects when low CDs were applied in combination with ozone for *E.coli* disinfection [18].

As has been discussed in Chapter 4 that when disinfection of secondary treated effluent was carried out using chlorine alone, the required high CD to achieve the WHO standard was 4.0 mg/L for 20 minutes (Ct 80 mg/L), whereas the TOD required alone was 30 mg/L. In contrast to this, sequential use of Cl_2 and O_3 in hybrid disinfection strategy produced a synergistic effect and was the most effective option in achieving the norms for TC and for complete inactivation of pathogens as well. These results verified that the synergistic effect of Cl_2 and O_3 increased the inactivation rate of chlorine resistant species and lowered the required CD by 47% when compared to chlorine as a single disinfectant. This in turn would reduce total THM formation also as concentration of THMs is basically dependent on CD and reaction time provided [19]. Thus, the optimization of chlorination process reduced the CD and reaction time both as well as the ozone dose required for disinfection. Ozone dose was reduced by 73% when compared to individual ozone process (30 mg/L), this in turn may help to marginally reduce overall cost of the process.

The results of the present study support our hypothesis that hybrid disinfection was more effective for the reduction of TC and for pathogen removal than the single disinfection. This may be due the fact that the conventional method of disinfection i.e. chlorination removed the major coliform species *(E.coli, Klebsiella, Citrobacter)* and then ozone had the potential to remove chlorine resistant species. The synergistic action can be explained by the mechanism of multiple damage, where two different

disinfectants (Cl_2 and O_3) cause damage to different types of microorganisms by different mechanisms, and therefore, promote a more effective inactivation [13].

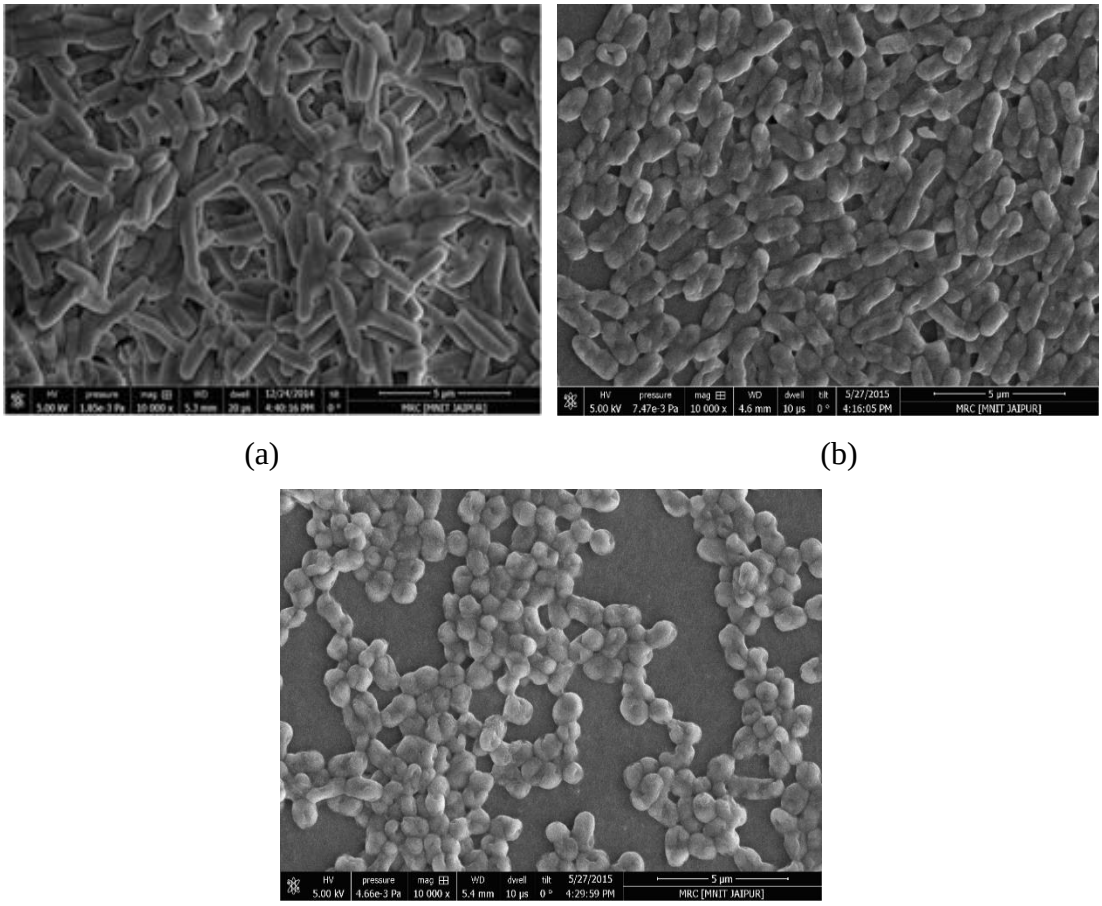
Table 7.2: Effect of different hybrid dose 'A' (Cl_2/O_3) on removal of TC in terms of CFU/100 mL for secondary treated effluent

S. No.	***TCC in sec. treated effluent***	***TCC at optimized CD***	***Hybrid dose Cl_2/O_3 (mg/L)***	***TCC after hybrid dose***	***Standard deviation***	***% Reduction***	***Log reduction***
1	128×10^5	32×10^2	$42Cl_2+6O_3$	16×10^2	32.12	99.98	3
2	128×10^5	32×10^2	$42Cl_2+8O_3$	80×10^1	26.68	99.99	4
3	128×10^5	32×10^2	$42Cl_2+10O_3$	10×10^1	12.00	99.999	5
4	128×10^5	32×10^2	$42Cl_2+12O_3$	<10	0	100	6

Tables 7.2 represent the effect of hybrid dose on removal of TC in terms of CFU/100 mL and table D.1 (Appendix D) represents data in terms of MPN/100 mL. It can be observed that up to initial hybrid dose of 42 Cl_2 (mg-min/L) + 6 O_3 (mg/L), only 3 log reduction was observed. On further increasing the dose up to 42 Cl_2 (mg-min/L) + 8 O_3 (mg/L), 4 log reduction was obtained in TCC. On further increasing the hybrid dose, 5 to 6 log reduction was also obtained. The results of inactivation of TCs by the pour plate method at hybrid dose are shown in Figure D.1 (Appendix D) and colilert results are presented in Figure D.2 (Appendix D), indicating removal of microbes in terms of MPN/100 mL.

7.2 SEM Analysis (Cl_2/O_3)

The surface cell morphology of bacteria was investigated by SEM to study the effect of hybrid disinfection [20]-[23]. The SEM images of coliforms before and after hybrid disinfection treatment are demonstrated in Figures 7.5 (a), (b) and (c), which show the changes in the cell morphology after treatment process and in turn give an idea of disinfection mechanism of disinfectants.



(a) (b)

(c)

Figure 7.5: TC (a) before chlorination, (b) treated with optimized CD 42 mg-min/L, (c) treated with hybrid dose 'A' Cl_2/O_3 42mg-min/L + 8 mg/L

Figure 7.5 (a) presents SEM image of coliforms in secondary treated effluent before disinfection. As already discussed in Chapter 4, 5 and 6 that before treatment bacterial cells remain turgid, swollen with smooth cell surface, which indicates that cells were healthy before they were disinfected. However, after chlorination, the morphology of cells had drastic changes as noticed in Figure 7.5 (b). Chlorine is electronegative disinfectant and therefore oxidises peptide links and denatures proteins present in microbial cell membrane [24]. Hence, when different strains of gram negative bacteria and pathogens were exposed to lethal doses of chlorine, it reduces ATP production [24]. The oxidising effect of chlorine inhibits cellular respiration in microorganisms, leading to cell inactivation [25]. Deep holes can be seen on the cell membrane as illustrated in Figure 7.5 (b). The rod shaped cells were shrunken and showed rough surfaces after chlorine disinfection. Chlorine exposure destroyed the cell wall by altering cells' physical, chemical and biochemical properties [24]. After

chlorine exposure most of the microorganisms were killed except the few resistant ones (*Serratia/Hafnia* and *Enterobacter*).

In the second step of hybrid disinfection strategy as discussed above the tertiary treated sample by chlorine was subsequently treated with low ozone dose for overall synergistic or combined effect on inactivation of chlorine resistant species. As both Cl_2 and O_3 are strong oxidants, it was observed that after complete hybrid treatment, high degree of disorder was observed in bacterial cell morphology. After ozone treatment the remaining bacterial cells shrunk to small size and now no more rod like structure of bacteria was seen as observed from Figure 7.5 (c) [26] [27]. OH^- ions saponify the lipid content in the enveloping membrane, leading to destruction of the superficial structure [24]. Due to damaged cell membrane and small size, they now appeared as cocci with rough surface. It has been reported that ozone beyond the bacterial cell membrane and cell wall, may affect nucleic acid of cells [28]. Hence, the use of two different disinfectants promotes the inactivation of microorganisms and synergistic effects occurred for more resistant microorganisms.

7.3 Determination of THMs (Cl_2/O_3)

The treated effluent by hybrid disinfection was evaluated to further determine the concentration of THMs. Samples with chlorination alone and chlorination followed by ozonation (hybrid disinfection strategy) were compared to determine the THM generation by GC-MS/MS.

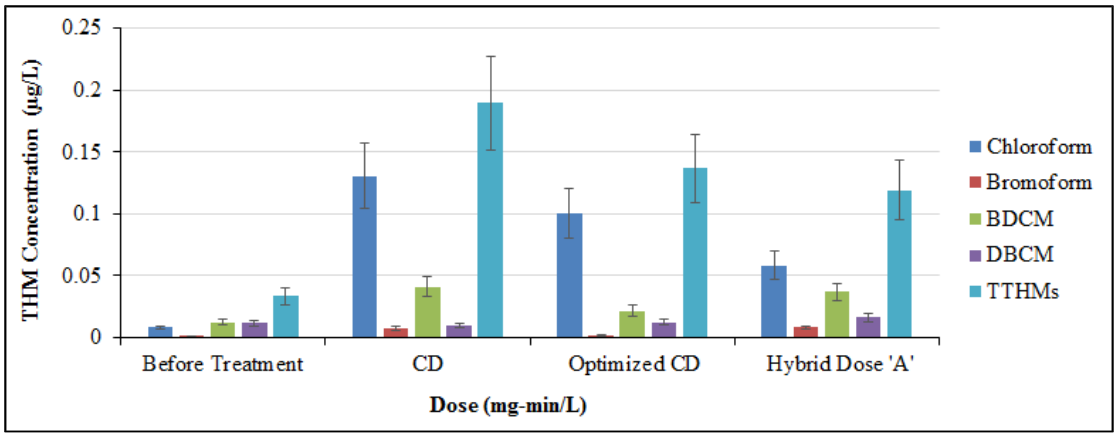


Figure 7.6: Changes in concentration of THM species with hybrid dose (47 mg-min/L CD + 8 mg/L O_3)

It may be noted from the above Figure 7.6 and as also discussed in previous chapters that a distinct benefit was obtained on replacing individual CD of 4 mg/L for 20 min (Ct 80 mg-min/L) with the hybrid process. In the first step of hybrid disinfection i.e. at optimized CD (2.5 mg/L for 16.83 min CD), formation of TTHMs reduced by 27% when compared to chlorination alone at 2.5 mg/L for 20 minutes. After second step of hybrid disinfection i.e. after ozonation there was a sharp reduction in the TTHMs by 37% when compared to chlorination alone. This was primarily due to not only lesser formation of the dominant THM, $CHCl_3$ at optimized CD (23%) but also due to its significant scavenging during the sequential process by 55% when compared to chlorination alone. Validated statistically by applying two tailed t-test at 95% confidence interval where the p value is 0.0092. It was noted that suitable combination of optimized CD followed by minimum ozone dose in hybrid disinfection strategy was superior to chlorination alone in order to control THMs.

It was also observed that the concentration of brominated species remained more or less similar after hybrid disinfection. One possible reason is that due to the presence of slightly high bromide concentration in effluent and as it has been reported in Chapter 5 also that ozone increases the concentration of brominated THMs if Br^- is present in sample due to their fast oxidation [29] [30]. It was observed that the same mechanism of oxidation of organic moiety was observed in all the three disinfection processes where disinfectants break larger organic moieties and release Br^-, which further react and increase concentration of brominated species in disinfected water.

It was further observed in this study that in the secondary treated effluent of RBC the total concentration of three bromo products (75%) is high when compared to $CHCl_3$ (24%) in samples before disinfection. It was also reported that brominated compounds formed a large part of the chlorination products in their study [31]. But overall, hybrid disinfection lowered TTHM concentration due to tremendous reduction in the concentration of the most potent carcinogenic THM i.e. $CHCl_3$, which was reduced by 55% when compared to chlorination alone and also reduced TTHM concentration in hybrid disinfection strategy. It might be possible that if effluent had lesser Br^- concentration, then these three brominated species of THMs would also be reduced after hybrid disinfection strategy. This statement is supported by reports that state that the use of ozone can reduce the formation of halogenated by-products containing low concentration of Br^- [12] [32].

The chromatograms for sample treated with optimizes CD and hybrid dose 'A' are presented in Figures 7.7 and 7.8. It can be observed from Figures 4.18, 4.20 and Table D.2 (Appendix D) that on increasing CD, an increment in the area of subsequent peaks occurred, while at optimum CD (Figure 7.7) the area got reduced, and finally on treatment with hybrid dose 'A' the area got reduced further as presented in Figure 7.8.

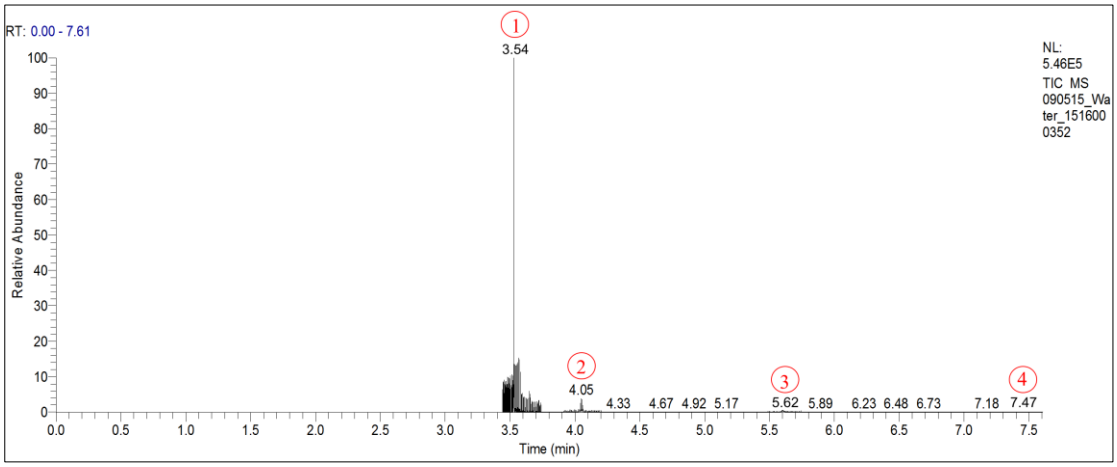


Figure 7.7: GC-MS/MS chromatogram obtained from MTBE extracts prepared from a representative wastewater sample after optimized CD

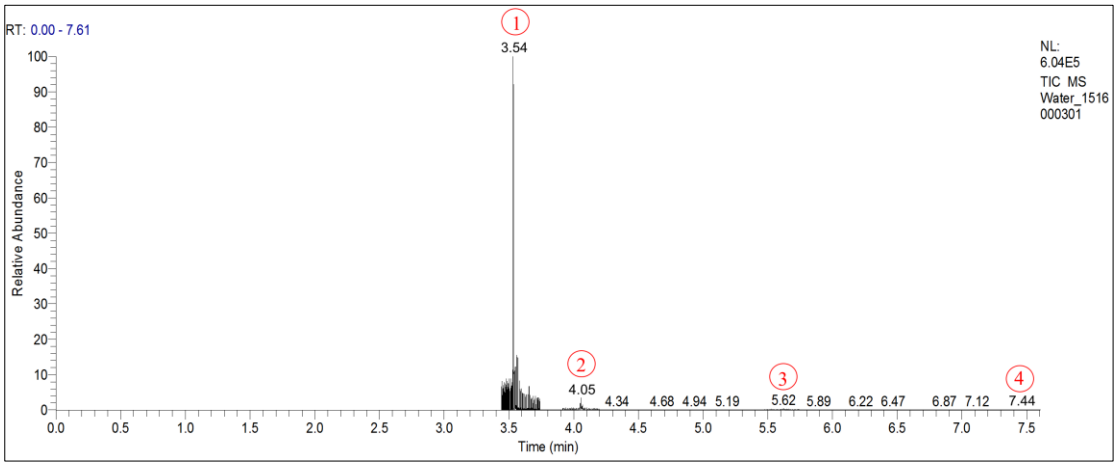


Figure 7.8: GC-MS/MS chromatogram obtained from MTBE extracts prepared from a representative wastewater sample after hybrid treatment 'A'

From Table H.2, it is concluded that the higher concentration of an analyte is confirmed by higher area of the corresponding analyte peak in chromatograph. It was noticed that in all the tested samples, the $CHCl_3$ concentration and total THM concentration did not exceed the maximum permissible value (100 µg/L) [31].

7.4 Hybrid Disinfection Strategy 'B' using Cl_2 and UV for Secondary Treated Effluent

A combination of Cl_2 followed by UV radiation might be another effective way to reduce the use of high CD, which in turn may reduce THMs and even can modify the overall economy of the process. In this strategy the samples were first treated with optimised CD and then further subjected to UV radiations (UV-C) disinfection. Chlorination at a relatively low CD as the first step was carried out to remove bulk of the coliform population susceptible to it, followed by another process i.e. UV radiations with which chlorine resistant species i.e. *Serratia/Hafnia* were removed. As it has been reported that UV radiation is efficient in inactivating resistant forms of bacteria due to its direct attack on nucleic acid, it was perceived that post chlorination UV treatment would require lower dose of disinfectant against the remaining chlorine resistant bacteria [28] [33]. In this hybrid disinfection strategy an optimized CD 2.5 mg/L for 16.83 min (Ct 42 mg-min/L) was adopted as a primary disinfectant dose followed by a low UV dose of 75 mJ/cm^2.

7.4.1 Hybrid Disinfection for TC Removal

The effect of the hybrid dose was observed on the five dominant microbial species and TCs. The results of hybrid disinfection strategy 'B' are represented in Figure 7.9.

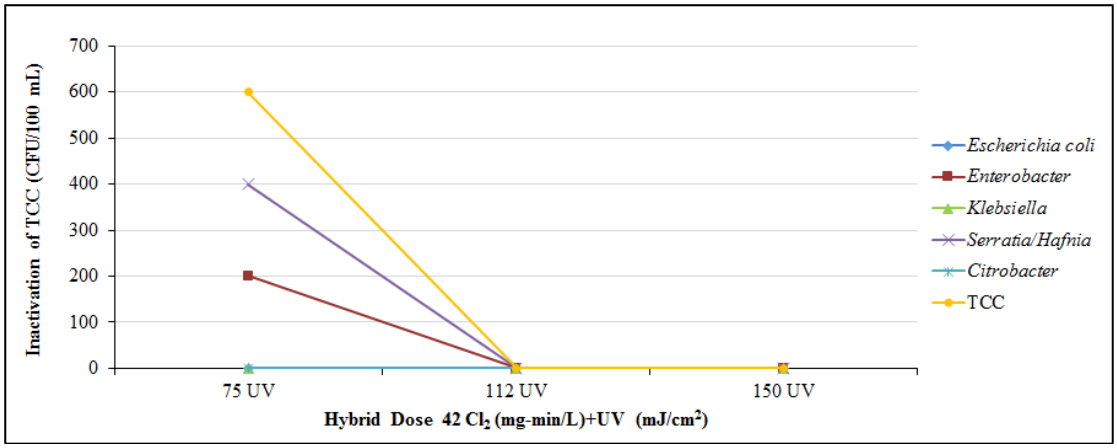


Figure 7.9: Inactivation of TC by hybrid dose Cl_2/UV

The chlorinated sample was then passed through UV chamber for exposure to UV radiations at a constant dose of 75 mJ/cm^2. It was observed that UV exposure was very

effective in reducing the counts of the species, which were resistant to chlorination in our earlier experiments [13]. The results of microbial analysis of final effluent after hybrid disinfection as shown in Figure 7.9, indicate that counts for *E.coli* and *Klebsiella* were reduced to zero only at optimized CD. After UV treatment, *Citrobacter*, also reduced to zero and the counts for *Enterobacter* and *Serratia/Hafnia* were low. This in turn reduced the TCC to the desired WHO norms for reusing the treated wastewater. Hence, it was concluded that chlorine first acts on the outer membrane of microbes resulting in an increase in its cell permeability which was followed by plasma membrane disintegration and cytoplasm damage due to its excessive oxidant nature, allowing UV in penetration inside these weaker microbes.

The results of the present study are supported by the findings of Caretti and Lubello [41] who reported synergistic benefits of PAA/UV radiations in the disinfection of total coliforms, *E.coli* and *Pseudomonas* in wastewater. Similar results were reported by Cho et al. [42], Gil Grozes [11], Finch et al. [9], Sobsey et al. [43], Cecilia and Claudio [44], Jang et al. [15], and Beber et al. [45]. Wang et al. [12] reported sequential disinfection strategy for TC removal using UV/Cl_2. The most recent study on sequential disinfection was carried out by Medeiros et al. [13] who studied synergy of Cl_2/UV radiation for the inactivation of protozoa and indicator microorganisms in wastewater.

7.4.2 Hybrid Disinfection for Pathogen Removal

Experiments were carried out to understand the efficiency of hybrid disinfection strategy 'B' i.e. using Cl_2 and UV on different pathogenic bacteria. The experiments were continued till the complete removal of pathogens was achieved.

From the Figure 7.4, it is observed that optimised CD was able to reduce the counts of most sensitive pathogen species to zero. The counts of total pathogens was high after optimized CD especially for *Pseudomonas* and *Shigella.* But when this chlorinated water was immediately exposed to specific dose of UV radiation (75 mJ/cm^2), it was observed that the counts for all the three pathogens dropped down to a very low level as shown in Figure 7.10.

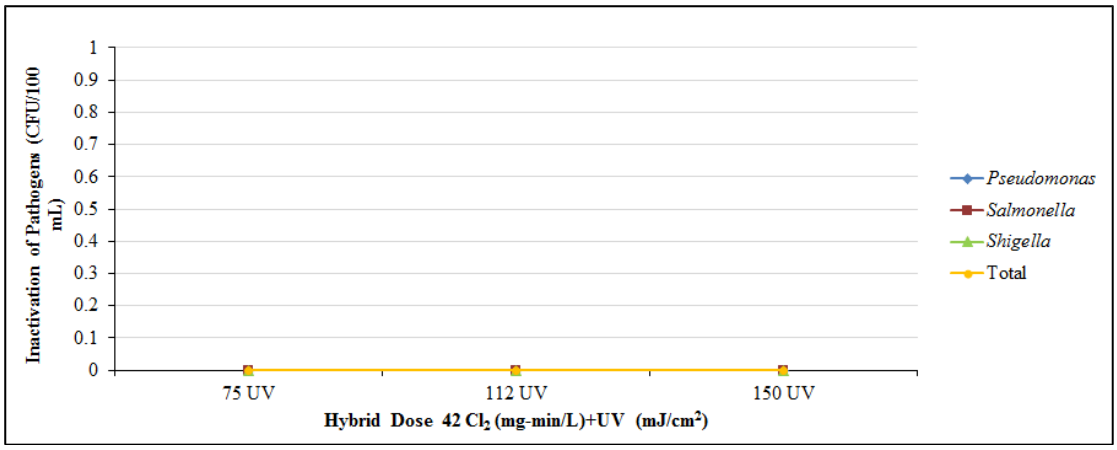


Figure 7.10: Inactivation of pathogens by hybrid dose Cl_2/UV

The hybrid strategy 'B' reduces the use of CD approximately by half (47%) as instead of 4.0 mg/L for 20 min (Ct 80 mg-min/L), the optimized dose used was 2.5 mg/L for 16.63 min (Ct 42 mg-min/L). This in turn reduces the negative effect of using too much chlorine in the formation of DBPs. In the same way UV dose was reduced from a standalone requirement of 150 mJ/cm^2 to 75 mJ/cm^2 (50% reduction). The exposure of secondary treated effluent to the hybrid dose was capable of reducing TCC up to 99.999% i.e. 5 log reduction as shown in Tables 7.3 and H.3. Hence, it was concluded that hybrid or combined disinfection technology is an effective advanced disinfection strategy, which reduces the use of high CD and effectively eliminates most microorganisms in treated water [46]. A combination of disinfectants such as Cl_2 and UV radiations produced greater inactivation when the disinfectants were added in a series rather than individually [41] [47] [48]. This can be explained by the fact that chlorination as the primary disinfectant increases the permeability of the outer membrane, leaving the remaining resistant bacterium vulnerable to destruction by UV radiations [49].

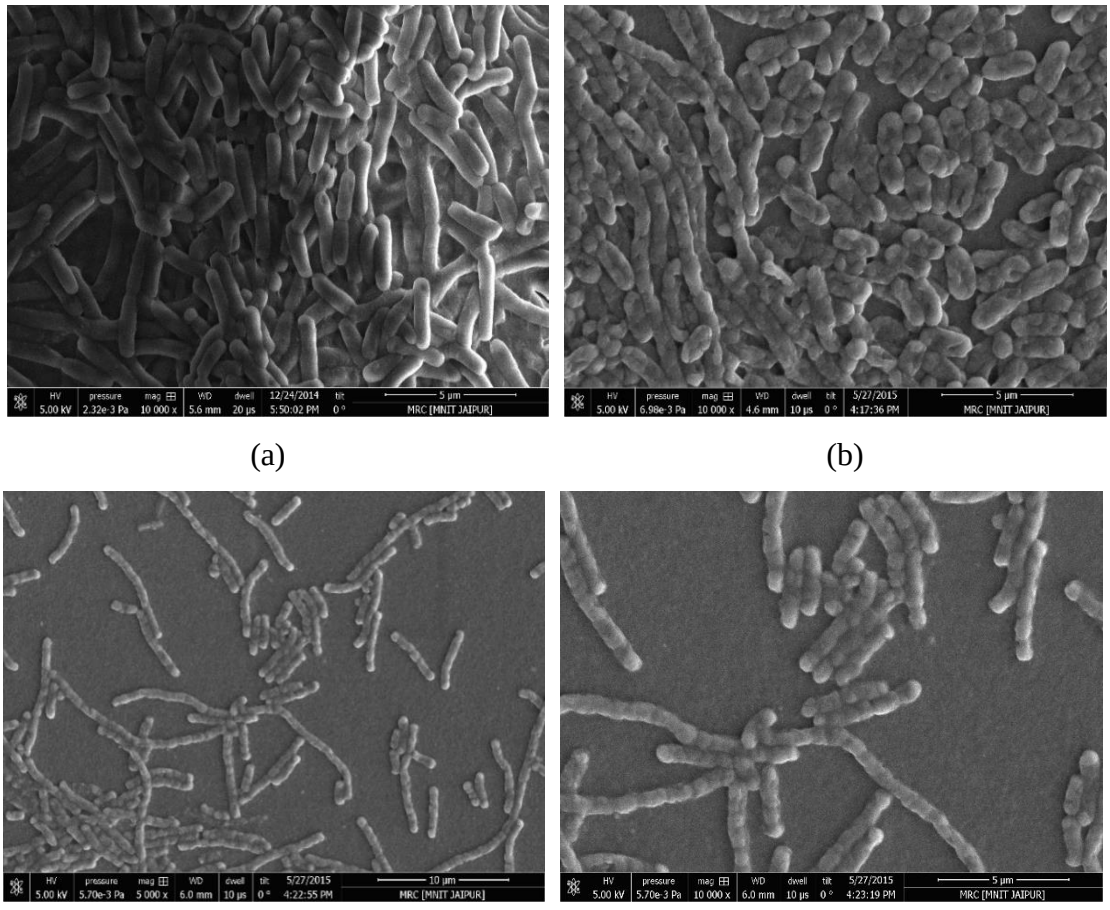
Table 7.3: Effect of hybrid dose 'B' (Cl_2/UV) on TC in terms of CFU/100 mL in secondary treated effluent

S. No.	***TCC in sec. treated effluent***	***TCC at optimized CD***	***Hybrid dose***	***TCC after hybrid dose***	***Standard deviation***	***% Reduction***	***Log reduction***
1	128 x 10^5	32 x 10^2	42 mg/L + 75 mJ/cm^2	40 x 10^1	22.03	99.999	5

The effect of hybrid disinfection dose on removal of TCs in terms of CFU/100 mL and MPN/100 mL is presented in Tables 7.3 and D.3 (Appendix D). It can be observed that at hybrid dose of 42 mg-min/L + 75 mJ/cm^2, 5 log reduction was observed in TCC. Hence, there was no need of further increasing the dose as the desired requirement of TCC prescribed by WHO norms for reusing treated wastewater was fulfilled.

7.5 SEM Analysis (Cl_2/UV)

The samples were analyzed with SEM before and after hybrid disinfection treatment to observe the total cell damage caused by two disinfectants in hybrid disinfection strategy.



(a) (b)

(c) (d)

Figure 7.11: TC (a) before treatment, (b) treated with optimized CD 42 mg-min/L, (c) treated with hybrid dose 'B' Cl_2/UV 42 mg-min/L + 75 mJ/cm^2 and (d) close view after hybrid disinfection

The destructive or lethal effect of two disinfectants i.e. Cl_2 and UV used in hybrid disinfection mode is clear from the above SEM images. It can be seen from Figures 7.11 (a) and (b) that chlorine damaged the external membrane of the microorganisms. The smooth and swollen appearance of bacteria in secondary treated effluent as shown in Figure 7.11 (a) changed to rough and flaccid one after chlorination as depicted from Figure 7.11 (b). Following chlorination, final inactivation in microbes was achieved by UV radiation, which directly targets purines and pyrimidine bases in nucleic acid [38]. At this stage inactivation of microbes by UV radiation was comparatively fast and more efficient as microbes were already damaged by chlorine in first step [13]. It was observed from Figure 7.11 (c) and (d), that few resistant microbial species that survived after chlorination tried to join together and form long filaments. A possible explanation of this phenomenon may be the effort shown by bacteria for their survival during drastic conditions, as chain formation would help them in single transduction and in transferring messages [50]-[52]. This phenomenon is already explained in Chapter 6 though it needs further examination.

Reactivation studies of samples after hybrid disinfection showed negligible regrowth of microbial species. This proves that hybrid disinfection strategy was more effective in destroying microbes effectively when compared to stand alone disinfectants.

7.6 Determination of THMs (Cl_2/UV)

The treated effluent produced by hybrid disinfection strategy 'B' and samples after chlorination were analysed to determine their THM concentrations using GC-MS/MS.

Figure 7.12 represents effect of hybrid dose on formation of THMs. In this hybrid disinfection strategy 'B', the first step i.e. optimized CD (42 mg-min/L), reduced TTHMs by 27% when compared to chlorination alone for desired level of disinfection. In the second step, it was analysed that TTHM concentration after hybrid strategy 'B' reduced by 44% when compared to chlorination alone. In this strategy similar to above one at optimised CD lesser formation of the dominant THM, $CHCl_3$ (23%), occurred followed by its significant scavenging (49%) due to UV radiations. Thus hybrid disinfection strategy 'B' of using chlorination followed by UV radiations was also found to be superior to chlorination alone in order to reduce TTHMs.

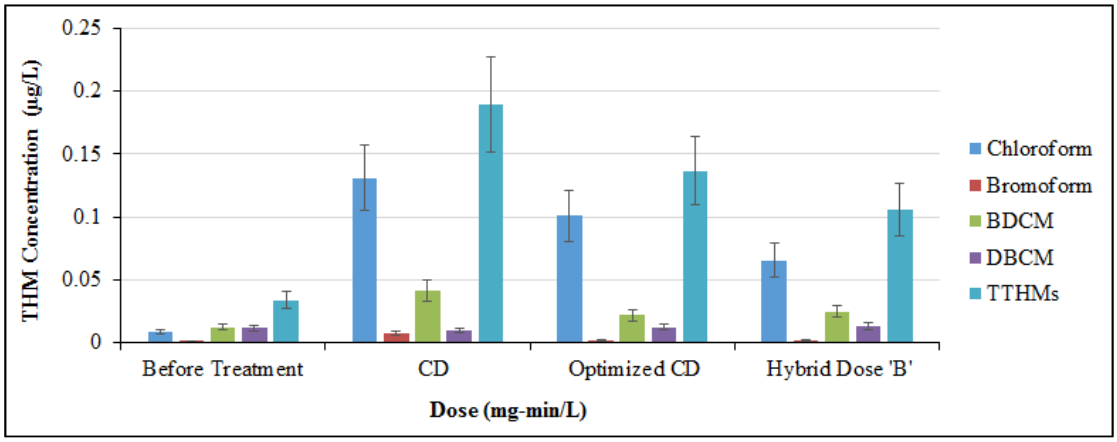


Figure 7.12: Changes in concentration of THM species with hybrid dose (42 mg-min/L + 75 mJ/cm^2)

It was assumed that due to the presence of slightly high bromide concentration in the effluent, the concentration of THMs having bromo compounds increased negligibly but reduction in $CHCl_3$ was about 49%, which in turn reduced TTHM concentration in hybrid disinfection strategy 'B'. This is because UV removes Br atoms from larger molecules that participate in THM production. UV radiations helps to cleave the bonds between larger organic compounds and bromine, so that bromide was liberated. This occurs because C-Br bond is weaker than the C-Cl bond [53] [54]. Hence, Br is transferred from larger brominated molecules to smaller volatile compounds like BDCM, DBCM and $CHBr_3$ due to which their concentration increases [53]. It might be possible that if effluent had lower bromide concentration, then these three brominated species of THMs would also be reduced after hybrid disinfection. Validated statistically by applying two tailed t-test at 95% confidence interval where the p value is 0.0103.

A comparison of chromatograms for untreated (Figure 4.18) and chlorinated (Figure 4.20) sample is presented in Chapter 4, which shows that increase in concentration of an analyte as represented by increment in the area of peak. Whereas, at optimized CD (Figure 7.7) and hybrid dose 'B' (Figure 7.13) the area of analytes peak reduced with decrease in their concentration.

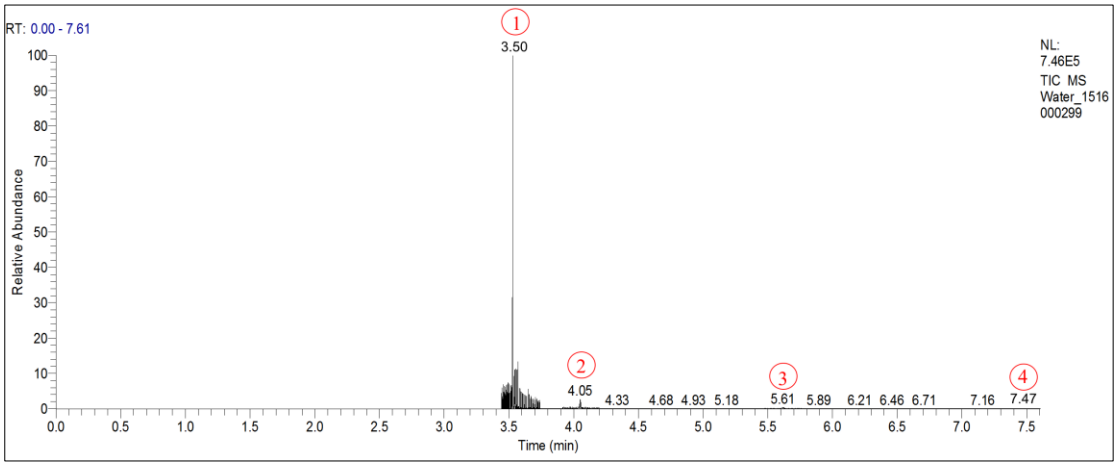


Figure 7.13: GC-MS/MS chromatogram obtained from MTBE extracts prepared from a representative wastewater sample after hybrid treatment 'B'

From Table D.4 (Appendix D), it can be concluded that the higher concentration of an analyte with increasing dose is confirmed by higher area of the corresponding analyte peak in chromatograph [31].

As it was also observed in Chapter 4, that when the CD was increased to 80 mg-min/L for controlling chlorine- resistant pathogenic microorganisms during the disinfection process, the risk of the formation of DBPs, such as THMs also increased. Thus, the present hybrid disinfectant strategies can not only result in reduced formation of THMs and scavenge $CHCl_3$, these can simultaneously ensure a better removal of pathogens due to synergistic action of a combinations of disinfectants. There are benefits from 2 or more disinfectants in dealing with a range of different types of microbes of different sensitivities to disinfection. For example, in the present study chlorine was effective for *E.coli*, *Klebsiella* and *Citrobacter* whereas ozone and UV were more effective for *Enterobacter* and *Serratia/Hafnia*, which were resistant to low doses of chlorine.

Thus, hybrid systems demonstrated distinct potential advantages compared to standalone systems such as better overall treatment efficiency with reduced DBPs. The synergistic effect of sequential hybrid disinfection on microorganisms has been confirmed by number of previous studies, which used combinations like Cl_2/Cl_2, O_3/Cl_2, UV/Cl_2 etc. for drinking water treatment [11] [12] [15], but the present combinations of disinfectants (reverse series) were not studied for sewage disinfection in details. Accordingly, the use of alternative oxidation/disinfection systems should be evaluated as possible alternative to chlorine. The main advantage of the hybrid

disinfection technology is that it produces fewer TTHMs than those by chlorine alone [55], which can be important for long term ecological management of water bodies receiving such wastewaters. On the other hand in order to optimize the efficiencies of individual disinfection treatments and modifying the economy of the overall disinfection process, hybrid treatments can offer an attractive alternative to the current conventional strategies. A comparative analysis of hybrid disinfection strategy with the three conventional disinfection processes has been compared and represented in the next chapter in Table 8.1.

7.7 Cost Analysis

A preliminary cost analysis (for a 200 MLD wastewater treatment plant), for all five disinfection processes, considered in the present study i.e. chlorination, ozonation, UV radiation, hybrid strategy 'A' (Cl_2/O_3) and 'B' (Cl_2/UV), is presented here. The unit costs were obtained through linear extrapolation from a report on wastewater treatment plant of capacity 196.84 MLD [56].

The different processes of disinfection were investigated in terms of their capital, operational and maintenance (O and M), and overall capitalized cost. The annualized costs were normalized to the volume of treated effluent that can be produced during 20 years of operation and at 10% interest rate [57]. It was assumed that 50% of the calculated cost is fixed as there will not be significant changes in civil works. Rest 50% of the cost was assumed to vary linearly with respect to doses, contact time, due to certain variable factors such as electricity consumption, chemicals used and variation in wear and tear of instruments [11] [58]. Future value (FV) and present value (PV) for all the processes were calculated using Equation 7.1 and 7.2.

$$\mathrm{FV} = \mathrm{A}\left(\frac{(1+\mathrm{i})^{\mathrm{n}}-1}{\mathrm{i}}\right) \tag{7.1}$$

$$\mathrm{PV} = \mathrm{A}\left(\frac{(1+\mathrm{i})^{\mathrm{n}}-1}{\mathrm{i}(1+i)^{n}}\right) \tag{7.2}$$

Where, annuity (A) = yearly operating and maintenance cost; rate of interest (i) = 10%; duration (n) = 20 years

Capitalized cost for all the processes was calculated by adding capital cost and PV for each process. It is important to mention that cost of dechlorination was added in the capitalized cost of chlorination process by multiplying the cost of chlorination with factor 1.4, assuming that dechlorination costs additional 40% of the cost of chlorination as has been mentioned in literature [58] [59]. Cost calculations are presented in

Appendix E. The capital, operating, and total capitalized costs are compared for the different disinfection processes under investigation, which are represented in Table 7.4. These results have been obtained for the Ct doses needed to eliminate 99.99% TC.

Table 7.4: Comparison of cost estimate for the investigated disinfection technologies

S. No.	***Disinfectant***	***Optimum dose***	***Unit***	***Capital cost ($)***	***O and M cost ($)***	***Capitalized cost ($)***
1	Chlorine	80	mg-min/L	2,435,242	586,372	10,398,311
2	Ozone	30	mg/L	38,392,408	1,192,739	48,546,870
3	UV	150	mJ/cm^2	43,182,060	1,117,653	52,697,273
4	Cl_2/O_3	42/8	mg-min/L and mg/L	32,608,924	1,487,163	45,269,982
5	Cl_2/UV	42/75	mg-min/L and mJ/cm^2	32,488,082	1,326,688	43,782,928

Economic analysis of each disinfection process as represented in Table 7.4 reveals that chlorination is much more economical than ozone and UV radiation. However, it has been reported in previous studies that residual chlorine, even at lower concentration is toxic to aquatic life, certain chlorine resistant species required high CDs, which results into formation carcinogenic DBPs in high concentrations; furthermore, all forms of chlorine are highly corrosive and toxic thus pose safety risks [59]. On the other side, UV and ozone systems are relatively expensive compared to chlorination systems and another major disadvantage of UV disinfection is photo reactivation of certain microbial species [38] [60]. Hence, we experimented with two hybrid disinfection strategies to optimize the overall disinfection technology for wastewater.

From Table 7.4, it can be noticed that, the disinfection cost of two hybrid disinfection technologies (A and B), using low CD followed by low dose of O_3/UV have much lesser cost when compared to individual process of O_3 and UV though slightly higher than that of chlorination alone. But due to immense other advantages mentioned above especially to reduce the risk associated with THMs and concerns of chlorine residuals on ecology may offer attractive alternatives to the existing disinfection approach.

It is important to mention that the cost estimates presented here may not be very realistic as the reference from which unit costs have been derived assumes a very high contact time (106 min) for chlorination, whereas a contact time of 20 minutes is widely practiced in field. Besides many assumptions in arriving at the total costs have been applied uniformly to all the processes, which may not hold true as cost of every treatment process will vary depending on different field parameters.

Chapter Summary

This chapter addresses research objectives 6, 7, and 8. It concludes that the adoption of hybrid disinfection processes in reverse sequence (a two-step treatment) for wastewater treatment can result in significant reduction in doses of disinfectants, decrease in the formation of DBPs, and a higher inactivation level of microorganisms including the most resistant types. The first step is intended to remove all major susceptible coliform species using chlorination followed by a serial step of another disinfectant that has the potential to remove chlorine resistant species at relatively low doses to optimize the overall disinfection process.

It was observed from the experimental data that a 4 log reduction in TCC was achieved where reduction in CD was 47% and TTHMs concentration was reduced by 37% in hybrid disinfection strategy 'A' (Cl_2 /O_3) when compared to chlorination process alone. At the same time reduction in ozone dose was 73% when compared to alone ozone dose. Hybrid disinfection strategy 'B' (Cl_2 /UV) reduced TCC by 5 log where reduction in CD was 47% and UV dose was reduced by 50% when compared to their stand-alone doses. On the other side, reduction in TTHMs after hybrid disinfection strategy 'B' was approximately 44%. Economic analysis of all disinfection processes revealed that ozonation and UV treatment costs are much higher compared to chlorine disinfection. While the two hybrid disinfection strategies 'A' and 'B' are relatively much cheaper than the two costly standalone processes (O_3 and UV), they are marginally costlier than chlorine but with lot of additional benefits. Hence hybrid disinfection strategies are recommended for disinfection of wastewater.

Thus by adopting a new hybrid disinfection strategy, we succeeded in achieving the main objective of the present study that can help mitigate serious environmental consequences associated with current practices of sewage chlorination and reduce the overall cost of disinfection despite using a costlier disinfectant in series, which is intended to remove only chlorine resistant coliform species with the additional benefit of lesser DBP formation.

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Chapter 8

Chapter 8

Conclusions

The disinfection of secondary treated effluent from STP located in MNIT Jaipur, based on RBC process was carried out using three different widely used disinfectants, namely, Cl_2, O_3, and UV radiations. It was concluded from the experimental results that all the three conventional disinfection processes when used individually have certain limitations, which cannot be neglected. To address these issues two optimization strategies for disinfection process were designed using combinations of disinfectants Cl_2/O_3 and Cl_2/UV, which could lead to higher inactivation efficiency for coliforms and pathogens when added in series.

The novelty of the present research lies in the application of a reverse sequence of disinfectants to those which are generally practiced in hybrid disinfection technologies for drinking water, where chlorine is used as the last step to maintain some residual in the distribution system. The logic was to evolve a hybrid disinfection strategy to avoid high doses of chlorine, which results in the formation of DBPs, many of which are proven carcinogens. In these two-step processes, the first step is intended to remove all major susceptible coliform species using chlorine followed by a serial step of costly secondary disinfectant that will take care of chlorine resistant species at relatively lower doses than those needed for their standalone usage. The results of hybrid disinfection strategy brought out benefits of reduced THMs especially $CHCl_3$, which is considered as a human carcinogen. This chapter summarizes the major findings of present study, based on which the following conclusions are drawn.

- Microbiological analysis of secondary treated sewage showed that five coliform species *E.coli, Enterobacter, Klebsiella, Citrobacter,* and *Serratia/Hafnia* were dominant constituting more than 90% of the bacterial population. These gram negative microbial species were cultured and enumerated in different samples to assess the efficacy of a disinfectant to achieve the objective of bringing the disinfected effluent to the desired standard of WHO for achieving TCC of 1000 CFU/100 mL for reuse.
- The batch process studies for chlorine disinfection reveals that 4 mg/L CD for 20 min was sufficient to achieve the desired standards. It was concluded that

such high CD was required due to *Serratia/Hafnia,* which were resistant to low CD. If the counts of these resistant species were ignored then at 2.5 mg/L of CD most of the other microbial species reached low counts.

- It was observed from inactivation kinetics that CD and CT were the best process control parameters in chlorine disinfection process, which may be simultaneously manipulated for achieving optimization.
- The mechanism of chlorine disinfection of microbial cells was analyzed by SEM images, which showed that chlorine attacks on the outer membrane of the microbes resulting in deformation of outer membrane of cells and finally into cell lysis at high doses.
- Optimization of chlorination process was carried out using CCD of Design of experiments in order to achieve counts for rest of the species to be brought within 1000 CFU/100 mL except for the resistant species *Serratia/Hafnia*. The two independent variables considered for CCD were dose and contact time for a response i.e. TC reduction.
- The optimized results suggested by the software were 2.5 mg/L CD for a contact time of 16.83 min (Ct 42 mg-min/L), where residual TCC will be 3200 CFU/100 mL. The predicted results by the software were in agreement with the experimental results. The optimized results represented a reduction in CD of about 47%, which helped in designing hybrid disinfection strategy.
- The effect of chlorine disinfection was also studied on three common pathogens *(Pseudomonas, Salmonella* and *Shigella)* and it was concluded that TCC and pathogens have similar sensitivity to a given disinfectant dose. But for complete removal of pathogens a dose of 4.5 mg/L was required. *Pseudomonas aeruginosa* was the most resistant species to chlorination.
- Similar results were obtained for chlorine disinfection in continuous process but it was found that a higher dose of 6.5 mg/L for 20 min was required to achieve the same disinfection standard as more chorine escaped in continuous process. Chlorine reduced organic matter present in water, which was evidenced by reduction in BOD and COD concentration.
- Analysis of four THMs ($CHCl_3$, $CHCl_2Br$, $CHClBr_2$, and $CHBr_2$) was carried out by highly sophisticated process, namely, GC-MS/MS, which provides more accurate results compared to GC-ECD or GC-MS. It was concluded that the

secondary treated effluent of RBC had fairly higher concentration of bromide compounds compared to that found in municipal sewage. Chlorination of this water resulted into increment in all four THMs on increasing CD. $CHCl_3$ showed the highest concentration among all four. TTHMs increased by 8 times when compared to secondary treated water. Hence, to improve disinfection process and to reduce toxicity due to DBPs alternative methods such as $O_3$and UV were studied.

- Disinfection study on ozonation showed that TOD of 30 mg/L was sufficient to achieve the desired TCC of 1000 CFU/100 mL. TOD was 62% lesser than the total Ct of 80 mg-min/L (4 mg/L for 20 min). Out of the five dominated species *Enterobacter* was observed to offer the highest resistance to ozone.
- Results of COD variation as a function of TOD revealed that with increase in TOD more organic matter is oxidized, which results into lowering of COD values. Reduction in the values of COD, turbidity, TSS exemplifies excellent oxidative nature of ozone, but also shows its competitive consumption with disinfection requirement.
- It was evident from SEM images that the due to excellent oxidation nature of ozone, ultrastructure of bacteria was affected. Bacterial cells collapsed and get shrunken. High ozone doses resulted into cellular lysis with complete destruction of cells.
- One factorial design method of RSM was used for statistically obtaining optimum ozone dose that satisfies the norms for TCC and COD simultaneously. Ozone dose was taken as independent variable, TCC and COD as its response.
- Actual v/s predicted response graphs were plotted to verify the obtained equations. The model was verified through ANOVA. The R^2 value higher than 95% for TCC and COD confirmed that the model was fit for the study. The optimized ozone dose suggested by the model was 30 mg/L to bring TCC within norms and the value of COD at this dose was 21mg/L.
- Efficacy of TOD was also tested for fewer pathogens and it was concluded that at 30 mg/L TOD complete removal of pathogens was achieved. *Shigella* was found to be more resistant to low doses of ozone.
- Analysis of THMs revealed that reduction in $CHCl_3$ was 85% when compared to its concentration in secondary treated effluent. Marginal increment in three

brominated species was also observed due to presence of Br^- in the secondary treated effluent. Compared to chlorination formation of TTHMs was much lesser (80% less) in ozonation.

- Ozonation technology is much costlier than chlorination and the mechanism for disinfection also involves its reaction with cell wall constituents, which found a strong competition with the COD of secondary treated wastewater technology. Hence, disinfection study was also carried out using the third alternative i.e. UV radiations, which is based on deactivation of DNA/RNA avoiding chemical reactions with organics/cell constituents.
- Experimental results with UV showed that a dose of 150 mJ/cm^2 was sufficient to achieve the desired level of disinfection. *Citrobacter* and *Serratia/Hafnia* were comparatively more resistant against low UV doses. Pathogens had similar sensitivity as those of coliforms and the same UV dose was sufficient to achieve total removal of pathogens.
- Analysis of SEM images revealed that low UV dose did not cause severe deformation in the outer membrane of bacterial cells as UV radiations directly attacks the genetic material of cells. At higher UV doses, cells became twisted with shrunken outer membrane. An interesting phenomenon was observed, which revealed that UV exposed microbes attached with one another and try to form long filamentous chains perhaps to defend themselves.
- The results of photo reactivation studies revealed that in samples treated with low UV dose, microbes reactivated when exposed to visible light. Reactivation was observed in *Serratia/Hafnia* and *Citrobacter* among coliforms and *Shigella* among pathogens. High UV doses resulted into complete inactivation of microbes and no reactivation was observed at 150 mJ/cm^2 UV dose.
- The physicochemical analysis of UV treated samples revealed that a very low oxidation of organic matter occurred during the process and UV radiations had higher specificity for disinfection than the chemical processes. Hence minimal reduction was observed in BOD and COD values.
- Analysis of THMs revealed that reduction in $CHCl_3$ was 71% when compared to its initial concentration. The other three brominated species increased marginally due to presence of bromide ions. TTHMs were reduced by 28% after

UV treatment when compared to their initial concentration. When compared with chlorine, UV radiations reduced TTHMs by 91% and $CHCl_3$ by 98%.

- The results of UV disinfection indicated that though UV treatment reduced the toxicity caused by DBPs but still it resulted in environmental risk caused by reactivation of microbes. Thus, it cannot be considered as a standalone technology for disinfection, and hence hybrid strategies were designed.
- Hybrid disinfection strategy 'A' (Cl_2/O_3) in which optimized CD (42 mg-min/L) was used as a primary disinfectant for removal of susceptible coliform species followed by ozone at very low dose (8 mg/L) to take care of chlorine resistant species was sufficient to achieve the desired level of disinfection. Pathogens were also completely reduced at this hybrid dose.
- It was concluded from SEM images that the two disinfectants with excellent oxidizing capacity, promoted inactivation of microorganisms with a high degree of disorder in bacterial cell ultrastructure.
- Analysis of THMs revealed that at this hybrid dose TTHMs were reduced by 37% and $CHCl_3$ by 55% when compared to only chlorination.
- Results of hybrid disinfection strategy 'B' which used synergy of Cl_2 and UV revealed that a dose of 42 mg-min/L Cl_2 with 75 mJ/cm^{2}of UV exposure was enough to meet the requirements. Pathogens also were completely eliminated at this hybrid dose.
- SEM analysis of the samples treated with hybrid dose 'B' revealed that it was very effective for destruction of microbes as no reactivation was noticed.
- Results of GC-MS/MS revealed that TTHMs were reduced by 44% when compared to chlorination alone.
- Physicochemical analysis of samples treated with hybrid doses revealed that hybrid strategy 'A' oxidized organic matter along with disinfection, as values for BOD and COD were reduced. On the other side a marginal reduction in the two parameters was observed in strategy 'B'.
- A brief comparative analysis of all the five disinfection technologies along with their cost comparisons is presented in Table 8.1

Table 8.1: Comparison between the different investigated disinfection processes

S. No.	***Parameter***	***Chlorine***	***Ozone***	***UV radiations***	***Hybrid disinfection 'B' (Cl_2/UV)***	***Hybrid disinfection 'A' (Cl_2/O_3)***
1	Optimum dose	80 mg-min/L	30 mg/L	150 mJ/cm^2	42 mg-min/L + 75 mJ/cm^2	42 mg-min/L + 8 mg/L
2	Log reduction	4	4	5	5	4
3	% Reduction	99.99	99.99	99.999	99.999	99.99
4	BOD (mg/L)	10.12	12.26	15.83	12.11	10.41
5	COD (mg/L)	58.88	26.22	92.56	55.33	46
6	TTHMs (µg/L)	0.1284	0.0245	0.0107	0.1058	0.1193
7	$CHCl_3$ (µg/L)	0.1144	0.0012	0.0023	0.0656	0.0581
8	Cost ($)	10,398,311	48,546,870	52,697,273	43,782,928	45,269,982
9	Environmental impact	High DBPs, toxic residual Cl_2, corrosive	Expensive, Hazardous	Expensive, reactivation of microbes	Safe	Safe

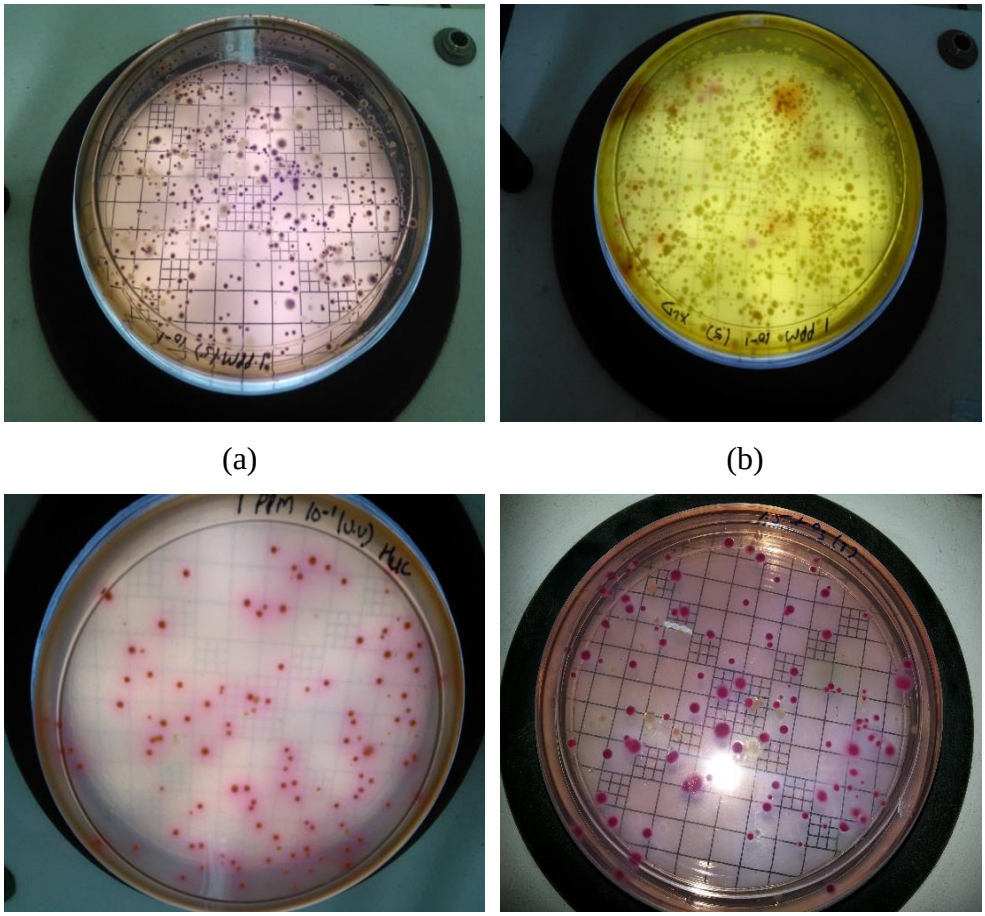
It is concluded from Table 8.1 that hybrid systems are better when compared to stand alone disinfection technologies as they provide higher treatment efficiency, reduced formation of TTHMs specially that of $CHCl_3$, and lesser risk of reactivation of microbes. Hence, the application of hybrid disinfection strategy has the potential to replace the existing approach of standalone technologies employed in wastewater treatment plants.

Recommendations for Future Research

The present study confirms that the hybrid disinfection proved superior to standalone systems, yet there is still a lot of scope for further detailed research in this area to establish it for field applications. The following recommendations may help extend the present study to further improve the process.

- Pilot scale studies should be carried out for developing scale up parameters.
- Samples from different treatment plants based on different treatment processes such as activated sludge process (ASP), moving bed biofilm reactor technology MBBR etc. can be tested to study the effect of hybrid disinfection doses required as per the characteristics of the secondary treated effluents.
- Detailed biochemical analysis can be carried out for identification and enumeration of different microbial species for a better process control. PCR technique can be used for confirmation of reactivation and repair mechanism of microbes.
- Ion chromatography can be used for analysis of bromide ions in water samples to provide support to the genesis of different THMs.
- A detailed study on HAAs along with THMs can be carried out using GC-MS/MS for getting a comprehensive idea about DPBs.
- Molecular techniques like PCR, random amplified polymorphic DNA (RAPD) should be used for fast detection of coliforms and new emerging pathogens to assess their fate under hybrid disinfection.
- Role of disinfection chlorination in altering antibiotic resistance among coliforms should be explored in details.
- Cost analysis made in the present study is quite preliminary in nature with a lot of assumptions. Detailed cost investigations for the treatment process, covering all aspects should be carried out to justify this new hybrid approach to disinfection.

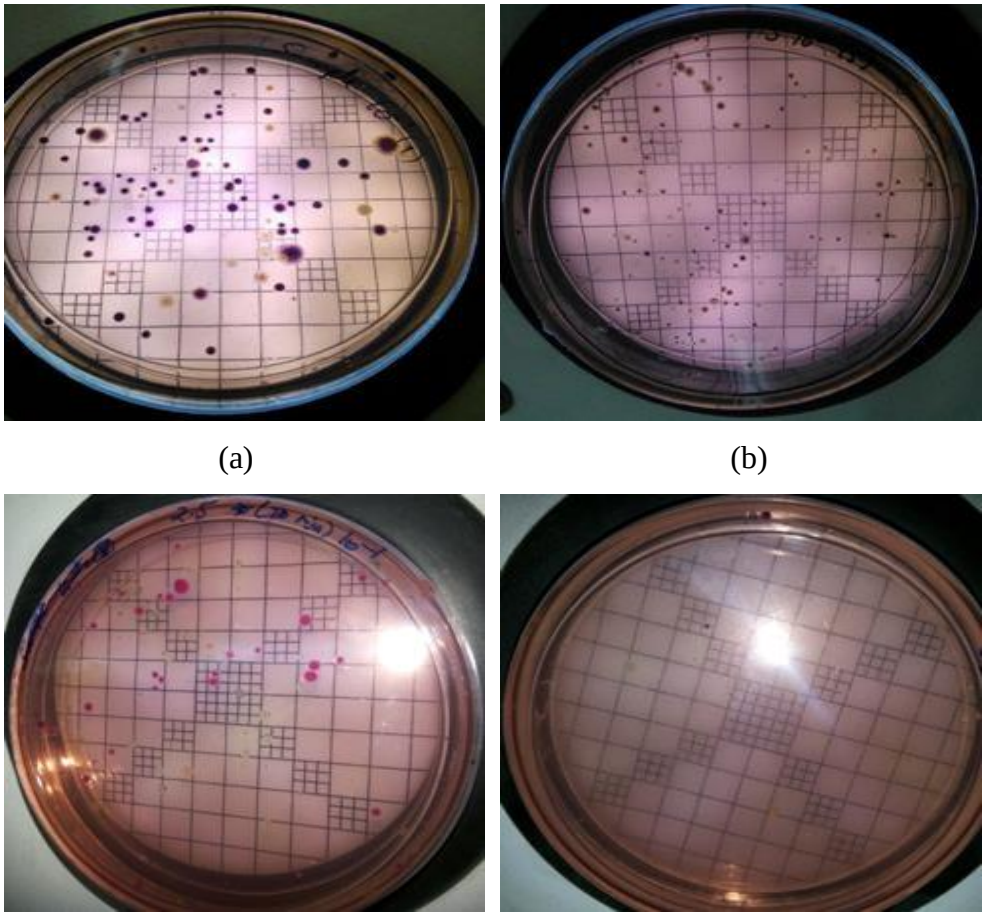
Appendix A



(a) (b)

(c) (d)

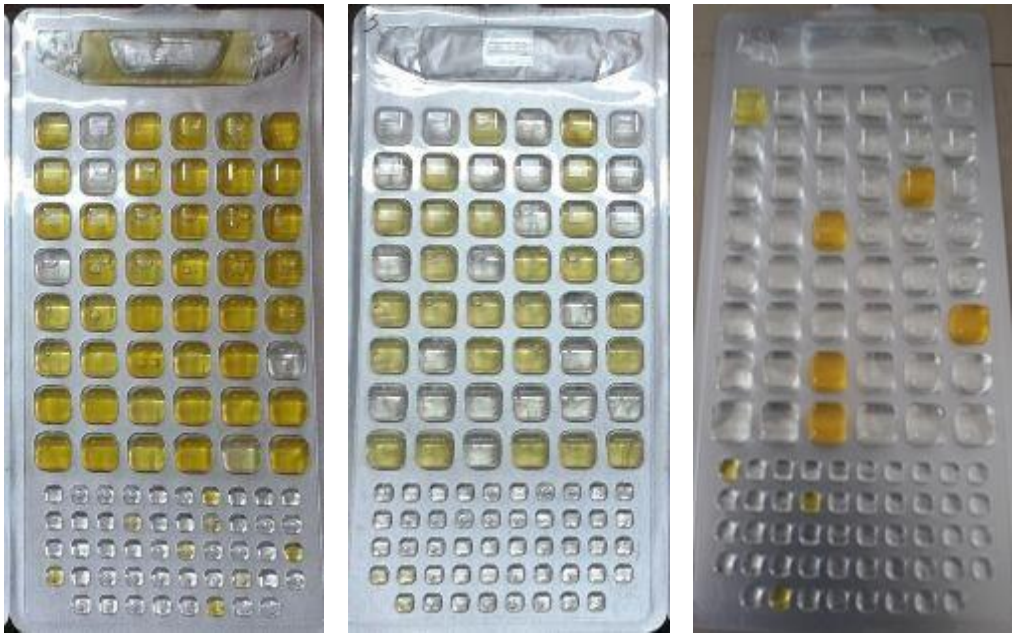
Figure A.1: Petriplates showing different colonies of bacteria on different specific agar media (a) EMB, (b) XLD, (c) Hekton, and (d) MacConkey



(a) (b)

(c) (d)

Figure A.2: Pour plate results of chlorination showing removal of TCs in terms of CFU/100 mL (a) secondary treated effluent, (b) effect of 1.5 mg/L of CD for 20 min, (c) effect of 2.5 mg/L of CD for 20 min, and (d) effect of 4 mg/L of CD for 20 min



(a) (b) (c)

Figure A.3: Colilert results of chlorination showing removal of TCs in terms of MPN/100 mL (a) secondary treated effluent, (b) effect of 2.5 mg/L of CD for 20 min, and (c) sample treated with 4 mg/L of CD for 20 min

Table A.1: Removal of microbial organisms (TC) at different CD in terms of MPN/100 mL

S. No.	***CD (mg/L)***	***TCC before disinfection***	***TCC after disinfection***	***% Removal of TC***	***Log reduction of TC***
1	1.0	16.9×10^5	1.0×10^5	94.08	1
2	1.5	16.9×10^5	8.4×10^4	95.02	1
3	2.0	16.9×10^5	1.0×10^4	99.40	2
4	2.5	16.9×10^5	3.1×10^3	99.81	2
5	3.0	16.9×10^5	8.4×10^2	99.95	3
6	3.5	16.9×10^5	4.1×10^2	99.97	3
7	4.0	16.9×10^5	4.1×10^1	99.99	4
8	4.5	16.9×10^5	3.1×10^1	99.99	4
9	5.0	16.9×10^5	2.0×10^0	100.00	5

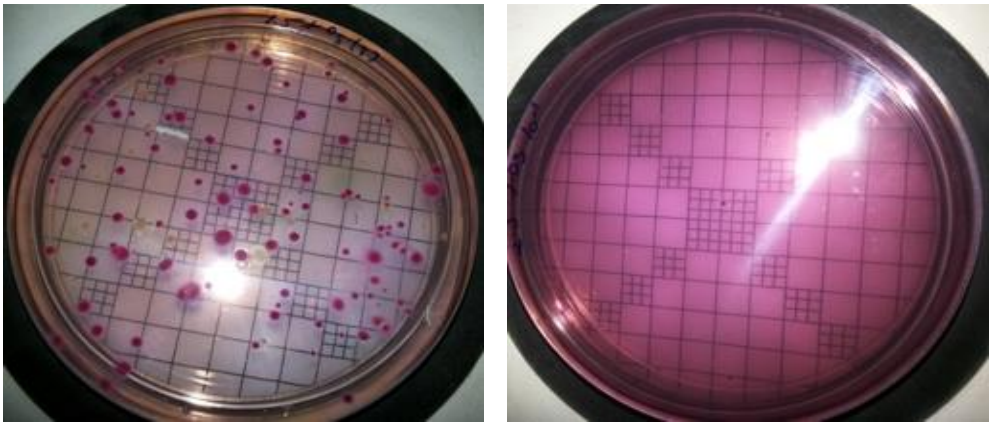
Table A.2: Removal of microbial organisms (TC) at different CD in terms of MPN/100 mL

S. No.	***CD***	***TCC before disinfection***	***TCC after disinfection***	***% Removal***	***Log reduction***
1	2.0	14.5×10^5	3.1×10^5	78.62	0
2	2.5	14.5×10^5	16.8×10^4	88.41	0
3	3.0	14.5×10^5	15.5×10^4	89.31	0
4	3.5	14.5×10^5	11.9×10^4	90.06	1
5	4.0	14.5×10^5	8.6×10^4	94.06	1
6	4.5	14.5×10^5	2.0×10^4	98.62	1
7	5.0	14.5×10^5	9.8×10^3	99.32	2
8	5.5	14.5×10^5	6.3×10^2	99.95	3
9	6.0	14.5×10^5	5.2×10^2	99.96	3
10	6.5	14.5×10^5	1.0×10^2	99.99	4

Table A.3: Analyte peak identification, retention time (RT) and concentrations for Figure 4.16

Peak	***Analyte***	***RT***	***Area***	***Calculated Amount (µg/L) (Instrument Reading)***
1	$CHCl_3$ (Before Treatment)	3.54	1816.14	0.4002
	$CHCl_3$ (1.5 mg/L)	3.53	15369.72	2.3140
	$CHCl_3$ (2.5 mg/L)	3.52	37142.87	5.7194
2	$CHCl_2Br$ (Before Treatment)	4.06	587.13	0.095
	$CHCl_2Br$ (1.5 mg/L)	4.06	720.52	1.4031
	$CHCl_2Br$ (2.5 mg/L)	4.04	956.52	0.1746
3	$CHClBr_2$ (Before Treatment)	5.61	3367.20	0.5912
	$CHClBr_2$ (1.5 mg/L)	5.61	4877.90	0.3105
	$CHClBr_2$ (2.5 mg/L)	5.61	6931.03	0.4303
4	$CHBr_3$ (Before Treatment)	7.48	59.82	0.6011
	$CHBr_3$ (1.5 mg/L)	7.46	88.39	0.0658
	$CHBr_3$ (2.5 mg/L)	7.33	208.11	0.0954

Appendix B



(a) (b)

Figure B.1: Pour plate results of ozonation showing removal of TCs in terms of CFU/100 mL (a) secondary treated effluent and (b) effect of 30 mg/L of TOD

(a) (b)

Figure B.2: Colilert results of ozonation showing removal of microbes in terms of MPN/100 mL (a) secondary treated effluent and (b) effect of 30 mg/L of TOD

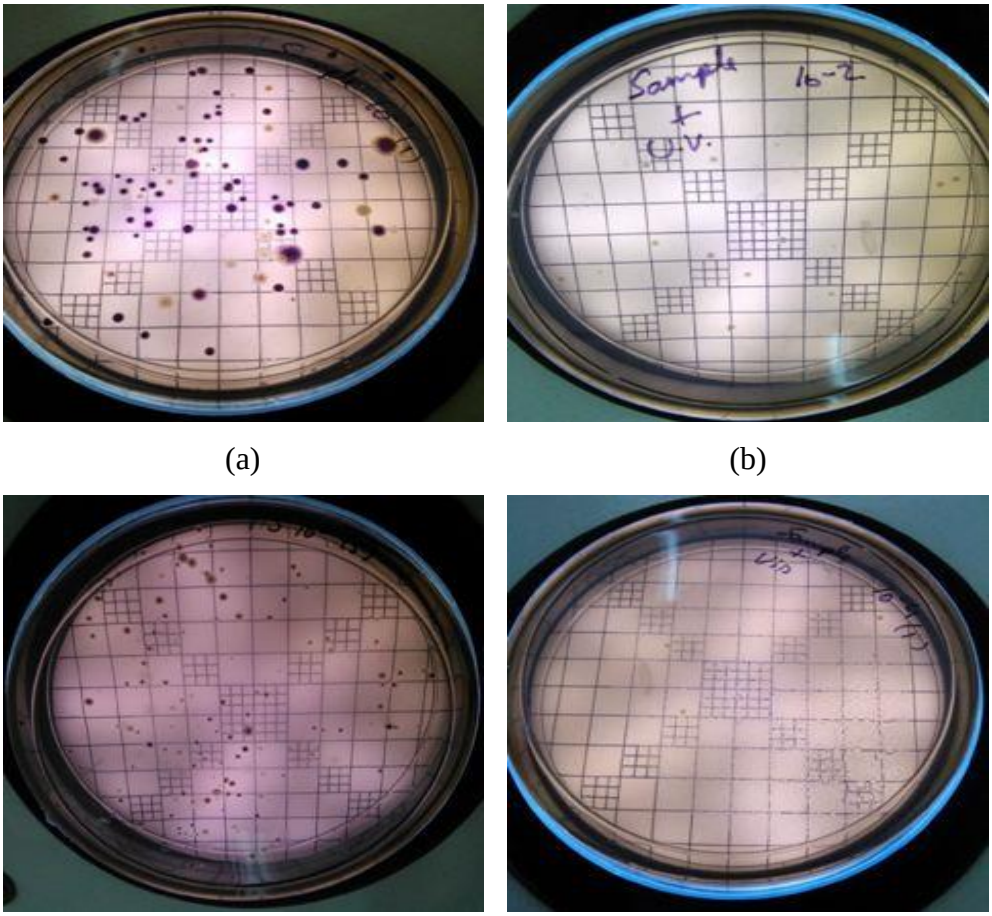
Table B.1: Effect of different ozone doses on TC in terms of MPN/100 mL on secondary treated effluent

S. No.	*TOD (mg/L)*	*TCC in sec. treated effluent*	*TCC in ozonated effluent*	*Standard deviation*	*% Reduction*	*Log reduction*
1	15	15.6×10^5	13.1×10^3	72.33	99.16	2
2	18	15.6×10^5	12.0×10^3	70.66	99.23	2
3	21	15.6×10^5	10.9×10^3	58.86	99.30	2
4	24	15.6×10^5	2.0×10^3	32.44	99.87	2
5	27	15.6×10^5	12.1×10^2	28.55	99.92	3
6	30	15.6×10^5	5.2×10^1	20.88	99.99	4
7	33	15.6×10^5	7.5×10^0	20.17	99.99	5
8	36	15.6×10^5	6.3×10^0	12.82	99.99	5
9	39	15.6×10^5	1.0×10^0	6.21	99.99	6
10	42	15.6×10^5	<1	0	100	6

Table B.2: Analyte peak identification, RT and concentrations for Figure 5.8

Peak	*Analyte*	*RT*	*Area*	*Calculated Amount (µg/L) (Instrument Reading)*
1	$CHCl_3$ (Before treatment)	3.54	1,816.14	0.4002
	$CHCl_3$ (30 mg/L)	3.54	596.40	0.0034
2	$CHCl_2Br$ (Before treatment)	4.06	587.13	0.0950
	$CHCl_2Br$ (30 mg/L)	4.05	638.95	0.7912
3	$CHClBr_2$ (Before treatment)	5.61	3,367.20	0.1912
	$CHClBr_2$ (30 mg/L)	5.61	4,864.56	0.3030
4	$CHBr_3$ (Before treatment)	7.48	59.82	0.0601
	$CHBr_3$ (30 mg/L)	7.48	109.81	0.0712

Appendix C



(a) (b)

(c) (d)

Figure C.1: Pour plate results of UV disinfection showing removal of microbes in terms of CFU/100 mL (a) secondary treated effluent, (b) effect of UV dose 75 mJ/cm^2 , (c) effect of UV dose 75 mJ/cm^2 + Visible light, and (d) effect of UV dose 150 mJ/cm^2 + Visible light

(a) (b)

Figure C.2: Colilert results of UV disinfection showing removal of microbes in terms of MPN/100 mL (a) secondary treated effluent and (b) effect of UV dose 75 mJ/cm^2

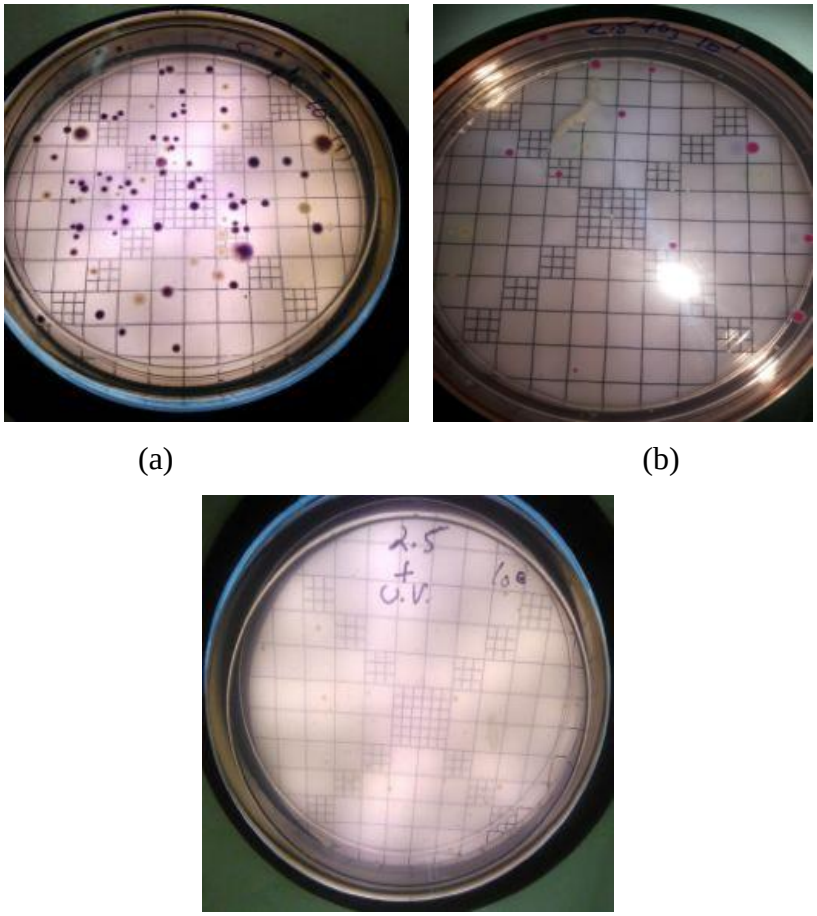
Table C.1: Effect of different UV doses on TC in terms of MPN/100 mL in secondary treated effluent

S. No.	***UV dose (mJ/cm^2)***	***TCC in sec. treated effluent***	***TCC in UV treated effluent***	***Standard deviation***	***% Reduction***	***Log reduction***
1	75	13.8×10^5	6.3×10^1	26.88	99.98	4
2	112	13.8×10^5	3.1×10^1	18.38	99.99	4
3	150	13.8×10^5	8.4×10^0	10.12	99.999	5

Table C.2: Analyte peak identification, RT and concentrations

Peak	***Analyte***	***RT***	***Area***	***Calculated amount (µg/L) (instrument reading)***
1	$CHCl_3$ (Before Treatment)	3.54	1816.14	0.4002
	$CHCl_3$ (75 mJ/cm^2)	3.54	1311.26	0.1152
2	$CHCl_2Br$ (Before Treatment)	4.06	587.13	0.0950
	$CHCl_2Br$ (75 mJ/cm^2)	4.05	631.33	0.1180
3	$CHClBr_2$ (Before Treatment)	5.61	3367.20	0.1912
	$CHClBr_2$ (75 mJ/cm^2)	5.61	3722.99	0.2236
4	$CHBr_3$ (Before Treatment)	7.48	59.82	0.0601
	$CHBr_3$ (75 mJ/cm^2)	7.48	80.14	0.0810

Appendix D



(a) (b)

(c)

Figure D.1: Pour plate results of hybrid disinfection showing removal of microbes in terms of CFU/100 mL (a) secondary treated effluent, (b) sample treated with hybrid dose 'A' (42 mg-min/L of Cl_2 + 8 mg/L O_3), and (c) sample treated with hybrid dose 'A' (42 mg-min/L of Cl_2 + 75 mJ/cm^2)

(a) (b) (c)

Figure D.2: Colilert results of hybrid disinfection showing removal of microbes in terms of MPN/100 mL (a) secondary treated effluent, (b) effect of hybrid dose ‘A’ (42 mg-min/L of Cl_2 + 8 mg/L O_3), and (c) effect of hybrid dose ‘B’ (42 mg-min/L of Cl_2 + 75 mJ/cm^2)

Table D.1: Effect of different hybrid dose 'A' (Cl_2/O_3) on removal of TC in terms of MPN/100 mL in secondary treated effluent

S. No.	*TCC in sec. treated effluent*	*TCC at optimized CD*	*Hybrid dose Cl_2/O_3 (mg/L)*	*TCC after hybrid dose*	*Standard deviation*	*% Reduction*	*Log reduction*
1	15.6×10^5	17.9×10^2	$42Cl_2+6O_3$	8.4×10^2	22.32	99.94	3
2	15.6×10^5	17.9×10^2	$42Cl_2+8O_3$	5.0×10^1	16.66	99.99	4
3	15.6×10^5	17.9×10^2	$42Cl_2+10O_3$	1.0×10^0	8.00	99.999	5
4	15.6×10^5	17.9×10^2	$42Cl_2+12O_3$	<1	0	100	6

Table D.2: Analyte peak identification, RT and concentrations for Figure 7.6

Peak	*Analyte*	*RT*	*Area*	*Calculated amount (µg/L) (instrument reading)*
1	$CHCl_3$ (Before Treatment)	3.54	1816.14	0.4002
	$CHCl_3$ (50 mg/L)	3.52	37142.87	5.7194
	$CHCl_3$ (Optimized CD)	3.54	25153.29	5.0353
	$CHCl_3$ (Hybrid Dose 'A')	3.54	22717.48	2.9055
2	$CHBrCl_2$ (Before Treatment)	4.06	587.13	0.095
	$CHBrCl_2$ (50 mg/L)	4.04	9560.52	0.1746
	$CHBrCl_2$ (Optimized CD)	4.05	3172.18	1.0794
	$CHBrCl_2$ (Hybrid Dose 'A')	4.05	4393.68	1.8422
3	$CHClBr_2$ (Before Treatment)	5.61	3367.20	0.5912
	$CHClBr_2$ (50 mg/L)	5.61	6931.03	0.4303
	$CHClBr_2$ (Optimized CD)	5.62	2247.17	0.6249
	$CHClBr_2$ (Hybrid Dose 'A')	5.62	8544.75	0.8142
4	$CHBr_3$ (Before Treatment)	7.48	59.82	0.6011
	$CHBr_3$ (50 mg/L)	7.33	208.11	0.0954
	$CHBr_3$ (Optimized CD)	7.47	115.69	0.0929
	$CHBr_3$ (Hybrid Dose 'A')	7.44	318.72	0.4001

Table D.3: Effect of hybrid dose 'B' (Cl_2/UV) on TC in terms of MPN/100 mL in secondary treated effluent

S. No.	*TCC in sec. treated effluent*	*TCC at optimized CD*	*Hybrid dose*	*TCC after hybrid dose*	*Standard deviation*	*% Reduction*	*Log reduction*
1	15.6×10^5	17.9×10^2	42 mg/L + 754 mJ/cm^2	4.0×10^0	15.21	99.999	5

Table D.4: Analyte peak identification, RT and concentrations for Figure 7.12

Peak	*Analyte*	*RT*	*Area*	*Calculated amount (µg/L) (instrument reading)*
1	$CHCl_3$ (Before Treatment)	3.54	1816.14	0.4002
	$CHCl_3$ (50 mg/L)	3.52	37142.87	5.7194
	$CHCl_3$ (Optimized CD)	3.54	25153.29	5.0353
	$CHCl_3$ (Hybrid Dose 'B')	3.50	16588.15	3.2842
2	$CHBrCl_2$ (Before Treatment)	4.06	587.13	0.095
	$CHBrCl_2$ (50 mg/L)	4.04	956.52	0.1746
	$CHBrCl_2$ (Optimized CD)	4.05	3172.18	1.0794
	$CHBrCl_2$ (Hybrid Dose 'B')	4.05	3645.52	1.2460
3	$CHClBr_2$ (Before Treatment)	5.61	3367.20	0.5912
	$CHClBr_2$ (50 mg/L)	5.61	6931.03	0.4303
	$CHClBr_2$ (Optimized CD)	5.62	2247.17	0.6249
	$CHClBr_2$ (Hybrid Dose 'B')	5.61	8815.85	0.6664
4	$CHBr_3$ (Before Treatment)	7.48	59.82	0.6011
	$CHBr_3$ (50 mg/L)	7.33	208.11	0.0954
	$CHBr_3$ (Optimized CD)	7.47	115.69	0.0929
	$CHBr_3$ (Hybrid Dose 'B')	7.47	418.72	0.1001

Appendix E

Table E.1: Cost analysis of chlorine disinfection

S. No.	*Chlorination*	*410 mg-min/L for 200 MLD plant*	*80 mg-min/L for 200 MLD plant*	*FV for experimental dose*	*PV for experimental dose*	*Capitalized cost*
1	Capital cost ($)	41,35,317	24,35,242	-	-	-
2	O and M cost ($)	9,95,727	5,86,372	33,584,505	4,992,111	10,398,311

Table E.2: Cost analysis of ozone disinfection

S. No.	*Ozonation*	*75 mg-min/L for 200 MLD plant*	*30 mg-min/L for 200 MLD plant*	*FV for experimental dose*	*PV for experimental dose*	*Capitalized cost*
1	Capital cost ($)	548,46,297	383,92,408	-		
2	O and M cost ($)	17,03,913	11,92,739	68,324,143	10,154,462	485,46,870

Table E.3: Cost analysis of UV disinfection

S. No.	*UV radiations*	*100 mJ/cm^2 for 200 MLD plant*	*75 mJ/cm^2 for 200 MLD plant*	*FV for experimental dose*	*PV for experimental dose*	*Capitalized cost*
1	Capital cost ($)	345,45,648	431,82,060	-		
2	O and M cost ($)	8,94,122	11,17,653	64,013,594	9,515,212	526,97,273

Table E.4: Cost analysis of hybrid disinfection A (Cl_2/O_3)

S. No.	***Hybrid disinfection "A"***	***42 mg-min/L + 8 mg/L for 200 MLD plant***	***FV for experimental dose***	***PV for experimental dose***	***Capitalized cost***
1	Capital cost ($)	326,08,924	-		
2	O and M cost ($)	14,87,163	851,77,264	216,61,057	$452,69,982

Table E.5: Cost analysis of hybrid disinfection B (Cl_2/UV)

S. No.	***Hybrid disinfection "B"***	***42 mg-min/L + 75 mJ/cm^2 for 200 MLD plant***	***FV for experimental dose***	***PV for experimental dose***	***Capitalized cost***
1	Capital cost ($)	324,88,082	-		
2	O and M cost ($)	13,26,688	759,86,076	112,94,846	437,82,928

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Optimization of ozone disinfection and its effect on trihalomethanes

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ABSTRACT

Ozone is an attractive disinfection alternative to chlorine as chlorine has some environmental consequences due to its disinfection byproducts potential. In the present study ozone was used for the disinfection of secondary treated effluent of sewage treatment plant which is based on rotating biological contactor, process, Malaviya National Institute of Technology (Jaipur). From the experimental results it was found that ozone dose of 30 mg/L was required to achieve WHO standards for Total Coliform Count (TCC) of 1000 CFU/100 mL for food crop irrigation and recreational impoundments. One factorial design method of response surface methodology (RSM) was used for statistically obtaining optimum ozone dose that satisfies the norms for COD and TCC simultaneously. The optimized ozone dose suggested by the model was 30 mg/L to bring TCC within norms and the value of COD at this dose was 21 mg/L. Experimental verification of the results was in good agreement with the predicted results of one factorial design. Analysis of scanned electron microscopy (SEM) images, shows the effect of ozone doses on bacterial cell membrane. Effect of ozone on four trihalomethanes was also studied using GC–MS/MS.

1. Introduction

Wastewater reuse has become an attractive option for protecting the environment and natural water resources in many regions of the world, particularly in water scarce areas where competition for water is high [1]. It is important that adequately treated wastewater should be disinfected, as the main objective when using reclaimed water is consumer's health and environmental protection [2].

Disinfection is the mechanism for the removal or reduction of pathogenic organisms to prevent the spread of water-borne diseases [3]. Historically, chlorination was the most widely used disinfectant to deactivate pathogenic microorganisms in wastewater. But alternative technologies have to be evaluated because of increasingly concern over undesirable disinfection byproducts (DBPs) of chlorination and its inefficiency in eliminating some of the resistant microorganisms at low chlorine doses (CD) [4–6]. Due to this concern, recently, there is an increasing use of ozone in wastewater reclamation.

Ozone is an attractive disinfection alternative as it is a potent germicide and simultaneously oxidizes organic matter thereby improving the wastewater quality [2,7–11]. It is an exceptionally good disinfectant that has faster disinfection kinetics and is more potent to eliminate most microorganisms when compared with other widely used chemical disinfectants [2,7]. It has been reported that ozone disinfection is very effective for removal of total coliforms (TC) and chlorine resistant microbes including pathogens which are especially resistant to most other disinfectants [8]. The germicidal effect of ozone results in total or partial destruction of the cell wall, resulting in cell lysis. In addition to this, ozone also breaks chromosomes, nitrogen carbon bonds between sugar and bases, DNA hydrogen bonds, as well as phosphate sugar bonds leading to depolymerisation and leakage of cellular constituents [3]. Thus, overall it helps in achieving higher effluent quality and better physicochemical and microbiological quality standards before it is discharged [13]. As opposed to chlorine, ozone does not even leave any trace of the residual product upon its oxidative reaction [4,14] and also avoids the environmental consequences caused by chlorinated DBPs as no harmful byproducts are formed after ozonation [15,16].

Abbreviations: AC, alternate current; ANOVA, Analysis of Variance; APHA, American Public Health Association; BOD, Biological Oxygen Demand; CFU, Colony Forming Unit; COD, chemical oxygen demand; DBP, disinfection by product; DNA, deoxyribose nucleic acid; HAA, halo acetic acid; MNIT, Malaviya National Institute of Technology; MPN, most probable number; NTP, normal temperature and pressure; ppm, parts per million; RBC, Rotating Biological Contractor; RSM, Response Surface Methodology; SEM, scanned electron microscopy; TCC, Total Coliform Count; TOD, transferred ozone dose; THM, tri halo methane; USEPA, United States Environment Protection Agency; UV, ultra violet; WHO, World Health Organization.

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Hybrid disinfection of sewage effluent—A comparative study of three secondary treatment plants of Jaipur, India

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ABSTRACT

Chlorination is one of the most widely used methods to disinfect wastewater, despite innumerable objections raised due to the resultant by-products. Any attempt to reduce the dosage of chlorine can be very useful in lowering the concentration of the disinfection by-products, many of which have been reported to be carcinogenic. This study examines the microbiological characterization of secondary treated sewage from three different sewage treatment plants based on different unit processes, namely activated sludge process, moving bed biofilm reactor and rotating biological contractor. The study also assesses the efficacy of using chlorine on major coliform species in order to achieve the objective of bringing the effluent to the desired standard for total coliform count (TCC) of 1,000 per 100 mL. The results indicate that 5 parts per million (ppm) chlorine dose (CD) was able to attain the TCC standard, if the counts for *Serratia/Hafnia* species were ignored. These species offered high resistance to chlorination due to which excessive overall doses were required to confirm to TCC standard. The chlorinated samples were further subjected to ultraviolet (UV-C) disinfection, the results of which can be employed to design a hybrid disinfection strategy with chlorination at a relatively low CD as the first step for removing bulk of the coliform population, followed by another process to which *Serratia/Hafnia* are susceptible. This can not only reduce the CD and thereby the by-products of chlorination but also bring down the overall cost of disinfection.

Keywords: Activated sludge process (ASP); Chlorination; Hybrid disinfection; Moving bed biofilm reactor (MBBR); Rotating biological contactor (RBC); Total coliform count (TCC)

1. Introduction

The volume of sewage effluent is increasing and safe disposal can often prove to be difficult due to the growth in population and urbanization [1,2]. The use of reclaimed wastewater for irrigation and other purposes is the obvious solution to this problem. However, several pioneering studies have provided the technological confidence for the safe reuse of treated effluent [3]. Primary treatment is essential to remove suspended solids and secondary treatment is designed to substantially degrade the soluble organic

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A review on sewage disinfection and need of improvement

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ABSTRACT

This paper presents a critical review on sewage disinfection process using chlorine, which is shrouded with lot of controversies. The review highlights the general applications and limitations of chlorination of sewage for disinfection including its low efficacy against resistant coliform bacteria that results in excess chlorine dosing. Excessive dose of chlorine is not only expensive, but it can also give rise to a series of DBPs many of which are proven carcinogens. Based on the results of the studies of our research group at MNIT, Jaipur, this paper explores as to how the chlorine doses can be optimized through step dosing and also how can the hybrid method of disinfection optimize the overall cost of desired level of disinfection. Combination of ozone in series with chlorine has been assessed for the possibility of reducing the overall disinfection cost and also at the same time getting rid of some of the carcinogenic DBPs of chlorination.

Keywords: Disinfection; Chlorination; Carcinogenic; DBPs

1. Introduction

India is facing acute water crisis with an ever growing population and as the demand outstrips the supply, which results in increased human exposure to wastewater discharged into the environment during the last two decades [1]. Conservation, watershed protection, and reclamation have become essential components of water management in the new millennium in order to meet the increasing demand [2]. Non-potable reuse of wastewater by the communities themselves for the applications such as irrigation, toilet flushing, industries is already widely practiced. Reuse of reclaimed water for potable purposes may be feasible after proper treatment and microbial reduction [3,4]. Primary, secondary, and even tertiary treatment cannot be expected to remove 100% of the incoming waste load, and as a result, many organisms still remain in the waste stream [5]. The conventional municipal sewage treatment plants, which generally do not include the disinfection process, reduce fecal microorganism's densities by 1–3 orders [6]. Thus, it becomes essential to develop an appropriate technology as to meet the standards in the receiving water bodies [4,7].

2. Sewage disinfection

Disinfection is the treatment of the effluent for the destruction or removal of all pathogens and microbes

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Brief Bio-data

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