TREATMENT OF PHARMACEUTICAL WASTEWATER CONTAINING β-LACTAM RING

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I hereby certify that the research work, reported in this thesis, has been performed by me and this work has not been submitted elsewhere for any other purpose (except for publication).

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ABSTRACT

Pharmaceutical industry is one of the major industries in Bangladesh which has its own strong manufacturing capabilities to produce and export pharmaceuticals products. Along with its products, it also generates wastewater which is a concern from environmental point of view. Pharmaceutical wastewater is generally characterized by high toxicity and BOD_5 and COD content, making it a potential threat to the environment and public health. Antibiotics in pharmaceutical wastewater are of particular concern, as they can induce bacterial resistance, even at low concentrations. Antibiotics generally have low biodegradability since they are biocidal substances and the degradation of these substances cannot be accomplished in the natural environment or biological treatment plants. The antibiotics containing liquid waste of pharmaceutical industries usually contain high amount of β-Lactam ring. The present study was undertaken to find out an effective decontamination process and also to evaluate the performance of Effluent Treatment Plant (ETP) in treating pharmaceutical wastewater containing β-Lactam ring so that it can comply with the national environmental standard. The main objective of the research was to provide a solution for the effective decontamination of β-Lactam residues in pharmaceutical wastewater using varying chemicals and reaction time and thus improving the efficiency of effluent treatment plant.

For decontamination, a trial was made in the present study to use organic CH_3COOH with NaOH in order to reduce the effects on microorganism in treating β-Lactam ring containing wastewater by biological treatment process along with the commonly used inorganic HCl with NaOH. The study result showed that both the combination of chemicals were effective in decontaminating β-Lactam ring for a 3-hr reaction time and no reformation of β-Lactam ring after neutralization by HCl or CH_3COOH was observed in the study by HPLC analysis. Regarding the effect of two acids on microorganism in biological treatment process, study result showed that the microbial count remained the same in Aeration tank for both the cases. However, the amount of CH_3COOH acid required in decontamination was higher than that of HCl, and from economic consideration, use of HCl is preferred to CH_3COOH, since cost of CH_3COOH is also higher (around 98%) than that of HCl.

The performance of different units of a Biological (Activated Sludge Process) CETP has also been studied in the present study after discharging decontaminated wastewater in CETP, as the concentration of decontaminated wastewater is higher than the allowable wastewater discharge limit set by ECR, 1997. From this research, it was observed that the contaminant in antibiotic containing wastewater was significantly reduced (reduction of BOD_5 62%, COD 68%, TSS 98% and TDS 28%) in Combined Tank with the mixing of relatively high volume and low concentration of wastewater from Solid Dosage Unit. Therefore, the dominant treatment in CETP is the Dilution process. Apart from dilution, removal of BOD_5 (66%) and COD (46%) mostly occurred in Aeration Unit of CETP. Having relatively less initial concentration of contaminants in Combined tank, the effluent discharged from the final point of CETP complies with the ECR, 1997 Standard limit regarding pH, TDS, TSS, BOD_5 and COD values, except DO concentration. It was observed from the laboratory analysis that COD, BOD_5, TDS, TSS and Turbidity of wastewater in Clarifier, DO Increase Tank and Filtration unit, has not been removed much compared to removal in Aeration unit. Therefore, regarding the performance of decontamination and CETP in the studied industry, it can be said that the β-Lactam ring is successfully decontaminated for both combination of chemicals (NaOH and CH_3COOH, NaOH and HCl) with 3-hr retention time and no reformation of β-Lactam in CETP for decontaminated cephalosporin wastewater and Combined Tank, Aeration Tank were found effective in CETP, other units such as Clarifier and Filtration unit was found ineffective in treating the industry's wastewater.

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LIST OF ABBREVIATIONS

AOP	Advanced oxidation processes
BAPI	Bangladesh Association of Pharmaceutical Industries
BBS	Bangladesh Bureau of Statistics
BAC	Biological Activated Carbon
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
CAGR	Compound Annual Growth Rate
CETP	Common Effluent Treatment plant
DO	Dissolved Oxygen
DGDA	Director General of Drug Administration
ECR	Environmental Conservation Rules
ECA	Environmental Conservation Act
ECR	Environmental Conservation Rules
ESBL	Extended-Spectrum β-Lactamases
MRSA	Methicillin Resistant Staph Aureus
NTU	Nephelometric Turbidity Unit
ORSA	Oxacillin Resistant Staphylococcus Aureus
PIWW	Pharmaceutical Industry's Wastewater
Pt-Co Unit	Platinum Cobalt Unit
PBP	Penicillin Binding Proteins
RBC	Rotating Biological Contactor
SBR	Sequencing Batch Reactor
UASB	Up-flow Anaerobic Sludge Blanket
WWTP	Waste Water Treatment Plant

Chapter 1
INTODUCTION

1.1 General

The Pharmaceutical industries are one of the promising sectors in Bangladesh which are currently contributing to the country's economy significantly. The National Drug Policy (NDP) in 1982 and 2005 has a major impact in the development and growth of the Bangladesh pharmaceutical industry. The Drug (Control) Ordinance in 1982 was the key factor of success of pharmaceutical industries in Bangladesh (Rich, 1994). About 250 pharmaceutical companies are now operating in the country, which are manufacturing about 5,600 brands of medicines in different dosage forms. According to a report of the International Management System (IMS) published in June 2015, the size of the pharmaceutical market of Bangladesh is estimated to be approximate 117 billion taka, with an annual growth rate of about 11.37% (Hossain and Shoaib, 2014). This rapid growth in the industrial sector also generates some problems associated with it. One ofthe major problems is the pollution caused by its solids and liquid wastes. Inefficient or lack of treatments of wastewater discharged from its production units, insufficient monitoring and lack of awareness to run treatment plants are making the pollution situation more adverse day by day.

Pharmaceuticals have been detected in effluent waters of wastewater treatment plants worldwide (Daughton, 2004). This is because of their increased use as well as not being targeted for their removal during wastewater treatment. This issue should be of concern because these trace amounts have the potential to cause harmful effects in aquatic life and possibly humans (EPA, 2001).

There have been a number of treatment options available for the treatment of pharmaceutical wastewater i.e. Up-flow Anaerobic Sludge Blanket (UASB) Reactor, Sequencing Batch Reactor (SBR), Biological Activated Carbon (BAC), Rotating Biological Contactor (RBC) etc. However, most of them are expensive and technology intensive and therefore the industry owners are reluctant to install or operate the treatment units. It is expected that, by developing a comparatively cost-effective treatment method it is possible to encourage the industry owners to treat the effluent of their industries and thus help improving the quality of water bodies in Bangladesh.

1.2 Statement of the Problem

Pharmaceutical wastewater is generally characterized by high toxicity, making it a potential threat to the natural environment. Antibiotics in pharmaceutical wastewater are of particular concern, as they can induce bacterial resistance, even at low concentrations (Hernández, 2007). The antibiotics residual in waste streams poses a great threat to receiving water bodies (streams, rivers, and ocean) (Pauwels and Verstraete, 2006). As a result, discharge of untreated wastewater causes damage to the environment and the resulting environmental pollution affects surface water, groundwater, plants, aquatic life and human health (Edokpayi et. al., 2017). Antibiotic resistance genes are now a concern and measures to prevent their further spread should be seriously taken care of (Pruden et. al., 2013). Antibiotics generally have low biodegradability since they are biocidal substances and the degradation of these substances cannot be accomplished in the natural environment or biological treatment plants (Richardson and Brown, 1985 and K¨ummerer, 2001). The antibiotics containing liquid waste of pharmaceutical industries is generally characterized by excessive amount of β-Lactam ring (Fukustsu, et. al, 2006). The decontamination of this β-Lactam is required to treat the wastewater before discharging into the environment.

As the number of pharmaceutical industries as well as generation of antibiotic wastewater from these industries is increasing day by day, it is very essential to know which method is the most suitable and cost effective for the treatment of effluent from pharmaceutical industries. If the present practice of designing ETP continues, within a short time we will have to suffer a very severe environmental pollution even if the ETPs are operated regularly.

Keeping this aspect in mind, an attempt has been undertaken to develop an effective decontamination process of β-Lactam ring residues and also to evaluate the performance of Effluent Treatment Plant (ETP) in treating pharmaceutical wastewater with respect to the National Standard of Wastewater Discharge (ECR, 1997).

It is expected that the findings generated from this study would provide an effective solution on decontamination of antibiotics in pharmaceutical wastewater to the professions working in the practical field of wastewater treatment.

1.3 Objectives

The main objective of the research is to develop an effective procedure for the better decontamination of β-Lactam antibiotic residues in Pharmaceutical wastewater to safeguard the water environment and thus improving the efficiency of effluent treatment plant. The specific objectives include:

i) Assessing the effectiveness of decontamination of β-Lactam residues in Pharmaceutical wastewater using varying chemicals
ii) Assessing the effects of reaction time and cost of chemicals on decontamination
iii) Evaluating the performance of different units of the industry's Effluent Treatment Plant (ETP) to treat mixed wastewater containing decontaminated wastewater and wastewater from solid dosage production unit.

1.4 Scope of the Research

The wastewater sample was collected from a Pharmaceutical industry and after collection laboratory analysis was carried out in Civil and Environmental Engineering Laboratory, BUET. The parameters selected for analysis are as follows: pH, BOD_5, COD, TSS, TDS, Color, Turbidity, EC, Cl^-, Zn, Cr, Cu and Cd. Decontamination with solutions of HCl, NaOH and CH_3COOH as agents for degradation was assessed. Decontamination process was done in Industry's decontamination tank. After that neutralization of decontaminated wastewater and its performance were evaluated by HPLC analysis using Industry's Quality Control Laboratory.

1.5 Organization of the Thesis

The present thesis comprises of five chapters.

Chapter 1'Introduction'gives an introduction on the research topic along with Rationale of the Study and objectives of the research work.

Chapter 2 titled 'Literature Review' contains a brief and selective review of the relevant literature. Characteristics of pharmaceutical wastewater, details of antibiotic contamination, treatment methods, pharmaceutical production flow etc. all are presented here.

Chapter 3 titled 'Methodology' describes the methodology for carrying out the present work. It includes collection of samples, experimental setup and procedures.

Chapter 4 titled ‘Results and Analysis’ examines the performance of decontamination and different unit of ETP.

Finally, Chapter 5 titled ‘Conclusion’ attempts to bring the major findings of the study and outlines the recommendations for the studies in this area.

Chapter 2
LITERATURE REVIEW

2.1 Introduction

In this Chapter, current scenario of pharmaceutical industry in Bangladesh, the general production flow in the pharmaceutical industry, characteristics of its wastewater specially antibiotic producing industries, sources of wastewater, introduction of antibiotics, effect of antibiotic contamination, the wastewater treatment methods commonly applied in this type of industry and review of treatment Process of Pharmaceutical Wastewater containing β-Lactam ring are briefly described for better understanding of the research.

2.2 Pharmaceutical Industry in Bangladesh

According to Bangladesh Association of Pharmaceutical Industries (BAPI) and Directorate General of Drug Administration (DGDA), approximately 257 licensed pharmaceutical manufacturers are in Bangladesh and about 150 are functional (BAPI, 2019). These manufacturing companies meet around 97% of local demand. Specialized products like vaccines, anticancer products and hormone drugs are imported to meet the remaining 3% of the demand. About 80% of the drugs produced in Bangladesh are generic drugs, rest 20% are patented drugs. According to DGDA, the industry has 3,534 generics of allopathic medicine, 2,313 registered Homeopathic drugs, 5,771 registered Unani Drugs and 3,899 registered Ayurvedic drugs (DGDA, 2019).

Domestic market of Pharmaceutical products in Bangladesh has shown an increasing trend over the past few years and the market size is BDT 187,566 million as on 2017 Q2 (IMS Health, 2016). However, this number does not reflect total market size because IMS report does not include homeopathic, unani, ayurvedic or herbal medicine information. According to Bangladesh Bureau of Statistics, the industry has contributed 1.85% to the GDP in 2016-17 (BBS, 2017). Pharmaceutical industry of Bangladesh is largely protected from external competition, as there is a restriction regarding import of similar drugs that is manufactured locally. This industry is the second largest contributor to national exchequer.

Pharmaceuticals industry of Bangladesh has grown significantly over the last five years. From 2012 to 2017, historical five years Compound Annual Growth Rate

(CAGR) was 15% and from 2014 to 2017, historical three years CAGR was 21%. According to industry experts, market size of pharmaceuticals may reach about BDT 330,000 million by 2020 (Acmeglobal, 2016).

2.3 Manufacturing Process in Pharmaceutical Industry

There are generally three production lines in pharmaceutical industries producing the lifesaving drugs in the form of tablet, capsule and dry syrup. Raw materials are imported from India, Europe, Korea, Japan, West Germany and many other countries of the world. The Flow Diagram of these production lines (capsule, dry syrup and tablet) is shown in Figure 2.1 to Figure 2.3.

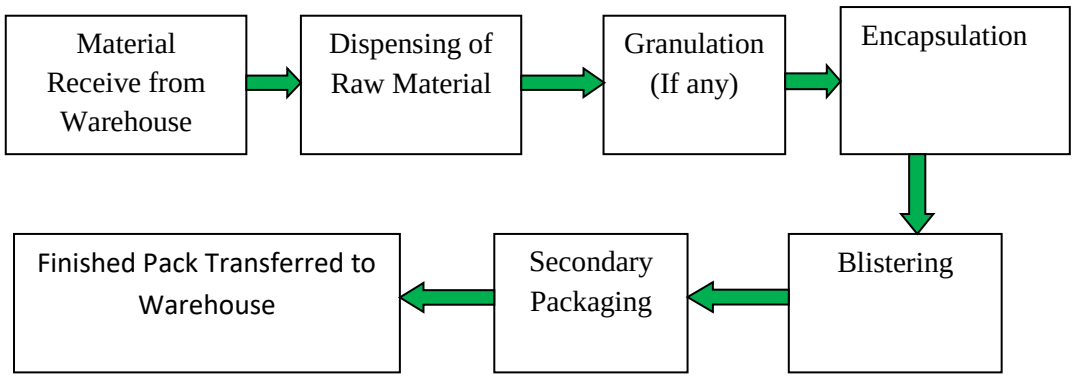


Figure 2.1: Production Process Flow Diagram – Capsule

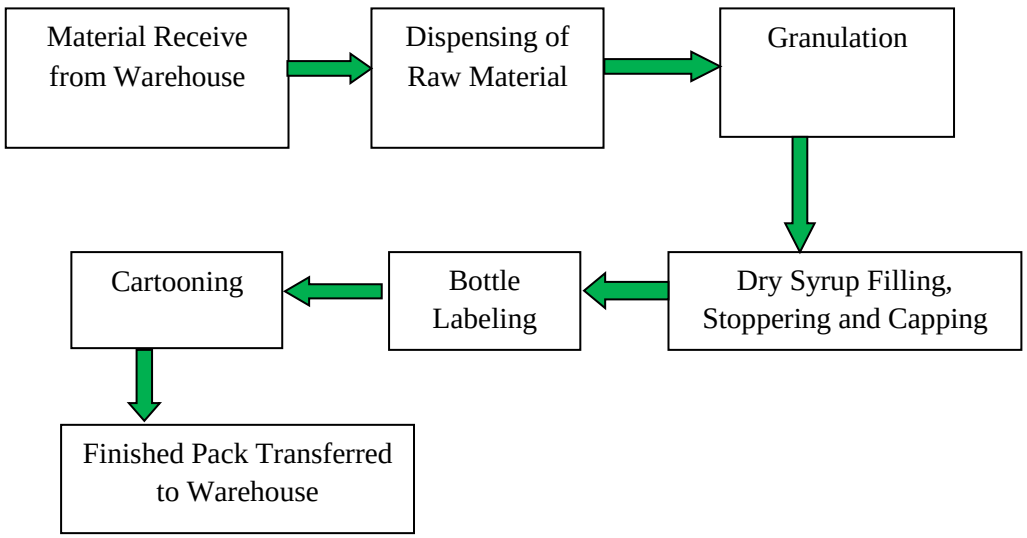


Figure 2.2: Production Process Flow Diagram – Dry Syrup

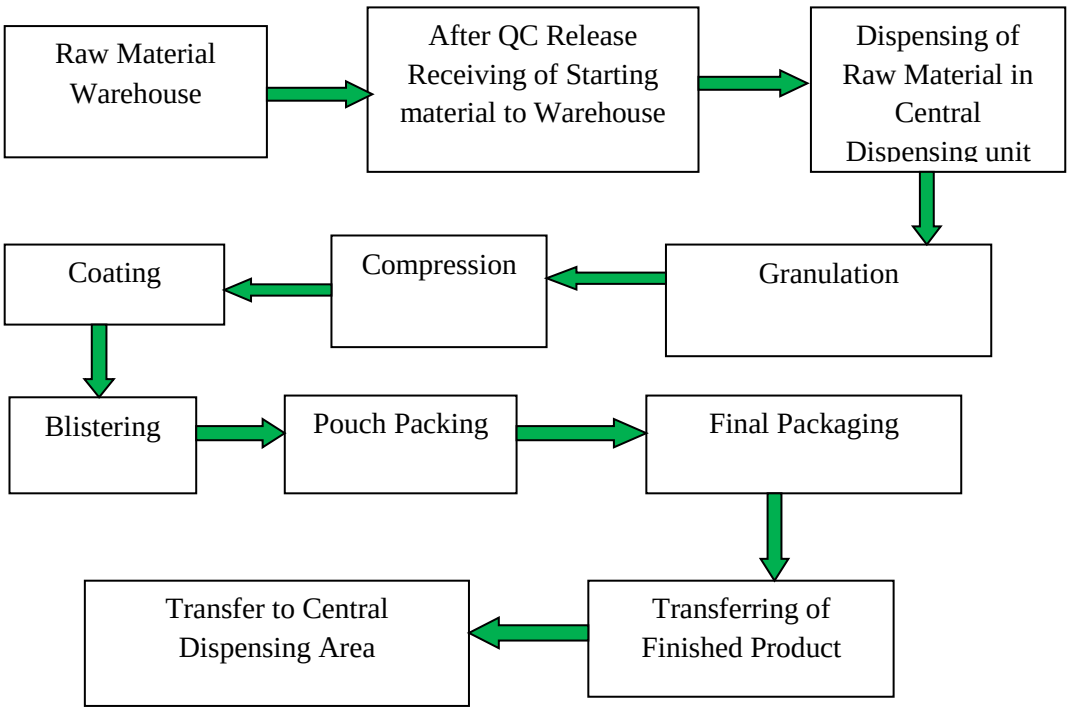


Figure 2.3: Production Process Flow Diagram – Tablet

2.4 Characteristics of Pharmaceutical Wastewater

In general, the composition of pharmaceutical wastewater is complex, which has high concentration of organic matter, microbial toxicity, high salt, and it's hard to biodegrade (Y et. al., 2017). Wastewater from pharmaceutical industry, producing penicillin and similar antibiotics are strong (high BOD and low pH) and generally cannot be treated with domestic wastewaters (Nemerow, 1984). In addition, most of pharmaceutical factories have batch process, which use different raw materials in their production processes, and produce wastewater of different varieties (Y et. al., 2017). Table 2.1 provides a summary of the characteristics of pharmaceutical wastewater in Bangladesh.

Table 2.1: Characteristics of Pharmaceutical Industry's Wastewater in Bangladesh

Parameters	Unit	References	
		(Rahman, 1997)	(Islam, 2012)
pH	-	6.87 (7.1-6.5)	6.95
BOD_5	mg/l	783 (2800- ≤ MDL)	190
COD	mg/l	1260 (4300-40)	409
Suspended solids	mg/l	770 (1414-135)	76
Chloride (Cl)	mg/l	2260 (4500-19)	-
Chromium (Cr)	mg/l	0.037 (0.047-0.028)	0.009
Copper (Cu)	mg/l	0.086 (0.131- 0.042)	0.084
Zinc (Zn)	mg/l	0.176 (0.215-0.138)	0.214
Cd	mg/l	-	0
Color	TCU/ Pt-Co	1100 (1200- 1000)	65
Turbidity	NTU	314 (586 - 48)	40
Total Solids	mg/l	38733(151993 – 15328)	-
DO	mg/l	-	1.23
TDS	mg/l	-	675

Table 2.2 shows the concentration of various parameters in Pharmaceutical Wastewater found in different literature in different countries. Table 2.3 presents the heavy metal content in Pharmaceutical Wastewater in different research papers around the world.

Table 2.2: Characterization of Pharmaceutical Industry's Wastewater

Parameters	References		
	(Gome and Upadhyay, 2013)	(Choudhary and Parmar, 2013)	(Wei et al.,2012)
pH	6.9	5.8–7.8	7.2–8.5
TSS (mg/l)	370	230–830	48–145
TDS (mg/l)	1,550	650–1,250	–
Total solids	1,920	880–2,040	–
BOD (mg/l)	120	20–620	480–1,000
COD (mg/l)	490	128–960	2,000–3,500
Biodegradability(BOD/COD)	0.259	–	0.20–0.39
Alkalinity (mg/l)	–	130–564	–
Total nitrogen (mg/l)	–	–	80–164
Ammonium nitrogen(mg/l)	–	–	74–116
Total phosphate (mg/l)	–	–	18–47
Turbidity (NTU)	–	–	76–138
Chloride (mg/l)	–	–	–
Oil and grease (mg/l)	–	–	–
Phenol (mg/l)	–	–	–
Conductivity(lS/cm)	–	–	–
Temperature (^oC)	–	–	–

Table 2.3: Metal contaminants in Pharmaceutical Industry's Wastewater

Parameters	References		
	(Ramola and Singh, 2013)	(Rohit and Ponmurugan, 2013)	(Vanerkar et al., 2013)
Iron (mg/l)	8.5–10.8	–	–
Chromium (mg/l)	0.12–0.31	0.01	0.057–1.11
Lead (mg/l)	0.158–0.262	0.03	0.559–6.53
Cadmium (mg/l)	0.16–0.56		0.036–0.484
Nickel (mg/l)	0.05–0.12	0.02	0.892–2.35
Zinc (mg/l)	1–1.3	0.20	0.583–0.608
Copper (mg/l)	–	0.02	0.649–1.67
Selenium (mg/l)	–	–	0.428–0.666
Arsenic (mg/l	–	–	0.0049–0.0076
Manganese (mg/l)	–	–	6.41–8.47
Sodium (mg/l)	–	–	155–266
Potassium (mg/l)	–	–	128–140
Calcium (mg/l)	–	–	–

2.5 Sources of Wastewater from Pharmaceuticals Industry

In Pharmaceutical industry, wastewater mainly generated for washing equipment of formulation and manufacturing associated process of various items like tablets, capsules, dry syrups etc. Wastewater also coming from quality control laboratory, product development and laundry room.

2.6 Antibiotics in Pharmaceuticals

Antibiotics are medicines that treat infections by killing bacteria. They don't work on viruses, like the flu (Webmd, 2017). The ‘antibiotic’ term qua generic is used to specify any class of organic molecule that blocks or ravage microbes by specific interactions with bacterial marks, without considering any compound or class (Michael et. al., 2013). Antibiotics are designed to act very effectively even at low doses and in case of intracorporal administration, to be completely excreted from the body after a short time of residence (Thiele-Bruhn, 2003(. They are non-biodegradable and can survive in aquatic environments for long periods (Alton et. al., 2004). The entrance of these compounds into the environment owing to anthropogenic sources can result in a potential risk for organisms. Although antibiotics exist at residual levels, they can cause resistance in bacterial populations, making them inactive in the treatment of several diseases in the near future (Schwartz et. al., 2006 and Homem, 2011). Moreover, they

can cause endocrine-disrupting effects when they are consumed by living organisms. They interfere with the synthesis, secretion, transport, binding action and elimination of hormones in the human body (EPA, 2001).

Antibiotics can be grouped according to their chemical structure or mechanism of action. There are various groups of chemicals that can be arranged to different subgroups, such as β-Lactams, quinolones, tetracyclines, macrolides, sulphonamides, and others. They are complicated molecules, which may have different functionalities within the same molecule. Consequently, they act as neutral, cationic, anionic, or zwitterionic under different pH conditions. Owing to different functionalities in a single molecule, their physico-chemical and biological properties (like octanol-water partition coefficients, sorption behavior, photo reactivity and antibiotic activity, and toxicity) may change with pH (Kümmerer, 2009).

2.6.1 β-Lactam Antibiotics

A beta-lactam (β-lactam) ring is a four-member lactam (A lactam is a cyclic amide). It is named as such because the nitrogen atom is attached to the β-carbon atom relative to the carbonyl. All members of the super family of antibiotics known as "β-lactams" have a core structure (highlighted in red) consisting of a 4-member "β-lactam" ring (Figure 2.4).

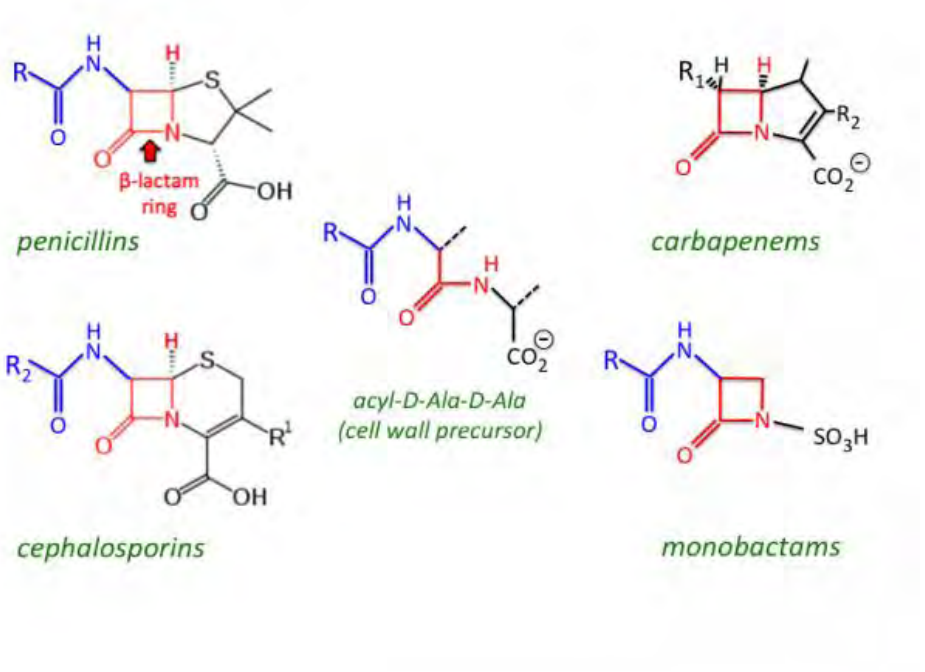


Figure 2.4: Core structure of β-lactam antibiotics

This ring mimics the shape of the terminal D-Ala-D-Ala peptide sequence that serves as the substrate for cell wall transpeptidases that form covalent bonds between different peptidogly can chains during periods of cell growth. The 4 ring structure and associated side groups result in tight binding to the active site of transpeptidases (also known as Penicillin Binding Proteins). Tight binding inhibits enzyme activity and consequent cell wall formation.

Modification of the structure of the naturally occurring penicillins (penicillin G and V) resulted in the development of both synthetic penicillin analogs, as well as new families of β-lactams (beginning with the cephalosporins) that have distinctly different side rings and side chains with different antibacterial spectrums of activity, greater resistance to beta lactamases, and different pharmacokinetic properties. At present there are four major beta-lactam subgroups that include:

1. Penicillins
2. Cephalosporins
3. Monobactams
4. Carbapenems

Mechanism of Bacterial Resistance to β-Lactams

Bacteria were exposed to naturally occurring "antibiotic" (inhibitory) compounds in their environment for at least a billion years before the modern use and development of antibiotics in medicine (Jose, 2009). As a result, over the eons of time, bacteria have developed resistance mechanisms for survival that our current medical use has selected for providing an evolutionary advantage to those bacteria capable of expressing such traits necessary for survival.

Resistance to an antibiotic can usually be attributed to one of many different "Protective mechanisms" that bacteria have developed to prevent the inhibitory effect of antibiotics (Alan, 2013):

A. Penetration
    a. Bacteria do not readily cross mammalian cell membranes
    b. Bacteria that are within the cytoplasm of human cells can be protected from exposure to high levels of antibiotics

B. Porins
    a. Gram-negative bacteria have an outer cell membrane that restricts drug access to the peptidoglycan cell wall to passage through porin channels
    b. The opening in porins is too small to allow diffusion of large molecules, such as vancomycin (resulting in an inherent or intrinsic form of gram-negative resistance against glycopeptide antibiotics)
    c. Some gram-negative bacteria express porins having an altered pore structure that does not permeation of smaller drugs (such as β-lactams or carbapenems).

C. Pumps
    a. Gram-negative bacteria can express P-glycoprotein-like ABC transporters in the inner plasma membrane that function as efflux pumps to transport antibiotics out of the cell, thereby protecting the organism from antibiotics having intracellular sites of action (e.g. topoisomerase or ribosomal subunits) (which is not a mechanism terribly relevant for cell wall synthesis inhibitors).

D. Penicillinases
    a. The most common mechanism for drug resistance to β-lactam antibiotics is bacterial synthesis of β-lactamases (Hall et al, 2003).
    b. Many bacteria synthesize β-lactamases that degrade β-lactam antibiotics before they reach the cell wall (Figure 2.5).
    c. Gram-positive bacteria that make β-lactamase excrete the enzyme into the extracellular space. Gram-negative bacteria excrete β-lactamase into the periplasmic space located between the cytoplasmic membrane and the outer membrane, where the cell wall is located.
    d. The genes that encode β-lactamases can be located on either: 1) the bacterial chromosome; 2) plasmids; or 3) transposable elements (which enhance the spread of β-lactamases among different bacterial species).
    e. Penicillinase is a specific subtype of β-lactamase (the first β-lactamase to be identified in 1940). It has a very limited specificity for inactivating penicillins. Resistance to penicillin due to expression of “penicillinase” led to the development of “penicillinase-resistant” β-lactams, such as

methicillin. Then some bacteria "struck back" and now "Methicillin Resistant Staph aureus (MRSA) (or ORSA) is relatively common.

f. Extended-Spectrum β-Lactamases (ESBLs) are enzymes produced by several types of gram-negative bacteria (E coli, Klebsiella, Enterobacter, Proteus) that endow bacteria with resistance to all penicillins, cephalosporins, and monobactams, but do not affect sensitivity to carbapenems (e.g. meropenem or imipenem).

E. PBPs

Bacteria can express a mutated penicillin binding proteins (PBP) that still has enzymatic activity for cell wall synthesis, but does not bind β-lactam antibiotics. These bacteria are drug-insensitive. This is a mechanism of resistance used by S.aureus (MRSA), which expresses the penicillin-insensitive PBP2a (expressed by the mecA gene) (Lowy, 2003 and Wikipedia: MRSA). The mecA gene is found within a transferable genetic element called mec. The mec containing mobile cassette has spread rapidly through the S. aureus population via horizontal gene transfer and selection from widespread antibiotic use (Roby et.al., 2015).

F. Peptidoglycan is absent

Some bacteria (e.g. mycobacteria) lack a cell wall, and can multiply in the presence of β-lactam antibiotics, potentially resulting in serious infection (e.g. atypical pneumonia).

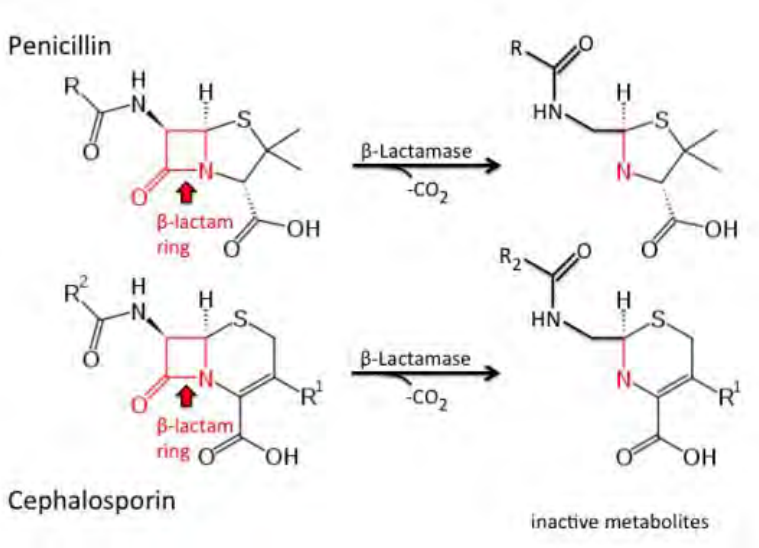
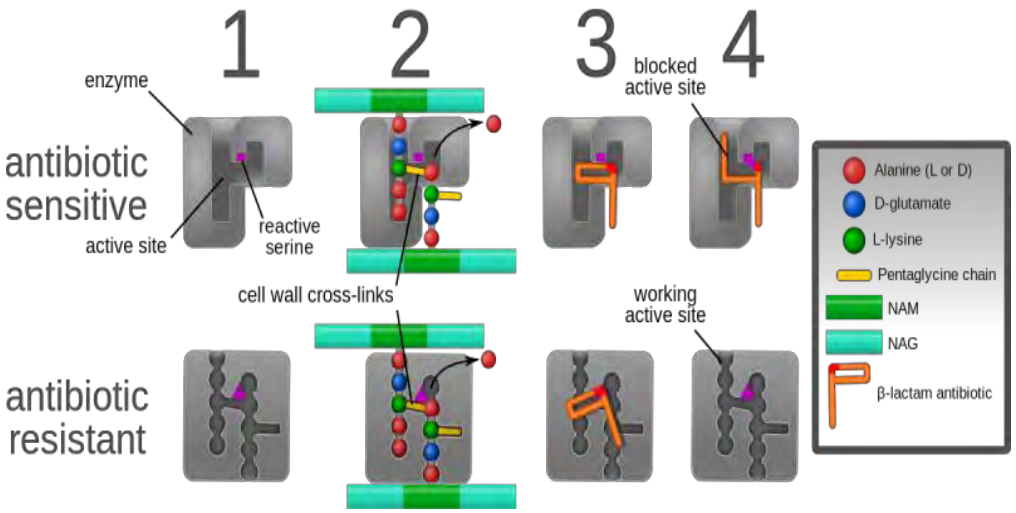


Figure 2.5: Hydrolysis of Penicillins and Cephalosporin by β-lactamase.

Penicillins and Cephalosporins share a common four-atom beta-lactam ring. Beta-lactamases are a family of enzymes produced by some gram-negative bacteria that provide a resistance to β-lactam drugs by breaking the ring open by hydrolysis, which eliminates the molecule's antibacterial actions. There are four different groups of β-lactamases that have different substrate (e.g. drug) specificities. Figure 2.6 shows the mechanism of MRSA (ORSA) resistance to β-lactam antibiotics**.**



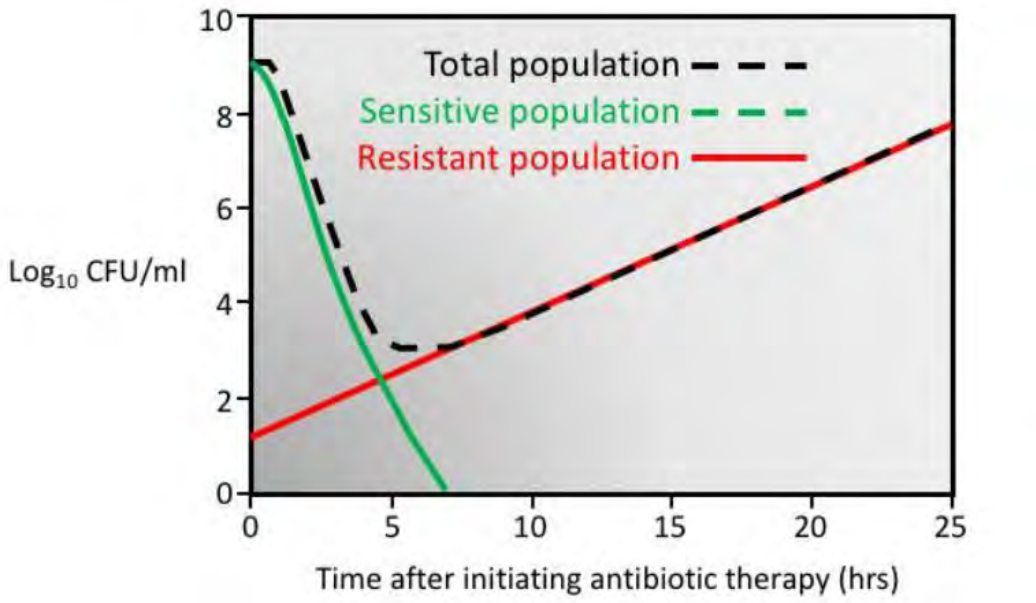
(Source: betalactam_pharm_TUSOM Pharmwiki)

Figure 2.6: Mechanism of MRSA (ORSA) resistance to β-lactam antibiotics**.**

In antibiotic-sensitive strains of bacteria, β-lactam antibiotics permanently inactivate PBP enzymes, which are essential for bacterial life, by permanently binding to their active site.

Top Row: Antibiotic Sensitive PBP. Once in the active site, the β-lactam ring springs open, permanently inactivating the sensitive enzyme.

Bottom Row: Strains of Methicillin Resistant S. aureus (MRSA/ORSA) express a PBP (PBP2a) that has an altered active site that will not allow β-lactam antibiotics to bind, resulting in resistance to this entire subclass of antibiotics. Figure 2.7 shows the Contribution of Broad Spectrum Antibiotics to Development of Antibiotic Resistance and table 2.4 represents the Cephalosporin Family.



(Source: Drusano Nature Rev Microbiol 2004)

Figure 2.7: Development of bacterial resistance**.**

Table 2.4: The Cephalosporin Family

Category	Parenteral Agents	Oral Agents
First Generation	Cefazolin	Cephalexin
Second Generation	Cefotetan, Cefoxitin, Cefuroxime	Cefuroxime axetil, Cefaclor
Third Generation	Cefotaxime, Ceftazidime, Ceftriaxone	Cefixime, Cefdinir
Fourth Generation	Cefepime	
Fifth Generation	Ceftaroline	

2.6.2 Sources of Antibiotics in the Environment

Recently the use of antibiotics in veterinary and human medicine has been widespread and consequently, the possibility of water contamination with such compounds has been increased (Xu et. al., 2007). These pollutants are continually discharged into the natural environment as parent compounds, metabolites/degradation products, or both forms by a diversity of input sources as shown in Figure 2.8 (Homem, 2011 and Ikehata, 2006).

Presence of antibiotics for human consumption in the environment occurs through discharge, entering in the sewer and reaching the Waste Water Treatment Plant (WWTP). Despite most of WWTPs are not projected to remove highly polar micro

pollutants (Xu, et. al., 2007), they can be transferred to surface waters and reach groundwater after leaching.

The sludge produced in WWTPs is utilized as soil manure and can cause problems when used as a fertilizer. Another significant pollution source is the direct delivery of veterinary antibiotics through the implementation in aquaculture. Inappropriate elimination of unused/expired drugs can also be considered as significant sources of contamination. These are derived directly from sewage discharge or landfills deposition, waste effluents from manufacture, or accidental spills during manufacturing or distribution (Xu, et. al., 2007).

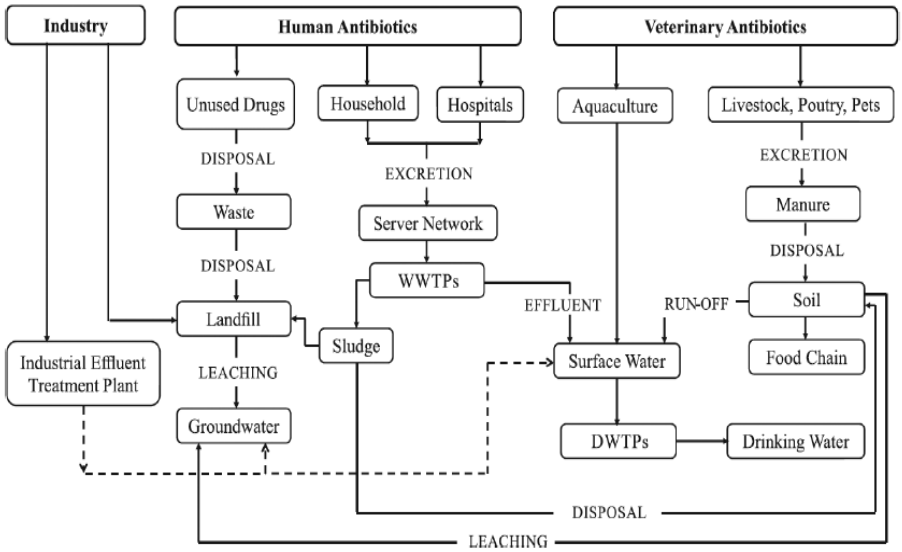


Figure 2.8: Pathways of Antibiotics in the Environment (Homem, 2011 and Ikehata, 2006)

2.6.3Effects of Antibiotic Contamination

Unlike the other conventional organic pollutants, antibiotics not only deteriorate environmental quality, but also result in the appearance and rapid spread of resistant bacteria in the environment (Neu, H., 1992). Therefore, antibiotic contamination is of increasing concern among general public, government officials and scientists about potential ecological and public health risks (Davies, 2010). Therefore, the research on antibiotic contamination will continue to be an important topic among scientific communities.

2.6.4 Antibiotic Contamination in Water and Food chain in Bangladesh

During the last two decades, Bangladesh is undergoing rapid advancement in industrial growth, especially in pharmaceutical sector. Since the enforcement of rules and regulations in complying the discharged wastewater quality is very dismal in Bangladesh, therefore there exists a risk of antibiotic contamination of pharmaceutical wastewater into surface water and land. Some recent news and research published related to this issue has stirred concern on this. This sub-section presents such information on antibiotic contamination in water and food chain in Bangladesh.

In Milk: Recently Dhaka University researchers found presence of antibiotics meant for humans, lead and detergent in packaged milk and dairy products of some of the top brands, including Milk Vita, Pran, Aarong, Igloo and Farm Fresh, among others. The antibiotics that were found in the milk include levofloxacin, ciprofloxacin and azithromycin, which are used mainly to treat bacterial infections in humans. A subsequent second test by the Dhaka University researchers on milk samples of the same brands, reconfirmed their findings, said Prof ABM Faroque, director of Biomedical Research Centre at DU, who led the research.

In River: Metronidazole, which is used to treat bacterial infections including skin and mouth, exceeded safe levels by the biggest margin, with concentrations at one site in Bangladesh 300 times greater than the safe level, the report published on the website of York Environmental Sustainability Institute at the University of York, England on May 27. The researchers detected a maximum total antibiotic concentration of 233 nanograms per litre (ng/l) in the River Thames and one of its tributaries in London. In Bangladesh, the concentration was 170 times higher, according to the research. Led by YESI's Professor Alistair Boxall, the study is first of its kind in global level, according to the report.

2.7 Pharmaceutical Wastewater Treatment Process

Pharmaceutical wastewater if disposed of with insufficient treatment may leads to great damage to the environment and groundwater resources. General treatment processes may not be effective in treating every pharmaceutical industry containing antibiotics due to its variable composition. Therefore, specific treatment is required for treatment of specific type of wastewater. The following sub sections describe the various treatment methods used in treating the pharmaceutical wastewater.

2.7.1 Hydrolytic Acidification

Hydrolysis acidification is the technology of controlling the anaerobic reaction in the hydrolysis and acidification stages. In hydrolysis acidification process, organic macromolecules and cell extracellular enzymes is decomposed into small molecules. These hydrolyzed products molecules can be dissolved in water through cell membrane synthesis of new cell material.

2.7.2 UASB (Up-flow Anaerobic Sludge Blanket) Reactor

The up-flow anaerobic sludge blanket reactor (UASB) is a single tank anaerobic process. Wastewater enters into the anaerobic reactor from the bottom, and flows upward. A suspended sludge blanket filters treats the wastewater as the wastewater flows through it (Figure 2.9).

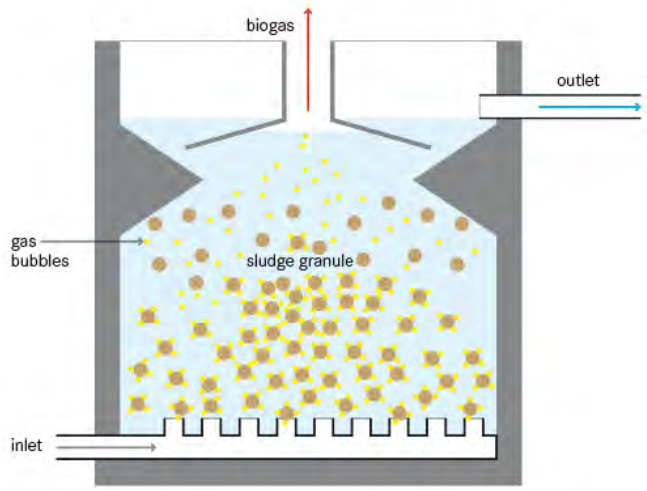


Figure 2.9: Up-flow anaerobic sludge blanket (UASB) Reactor

2.7.3 SBR (Sequencing Batch Reactor) Method

It is a fill and draw activated sludge system where wastewater is added to a single "batch" reactor, treated to remove undesirable components, and then discharged. Equalization, aeration, and clarification can all be achieved using a single batch reactor (Figure 2.10). To optimize the performance of the system, two or more batch reactors are used in a predetermined sequence of operations.

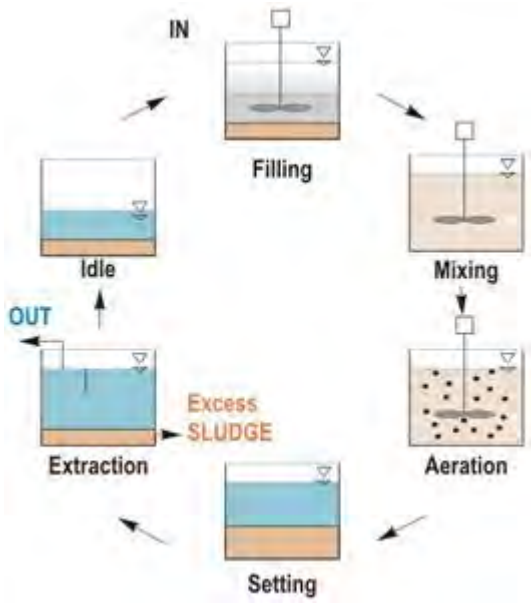


Figure 2.10: Sequencing Batch Reactor (SBR) System

2.7.4 BAC (Biological Activated Carbon) Method

In this method huge surface area and developed void structure of activated carbon acts as carriers for aggregation, propagation and growth of microorganisms under the condition of moderate temperature and nutrition, which exerted microbiological degradation simultaneously (Figure 2.11). Some bacteria that are widely found in the environment such as nitrifying bacteria or heterotrophic bacteria are responsible for metabolizing the biodegradable organic matter.

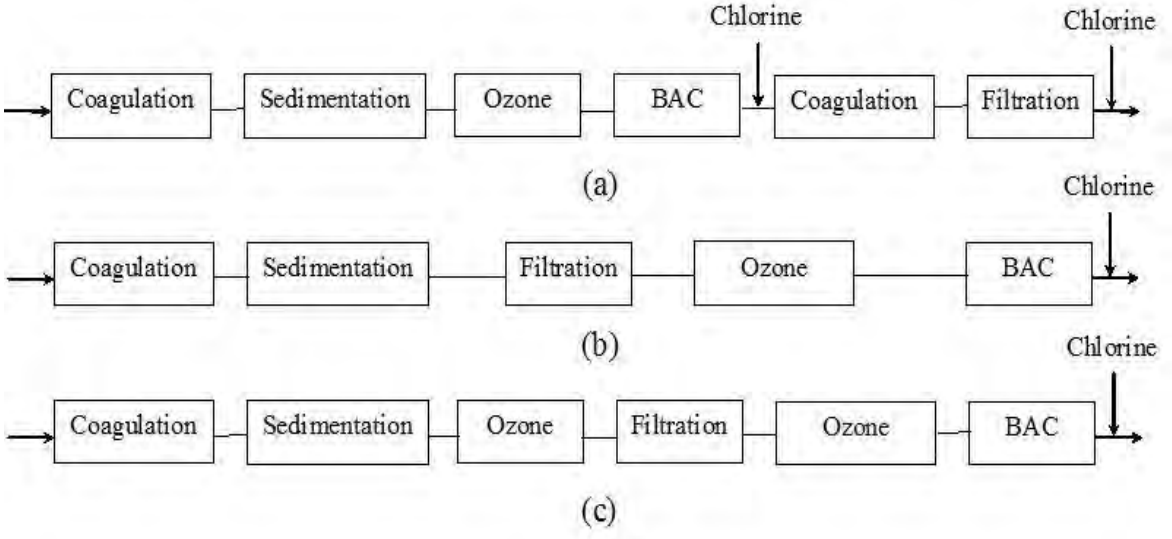


Figure 2.11: Biological Activated Carbon (BAC) Method

2.7.5 Advanced Oxidation Process (AOP)

Advanced oxidation processes involve the in-situ generation of highly potent chemical oxidants such as the hydroxyl radical, have emerged as an important class of technologies to accelerate the non-selective oxidation and thus the destruction of a wide range of recalcitrant organic contaminants in wastewater which cannot be eliminated biologically. Under proper conditions, the components to be removed are converted completely to CO_2, water and mineral salts. Advanced oxidation processes are characterized by the production of the hydroxyl radical (OH^-) and hydroxyl radicals are generally accelerated by the combinations of some commonly used oxidants such as H_2O_2, UV, O_3, TiO_2 and Fe^{2+}(Figure 2.12).

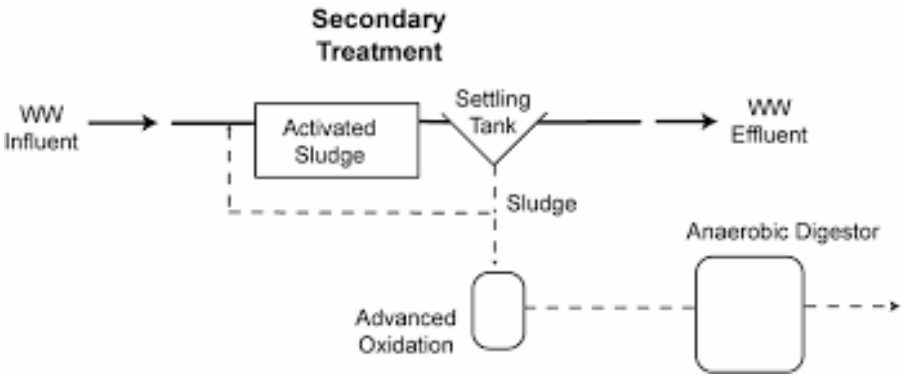


Figure 2.12: Advanced Oxidation Process (AOP)

2.7.6 Rotating Biological Contactor (RBC)

A Rotating Biological Contactor (RBC) is a secondary biological treatment process which utilizes a rotating shaft surrounded by plastic media discs (Figure 2.13). RBCs utilize a fixed film media system similar to a trickling filter. The microbial growth is passed through the wastewater, while the wastewater is passed through the microbial growth in a trickling filter. Biological growth attaches to the media discs and form a slime layer over the discs. The rotation of the shaft alternately exposes the biomass with the wastewater and then with the oxygen in the atmosphere.

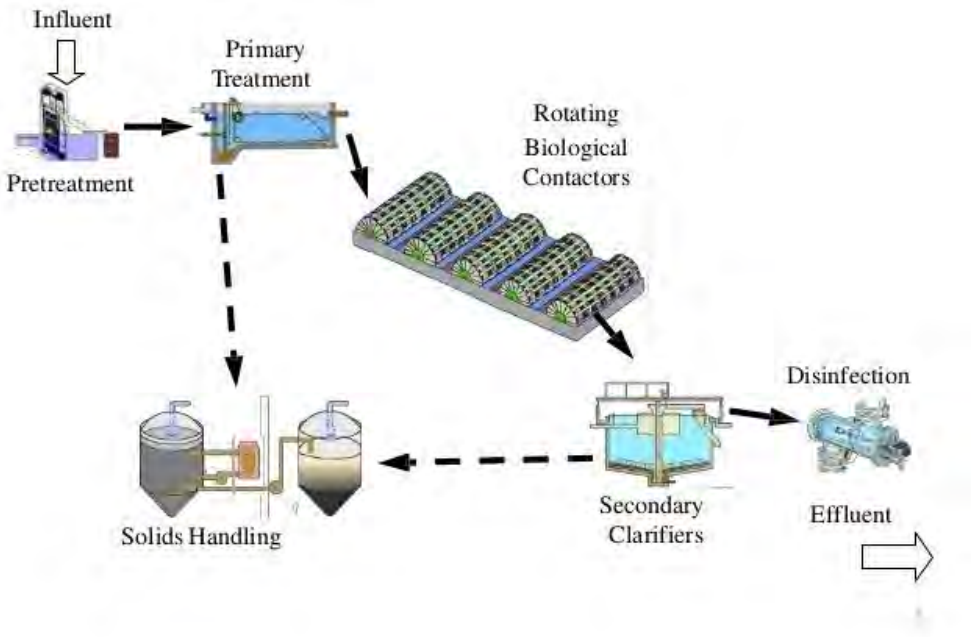


Figure 2.13: Rotating Biological Contactor (RBC) Flow

2.8 Review of Treatment Process of Pharmaceutical Wastewater containing β-Lactam ring

2.8.1 Biodegradation by Bacillus Subtilis 1556WTNC

In this process biodegradation of pharmaceutical active compounds (β-Lactam antibiotics) is treated in sewage effluents as a function of β-lactamase produced Bacillus subtilis 1556WTNC. The enzymatic biodegradation process was conducted under the optimal conditions for β-lactams production (5.9 $\log_{10}$ CFU mL^{-1}; pH 6.5; temperature 35 °C for 12 days). Bacillus subtilis 1556WTNC exhibited the potential to produce β-lactamase and biodegrade β-lactam antibiotic genetically (Adel, A. S. A., 2014).

2.8.2 Anaerobic Membrane Bioreactor (AnMBR)

An 180 L anaerobic membrane bioreactor (AnMBR) was operated for 253 days to treat real pharmaceutical wastewater, which mainly composed of β-lactams antibiotics including amoxicillin, ceftriaxone, cefoperazone and ampicillin. The operation was divided into three stages with hydraulic retention time (HRT) of 48 h, 36 h and 24 h, respectively, the corresponding average organic loading rates and antibiotics loading rates increased from 2.37 ± 0.28 to 4.46 ± 0.87 kg-COD·m^{-3}·d^{-1} and from 19.06 ± 0.67 to 37.91 ± 3.57 g-BLAs·m^{-3}·d^{-1}, respectively (Bin, et. al., 2018).

2.8.3 Photo-Fenton Process

Photo-Fenton process at near-neutral pH was applied for the removal of β-Lactam antibiotic oxacillin (OXA) in water using artificial and sunlight. Initially, the main variables of the process (Fe(II), H_2O_2, and light power) were optimized by a statistical factorial design (2^3 with center points). In the photo-Fenton system, the H_2O_2 alone, UV-light/ H_2O_2, and Fe(II)/ H_2O_2 subsystems presented a significant participation on antibiotic removal (Giraldo, et. al., 2018).

2.8.4 Advanced Oxidation Processes

Advanced oxidation processes can work as alternatives or complementary method in traditional wastewater treatment and highly reactive free radicals, especially hydroxyl radicals (OH) generated via chemical (O_3/H_2O_2, O_3/OH-), photochemical (UV/O_3, O_3/H_2O_2) reactions, serve as the main oxidant. It was found that most of the investigated advanced oxidant treatment processes for the oxidation of antibiotics in water are direct and indirect photolysis with the combination of H_2O_2, TiO_2, Ozone and Fenton's reagent (Ayse, et. al., 2017).

2.8.5 Degradation of β-Lactam Antibiotics with Hydroxylamine

The degradation of β-Lactam antibiotics with hydroxylamine is nucleophilic addition of hydroxylamine on the β-Lactam carbonyl carbon, the pH of the medium is considered to affect the nucleophilic reaction. Thus the effect of pH on the degradation was investigated with cefpodoximeproxetil and by adjusting the pH of 0.1% hydroxylamine solution to 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0. According to the results, the degradation progressed at pH beyond 7.0, while it only slightly progressed at pH 4.0 and 5.0. The residue of cefpodoximeproxetil was 99.5% at pH 4.0, and 65.8% at pH 7.0 and 40.3% at pH 10.0 (Fukustsu, et. al., 2006). The degradation also progressed with pH-unadjusted hydroxylamine, the residue of cefpodoximeproxetil being 53.0% after the degradation. Since the pH of the hydroxylamine solution was 9.2, the nucleophilicity of hydroxylamine was maintained and had reacted with the β-lactam carbonyl carbon.

2.9 Effluent Quality Standards for Industrial Unit of Bangladesh

The Bangladesh government has formulated effluent quality standards for the discharge of industrial waste water to safeguard the environment according to ECA, 1995. These standard values are described in ECR, 1997 is presented in Table 2.5.

Table 2.5: Standards for Wastewater Discharge from Industrial Units or Projects (ECR, 1997).

Sl. No.	Parameter	Unit	Standards for Wastewater from Industrial Units (ECR, 1997)		
			Inland Surface Water	Public Sewerage system connected to treatment at second stage	Irrigated Land
1	Ammonical Nitrogen (N molecule)	mg/l	50	75	75
2	Ammonia (free ammonia)		5	5	15
3	Arsenic		0.2	0.5	0.2
4	BOD_5 20°C	mg/l	50	250	100
5	Boron (B)	mg/l	2	2	2
6	Cadmium (Cd)	mg/l	0.05	0.5	0.5
7	Chloride (Cl^-)	mg/l	600	600	600
8	Chromium (total Cr)	mg/l	0.5	1	1
9	COD	mg/l	200	400	400
10	Chromium (hexavalent Cr)	mg/l	0.1	1	1
11	Copper (Cu)	mg/l	0.5	3	3
12	Dissolved Oxygen (DO)	mg/l	4.5-8	4.5-8	4.5-8
13	Electrical Conductivity	mg/l	1200	1200	1200
14	Total Dissolved Solids (TDS)	mg/l	2100	2100	2100
15	Fluoride (F)	micro	7	15	10
16	Sulfide (S)	mho/cm	1	2	2
17	Iron (Fe)		2	2	2
18	Total Kjeldahl Nitrogen (N)	mg/l	100	100	100
19	Lead (Pb)	mg/l	0.1	0.1	0.1
20	Manganese	mg/l	5	5	5

	(Mn)				
21	Mercury (Hg)	mg/l	0.01	0.01	0.01
22	Nickel (Ni)	mg/l	1	1	1
23	Nitrate (N molecule)	mg/l	10	Undetermined	10
24	Oil & grease	mg/l	10	20	10
25	Phenol compounds (C_6H_5OH)	mg/l	1	5	1
26	Dissolved Phosphorus (P)	mg/l	8	8	10
27	Radioactive materials:	To be specified by Bangladesh Atomic Energy Commission			
28	pH		6-9	6-9	6-9
29	Selenium (Se)	mg/l	0.05	0.05	0.05
30	Zn (Zn)	mg/l	5	10	10
31	Total Dissolved Solids	mg/l	2100	2100	2100
32	Temperature	Centig rade			
33	Summer		40	40	40
34	Winter		45	45	45
35	Total Suspended Solid (TSS)	mg/l	150	500	200
36	Cyanide (CN)	mg/l	0.1	2	0.2

Chapter 3
METHODOLOGY

3.1 Introduction

The present chapter describes the methods adopted in this thesis work to achieve its goals. The chapter includes the location of sampling point, collection of samples, laboratory methods for characterization of collected samples and the methods adopted in decontamination of β-Lactam ring through varying reagent and contact time.

3.2 Methodology

3.2.1 Location of Sample Collection

The sample was collected from a renowned Pharmaceutical Industry, located at BSCIC Industrial Estate, Tongi, Gazipur, Dhaka (Figure 3.1). The industry produces various pharmaceuticals products such as Tablet, Capsules and Dry Syrup. The pharmaceutical industry selected in the present study has a biological (Activated sludge process) CommonEffluent Treatment Plant (CETP) with a capacity of 24 m^3/ day.Figure 3.2 and Figure 3.3 shows the flow diagram and different units of CETP of the industry respectively.

Figure 3.1: Location Map of the industry

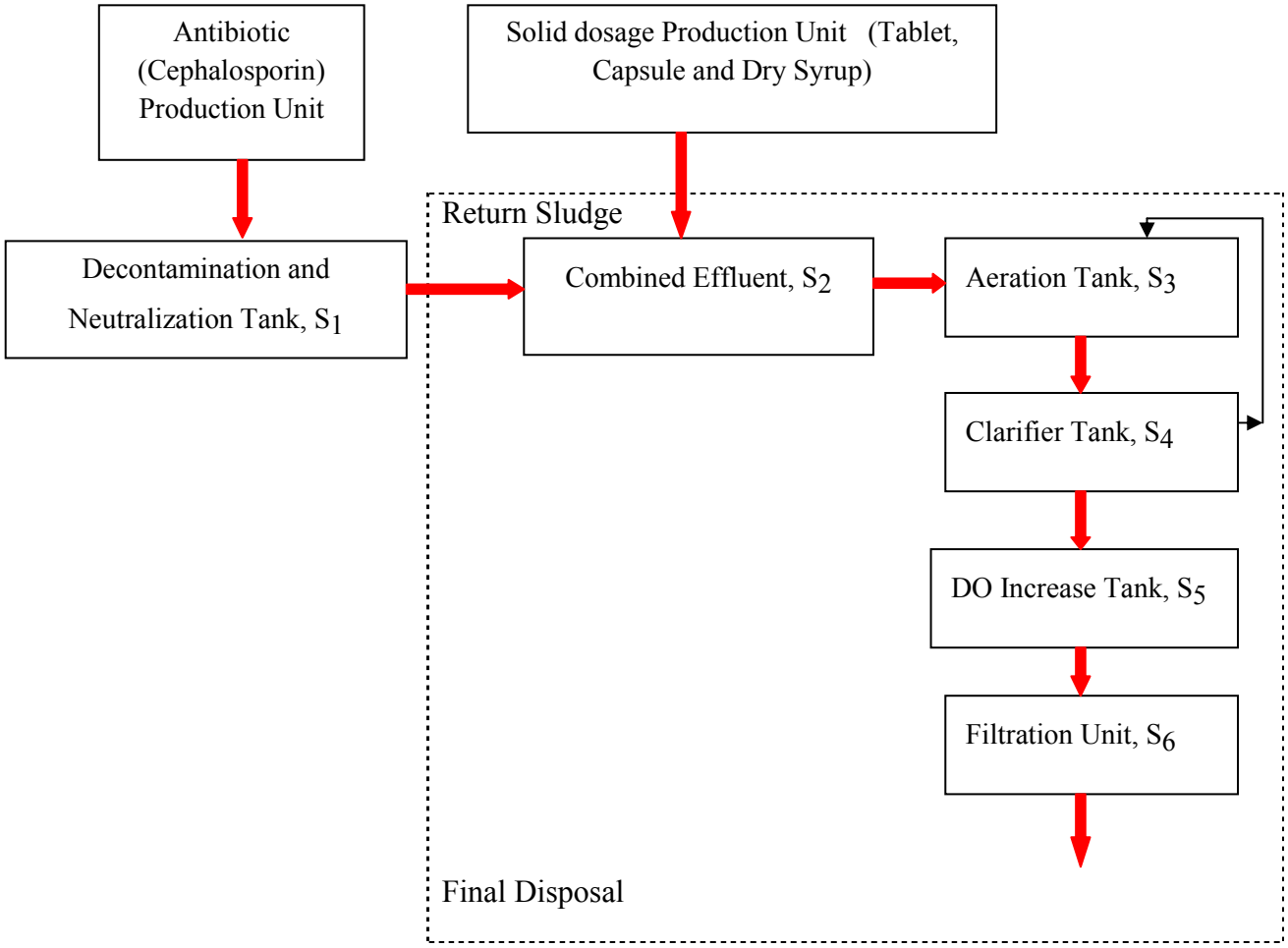


Figure 3.2: Process flow diagram of CETP

Figure 3.3: Effluent Treatment Plant(ETP) of a renowned PharmaceuticalIndustry.

Studied pharmaceutical industry has two types of production unit, solid dosage unit and cephalosporinantibiotic production unit. At first wastewater from cephalosporin unit is decontaminated by 10% NaOH and HCl with 6.0hr retention time, because of the presenceof β-Lactam ring coming from the cephalosporin production unit. Decontamination is necessary for saving the aquatic environment from antibacterial resistance. Wastewater samples were collected from the different units of CETP to characterize the wastewater and also to study the performance of those units in CETP. Samples were also collected from the decontamination tank and HCl and acetic acid were used separately for neutralizing the water in industry's quality control laboratory. The reason for using two types of acids in neutralization is to study the effects of acids in microbiological growth in aeration tank and the variation in cost. In this study,decontamination process was performed by dosing 10% NaOH to raise pH around 12.5 (stay time 3hr, 4hr and 5hr for study purpose) followed by Acetic acid and Hydrochloric aciddosing separately to attain pH around 7.0. Neutralization of decontaminated wastewater is necessary because high alkalinity can affect the bacterial culture of central ETP. Decontamination performance was evaluated by HPLC analysis. Collection of the sample (S_1)was performed after decontamination, allowed to stay 6.0hr,and neutralized and then transferred to combined tank where it was mixed with wastewater coming from other solid dosage form production units. After that,samples were collected from CentralEffluent Tank (S_2), Aeration Tank (S_3), Clarifier Tank (S_4), DO Increase Tank (S_5) and Filtration Unit(S_6) (Figure 3.4) at the same day for evaluation of effectiveness of the treatment. Finally effluent just before its final discharge into the surface water has been again analyzed in HPLC to test any presence of β-Lactam ring.Collection of those samples was done in plastic containers which are cleaned properly by distilled water. Six effluent samples collected from six units are follows:

S_1-Decontaminated Effluent	
S_2-Combined Effluent	
S_3-Aeration effluent	
S_4- Clarifier Effluent	
S_5-DO Increase Tank Effluent	
S_6- Filter Outlet (Sand Filter + Activated Carbon Filter)	**Figure 3.4**: Collected Wastewater Samples from different units of CETP

3.2.2 Decontamination of β-lactam Ring Wastewater

Primarily wastewater containing β-Lactam ring from Cephalosporin production unit is decontaminated by dosing 10% NaOH at pH 12.0-12.5 in decontamination tank of wastewater treatment plant with the help of automatic pH controller(ProMinent DULCOMETER Controllers and Sensors)(Figure 3.5) asβ-Lactam ring is successfully broken at highly alkaline condition (pH 12.0-12.5) which has found from literature (Hou and Poole, 1971, Deshpande, et. al., 2004, Eiichi and Hitomi, 2003).Dosing of NaOH is an online system (auto pH controller). It dosage the NaOH by a dosing pump (Figure 3.6)until the water attain the pH 12.0 to 12.5. When the wastewater reaches the required pH (12.0-12.5), it was hold in decontamination tank in circulation condition by a submersible pump. Then 50 ml decontaminated wastewater has been collected from the decontamination tank after 3hr, 4hr and 5hr time period. The collected 50ml decontaminated wastewater then neutralized by HCl and CH_3COOH to pH around 7.0 separatelyin factory's Quality Control Laboratory. For two different antibiotics such as Cefixime and Cefuroxime, samples were collected separately and neutralization using

HClwas performed separately. Similar procedure followedfor CH_3COOH neutralization. After neutralization the sample wastewater has been analyzed by HPLC (High Performance Liquid Chromatography) in factory'sQuality Control Laboratory for evaluation of decontamination performance. Figure 3.7 shows the flow diagram of decontamination and neutralization method adopted in the study. Industry'sHPLC machine (Figure 3.8) was used in the present study to assess the performance of decontamination of β-Lactam ring. HCl and CH_3COOH are used as a neutralization agent for decontaminated wastewater to analyze the comparative evaluation of their cost, time and effect on microbial culture on biological ETP.

Figure 3.5: ProMinent DULCOMETERpHControllers

Figure 3.6: NaOH dosing pump of decontamination unit

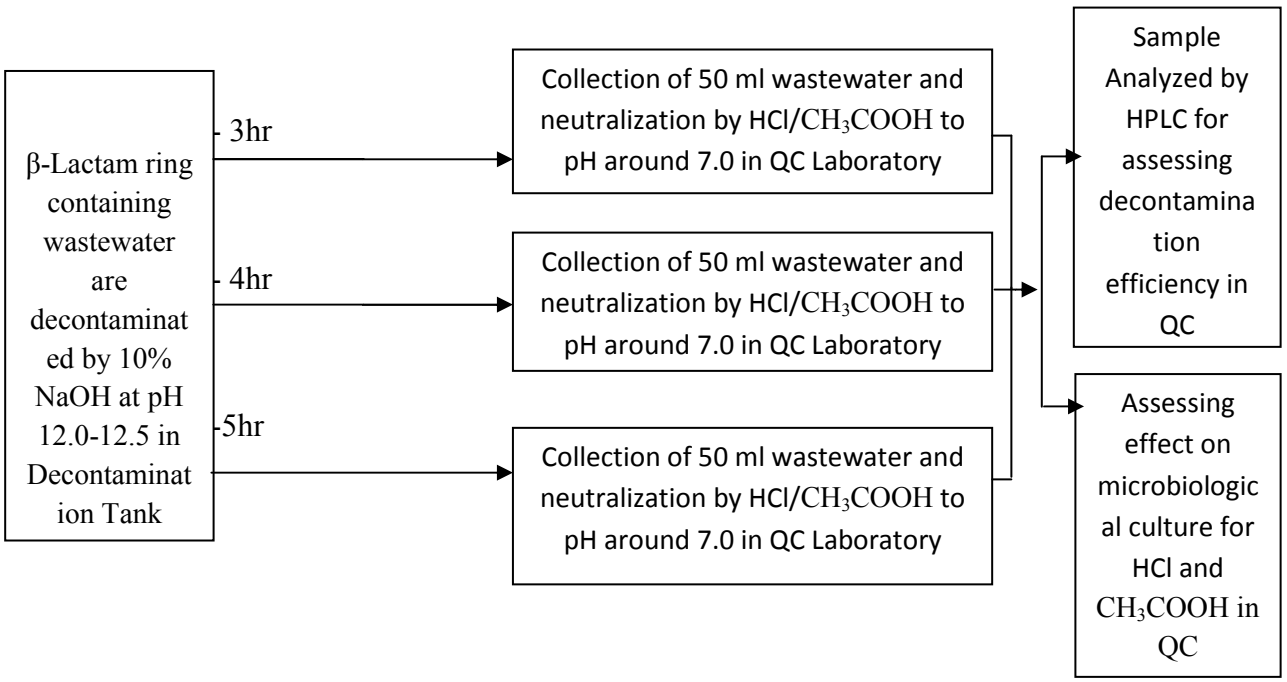


Figure 3.7: Flow diagram of decontamination analysis

Figure 3.8: High Performance Liquid Chromatography (HPLC) machine used in the study

3.2.3 Laboratory Analysis

The characteristics of collected wastewater samples and treated effluent were determined in the Environmental Engineeringlaboratory of BUET. In the present study pH, EC, turbidity, TSS, TDS, color, DO, COD, BOD_5, Cl^-, Cd, Cr, Cu, Zn were determined following the standard methods (AWWA, 1998) for wastewater sample (Figures 3.9) from the stated units ofETP. Table 3.1 presents the methods adopted along with the name of the apparatus used in determining the concentration of parameters.

Table 3.1: List of analyzed parameters and procedures

Parameters	Unit	Method	Apparatus
pH	-	SM 4500 H-B	pH Meter
EC	µs/cm	SM 5210	EC Meter
Turbidity	NTU	SM 2130 B	HACH
TSS	mg/L	SM 2540 D	Oven
TDS	mg/L	SM 2540 B-D	Oven
Color	Pt-Co	SM 2120C	DR 2010 UV Spectrophotometer
DO	mg/L	-	DO Meter
COD	mg/L	SM 5220C	DR 2010 UV Spectrophotometer
BOD_5 at 20^0C	mg/L	SM 5210	Winkler bottle
Cl^-	mg/L	Titration	Burette and Funnel
Cd	ppm	USEPA 213.2-SM3113B	Atomic Absorption Spectrophotometer
Cr	ppm	USEPA 200.9-REV2.2: SM31111B	Atomic Absorption Spectrophotometer
Cu	ppm	USEPA 200.9-SM31111B	Atomic Absorption Spectrophotometer
Zn	ppm	USEPA 200.9-SM31111B	Atomic Absorption Spectrophotometer

(a) (b)

Figure 3.9: Heavy Metal Analysis of Wastewater Samples in the Laboratory

3.2.4 Microbial Analysis (Aerobic bacterial count test)

a. At first sample was collectedin sterile sampling bottles.
b. Made the 10 fold serial dilution of sample up to 10^6 fold using sterile normal saline.
c. Then 1ml diluted sample was made in duplicate plates from each of 10^4, 10^5 and 10^6 dilution.
d. Poured sterile molten R2A agar (less than 50°C) in all the plates.
e. Made negative control by pouring sterile molten R2A agar (less than 50°C) in an empty sterile plate.
f. Allowing the plates to solidify then incubate the plates in inverted condition at 30-35°C for 5 days.
g. After incubation hose the plates having not more 300 cfu/plate for counting.
h. Counted the colonies of two duplicate plates and make the average.
i. Multiply the average counting with dilution fold and express the result in cfu/ml.

3.2.5 HPLC Analysis

High Performance Liquid Chromatography (HPLC) relies on pumps to pass a pressurized liquid and a sample mixture through a column filled with adsorbent, leading to the separation of the sample components. The active component of the

column, the adsorbent is typically a granular material made of solid particles (e.g., silica, polymers, etc.), 2–50 μm in size.

Analysis of Cefuroxime Axetil

Mobile Phase Preparation:

Buffer: 23.0 g of monobasic ammonium phosphate in1000 ml of water, adjusted to a pH of 2.4 with phosphoric acid.

Mobile Phase: Buffer: Methanol (62:38).

Chromatographic Condition:

Apparatus : HPLC with Spectrophotometer UV Detector

Column : Zorbax TMS, L13, 4.6-mm X 250-mm; 5-μm or equivalent

Flow Rate : 1.2 ml/minute

Wavelength : 278 nm

Inject Volume : 20 μl

Run Time : 20 minute (Approximately)

Analysis of Cefixime

Mobile Phase Preparation:

Solution (A):25ml of 0.4M tetrabutylammoniumHydorxide solution (20.8ml of 12.5% TBAH) in a 1000ml volumetric flask and volume with water. Adjust with 1.5M phosphoric acid to a pH of 6.5±0.05.

Mobile Phase:Solution A: Acetonitrile (1:3)

Solution (B):13.6g/l of monobasic potassium phosphate in water.

Solution (C):14.2g/l of anhydrous dibasic sodium phosphate in water.

Diluent: Adjust the pH of solution-C with solution-B to 7.0±0.05.

Chromatographic Condition:

Apparatus : HPLC with UV/PDA Detector

Column : L1; (12.5cmmx4.6mm); 4μm

Flow rate : 1.0ml/minute

Inject Volume : 10 μl

Column Oven : 40°C

Wavelength : 254nm

Run time : About 15 minutes

Chapter 4
RESULTS and ANALYSIS

4.1 Introduction

The experimental results obtained from the conducted laboratory analysis to achieve the goals of the present research have been described in this chapter. The characterization of the wastewater of pharmaceutical industry along with the decontamination process and effectiveness of different units in the treatment process is presented in the following sections. The cost comparison in decontamination process is also shown in this Chapter. A detail analysis has also been carried out here to interpret the results.

4.2 Flow Volume of Wastewater

The wastewater from the studied industry is discharged from two sources: one from decontamination tank (where decontamination of β-Lactam ring occurs) and other from solid dosage units and the two types of wastewater are then mixed in a common tank (CETP) (Figure 3.2).The flow-rate of the wastewater entering into the decontamination tank and CETP were measured separately using flow meter (Figure 4.1) and obtained results are discussed in the following sub-section.

(a) Flow Meter used in Decontamination Tank

(b) CETP inlet Flow Meter

Figure 4.1: Flow Meter of Decontamination Tank and CETP

4.2.1 Influent Wastewater flow volumes

The flow rate of the influent wastewater going to the Common ETP was measured over a seven-day period and shown in Figure 4.2. Figure 4.2 shows that the daily flow rate varies from 16-23 m^3/day with an average value of19.64 m^3/day.

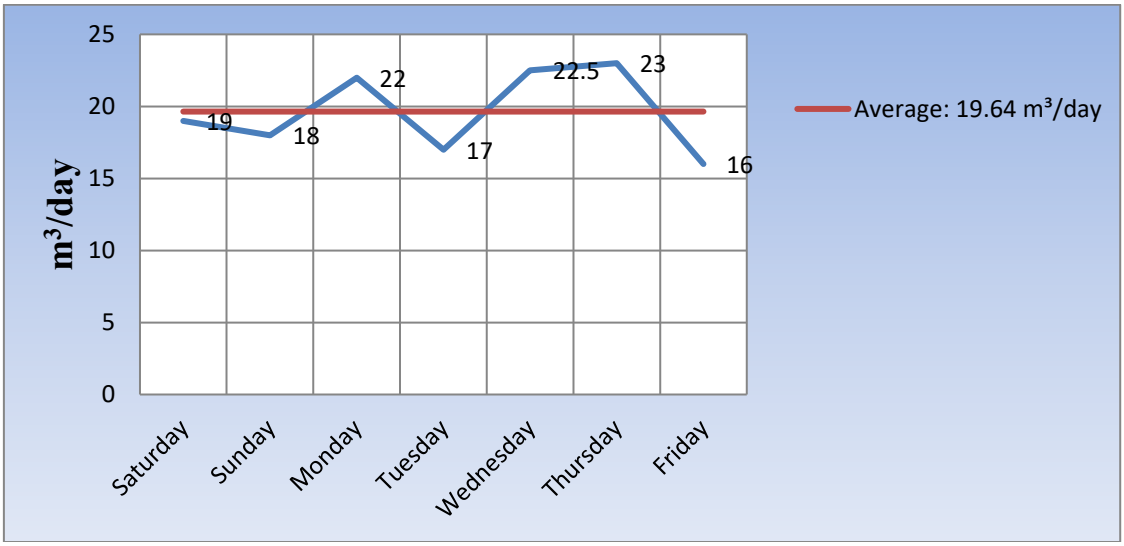


Figure 4.2: Typical daily flow rate of the influent to the CETP

The typical daily flow rate of the influent wastewater going to the decontamination tank was measured and is shown in Figure 4.3. Figure 4.3 shows that the daily flow rate of decontamination tank varies from 2.6-3.5 m^3/day with an average value of 3.07 m^3/day which is much less compared to Common ETP influent wastewater. Therefore, it can be said that the influent to the CETP from Solid dosage unit is much higher than the influent from decontamination tank.

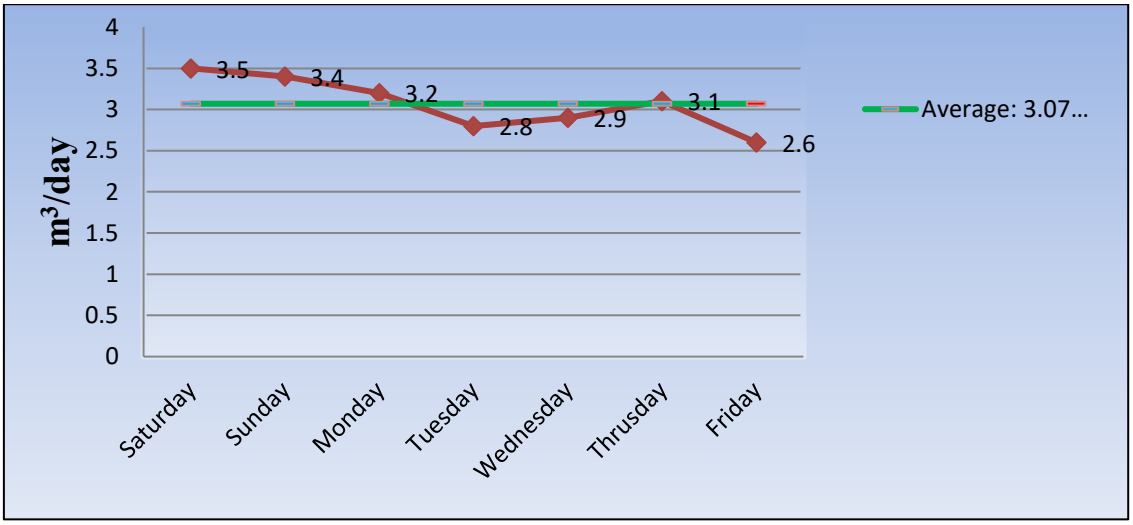


Figure 4.3: Typical daily flow rate of the influent to the decontamination tank

4.3 Characterization of Pharmaceutical Industrial Wastewater

The characterization of the collected effluent samples from industry's different treatment units (Decontamination tank, Common tank, Aeration tank, Clarifier, DO Increase tank and Filtration unit) has been performed. Fourteen parameters such as pH, BOD_5, COD, TSS, TDS, Color, Turbidity, EC, Cl, Zn, Cr, Cu and Cd have been tested to determine the characteristics of wastewater. Table 4.1 presents the characteristics of decontaminated wastewater (Sample, S_1) with average concentration of 3 samples.

Table-4.1: Characteristics of Decontaminated Wastewater (S_1)

Parameter	Unit	Average Concentration	Standards for Wastewater from Industrial Units (ECR, 1997)		
			Inland surface water	Public Sewerage System	Irrigated land
pH	-	6.05	6-9	6-9	6-9
EC	µS/cm	941	1200	1200	1200
Turbidity	NTU	25.5	-	-	-
TSS	mg/L	2621	150	500	200
TDS	mg/L	461	2100	2100	2100
Color	Pt-Co	124	-	-	-
DO	mg/L	0.29	4.5-8	4.5-8	4.5-8
COD	mg/L	504	200	400	400
BOD_5	mg/L	200	50	250	100
Cl^-	mg/L	96	600	600	600
Cd	ppm	0.005	0.05	0.5	0.5
Cr	ppm	0.006	0.5	1	1
Cu	ppm	0.008	0.5	3	3
Zn	ppm	0.205	5	10	10

From Table 4.1, it has been found that decontaminated wastewater is slightly acidic having high TSS, COD and BOD_5 with a very low concentration of dissolved oxygen (0.29 mg/l) which does not comply with discharge limit of ECR, 1997. Presence of heavy metals in this sample is not significant and they are within the discharge limits according to ECR, 1997.

The analysis of combined wastewater (mixed wastewater from decontamination tank and solid dosage units) was performed for 3 samples and average results is shown in Table 4.2

Table 4.2: Characteristics of Combined Wastewater (S_2)

Parameter	Unit	Average Concentration	Standards for Wastewater from Industrial Units (ECR, 1997)		
			Inland surface water	Public Sewerage System	Irrigated land
pH	-	7.46	6-9	6-9	6-9
EC	µS/cm	470	1200	1200	1200
TDS	mg/L	330	2100	2100	2100
DO	mg/L	0.394	4.5-8	4.5-8	4.5-8
COD	mg/L	444	200	200	200
BOD_5	mg/L	152	50	50	50
Turbidity	NTU	32.3	-	-	-
TSS	mg/L	49	150	150	150
Color	Pt-Co	106	-	-	-
Cl^-	mg/L	46	600	600	600
Cd	ppm	0.00	0.05	0.05	0.05
Cr	ppm	0.003	0.5	0.5	0.5
Cu	ppm	0.008	0.5	3	3
Zn	ppm	0.251	5	10	10

It is observed that although TSS, BOD_5 and COD of the mixed wastewater are much less compared to that of decontamination water, still their concentration is higher than the allowable wastewater discharge limit set by ECR, 1997. The reason of comparatively less amount of BOD_5, COD, and TSS in Common Wastewater Tank is due to the less flow volumes of decontamination tank than that of solid dosage unit.

Although the characteristics of pharmaceutical wastewater varies widely depending upon the type and volume of products, the concentration found in the present study (Table 4.2) shows conformity with the findings of other researchers (Table 2.1, Chapter 2), specially pH, BOD_5 and COD. Characteristics of combined wastewater (Table 4.2) especially pH, COD, BOD_5 are quite similar as found by Islam (2012) which has been discussed in Chapter 2, Zn content is almost same as found in literature (Rohit and Ponurugan, 2013). Rest of the parameter differs a wide range due to the variation of product formulation and type of material used for manufacturing.

4.4 Effectiveness of Decontamination Process

The industry produces antibiotic 'Cephalosporin' in Antibiotic poduction unit. Generally β-Lactam ring containing Cefuroxime Axetil and Cefixime are formulated in Cephalosporin production. The wastewater produced from this unit is stored in a Decontamination tank (Figure 4.4). Clothings worn by the Lab Personnels working in manufacturing area are washed separetly in the laundry room and this wastewater also enetres into the decontamination tank.

Before entering into the common ETP, this wastewater containing β-Lactam ring needs to be decontaminated properly by applying special treament method, since conventional ETP will not be able to treat it and if untreated, this antibiotic containing effluent discharged from ETP will pose a threat to the environment and public health.

To decontaminate this wastewater, commonly HCl and NaOH is used with 6.0 hr retention time. But 1.0% Hydroxylamine is found very effective in decontamination of Cephalosporin wastewater (Naoto et. al., 2006) but it is more costly than NaOH which is the main reason for using NaOH for decontamination of Cephalosporin wastewater. In this study, experiments were conducted using CH_3COOH with an objective to see its effectiveness in decontamination and also its effects in influencing microbial quality of water in biological treatment units in later stage of ETP. Along with this trial, reaction time was varied using both HCl and CH_3COOH to study the effect of time in decontamination process. The cost comparison using HCl and CH_3COOH in decontamination is also made at the end of this chapter.

Figure 4.4: Industry's Decontamination Tank

The flow diagram in Figure 4.5 shows the activities carried out in the present study to decontaminate the β-Lactam ring containing wastewater coming from Cephalosporin unit (Antibiotic production unit) in decontamination tank.

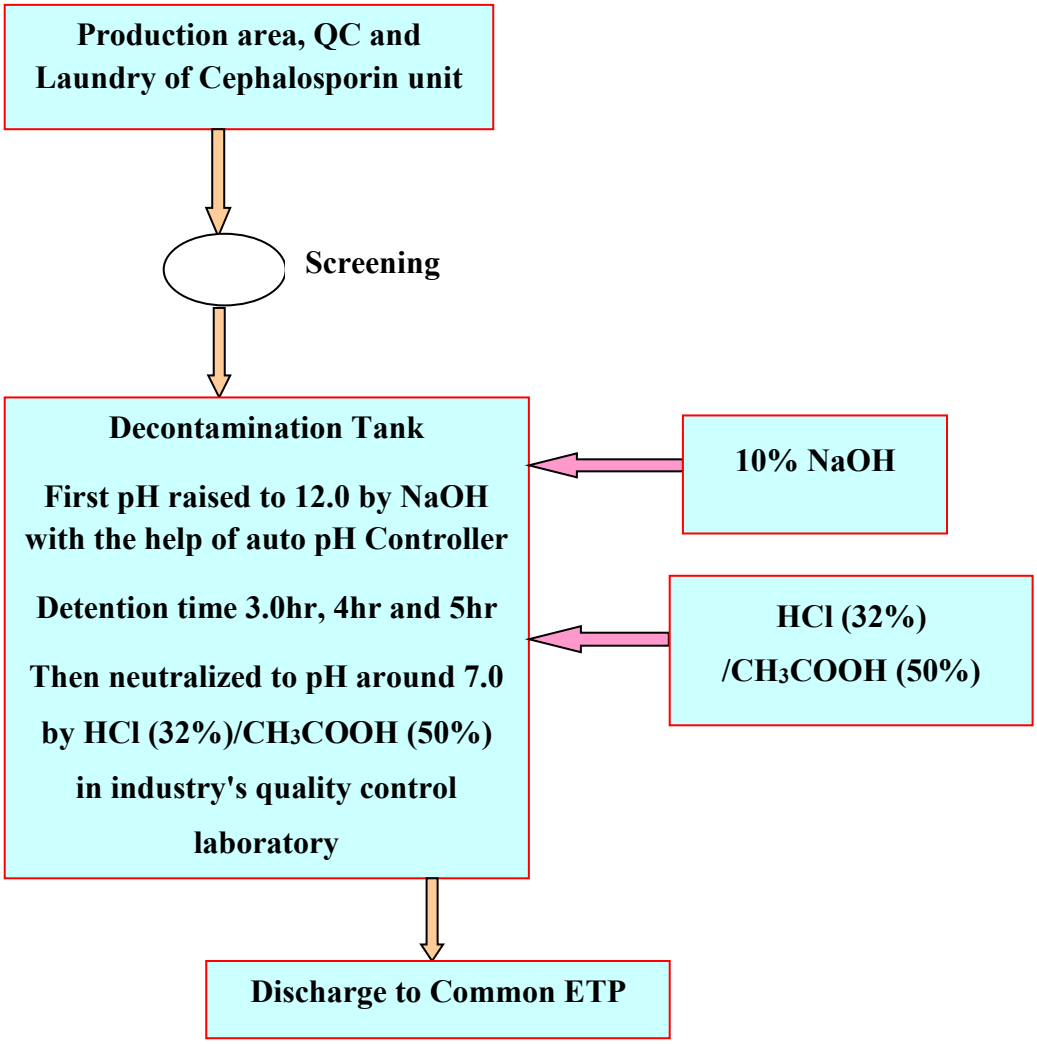
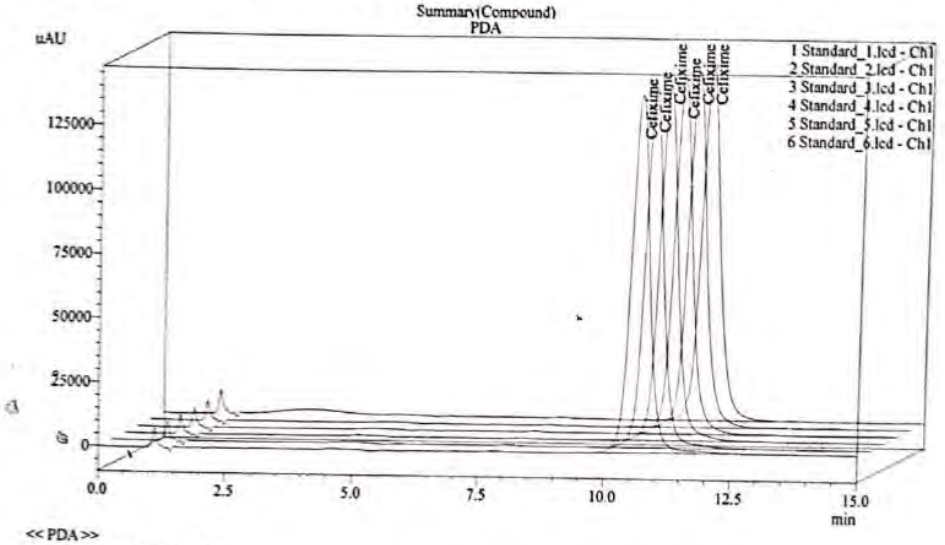


Figure 4.5: Flow Diagram of β-Lactam ring Decontamination Process

In thepresent study for decontamination process (breaking down of β-Lactam ring of two compound i.e. Cefixime and Cefuroxime), first 10% NaOH was used in wastewater of decontamination tank, then it was neutralized by HCl (32%) and CH_3COOH (50%) separately in industry's quality control laboratory allowing varying reaction time such as 3 hr, 4 hr and 5 hr in decontamination tank. After this, sample was taken and performance of decontamination has been analyzed by HPLC which is shown in following Chromatograms (Figure 4.6-Figure 4.21). The analysis of HPLC result is discussed in the following sub-sections.

System Configuration
<<Instrument>>
Instrument Name : CE-QC-HPL-001
Description : LC-2030C 3D

Sample Information
Acquired by : Ruksana Sultana
Project Name : Cefixime
Sample Name : Cefixime WS
Sample ID : Standard
Method File : Rofixim PFS.lcm
Data File : Standard_1.lcd
Vial# : 3
Injection Volume : 10
Date Acquired : 28/06/2018 14:15:59
Date Processed : 30/06/2018 08:29:20



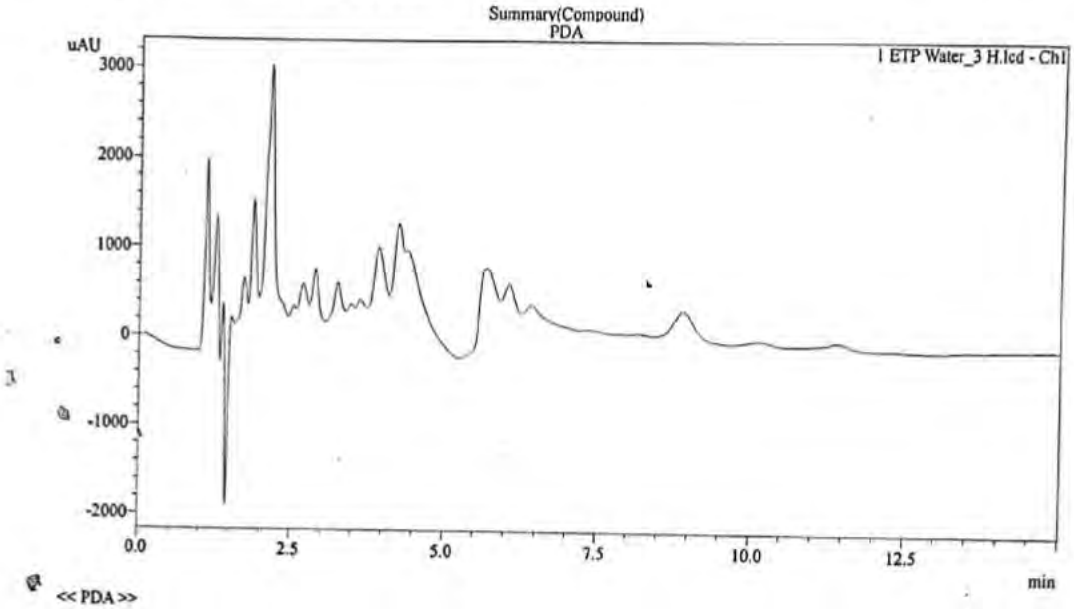
<< PDA >>
ID#1 Compound Name: Cefixime

Title	Sample ID	Ret. Time	Area	Area%	Height	Plates/meter(USP)
Standard_1.lcd	Standard	10.707	3588855	100.000	135794	27337
Standard_2.lcd	Standard	10.703	3580593	100.000	135487	27811
Standard_3.lcd	Standard	10.683	3582372	100.000	133434	27749
Standard_4.lcd	Standard	10.669	3581104	100.000	135400	27720
Standard_5.lcd	Standard	10.651	3581157	100.000	137606	27748
Standard_6.lcd	Standard	10.641	3571790	100.000	137473	27898
Average		10.676	3580978	100.000	135866	27711
%RSD		0.252	0.152	0.000	1.135	0.699
Standard Deviation		0.027	5452	0.000	1542	194

Figure 4.6: Chromatogram of Standard solution of Cefixime for NaOH and CH_3COOH Treatment

System Configuration
<<Instrument>>
Instrument Name : CE-QC-HPL-001
Description : LC-2030C 3D

Sample Information
Acquired by : Ruksana Sultana
Project Name : Cefixime
Sample Name : ETP Water
Sample ID : ETP Water
Method File : Rofixim PFS.lcm
Data File : ETP Water_3 H.lcd
Vial# : 6
Injection Volume : 10
Date Acquired : 28/06/2018 16:49:47
Date Processed : 30/06/2018 08:30:10



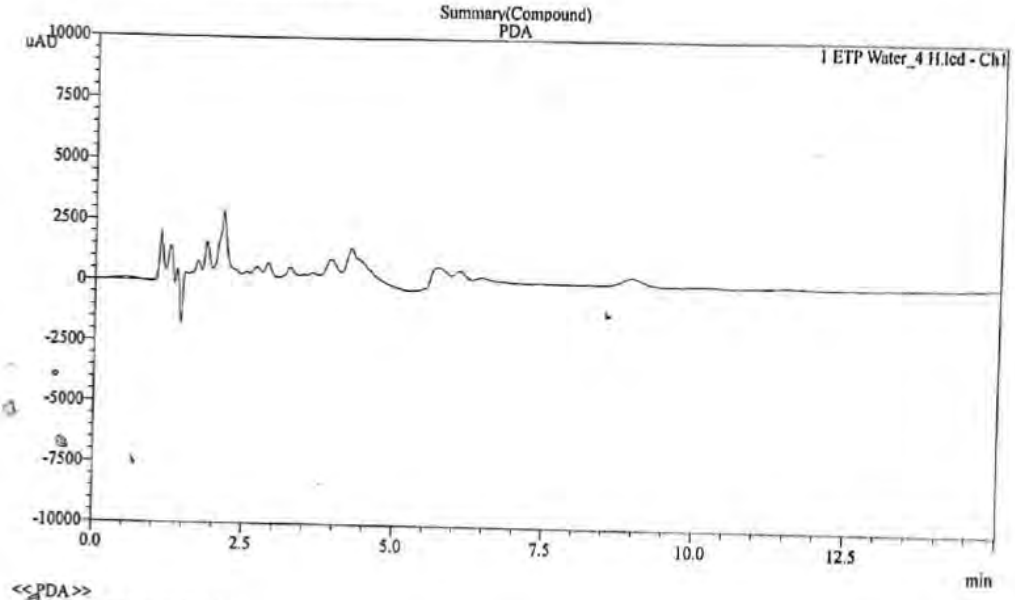
<< PDA >>
ID#1 Compound Name: Cefixime

Title	Sample ID	Ret. Time	Area	Area%	Height	Plates/meter(USP)
ETP Water_3 H.lcd	ETP Water	0.000	0	0.000	0	--
Average		0.000	0	0.000	0	--
%RSD		0.000	0.000	0.000	0.000	0.000
Standard Deviation		0.000	0	0.000	0	--

Figure 4.7: Chromatogram of 3hr tested decontaminated and neutralized by CH_3COOH for Cefixime wastewater

System Configuration
<<Instrument>>
Instrument Name : CE-QC-HPL-001
Description : LC-2030C 3D

Sample Information
Acquired by : Ruksana Sultana
Project Name : Cefixime
Sample Name : ETP Water
Sample ID : ETP Water
Method File : Rofixim PFS.lcm
Data File : ETP Water_4 H.lcd
Vial# : 7
Injection Volume : 10
Date Acquired : 28/06/2018 17:05:10
Date Processed : 30/06/2018 08:30:15



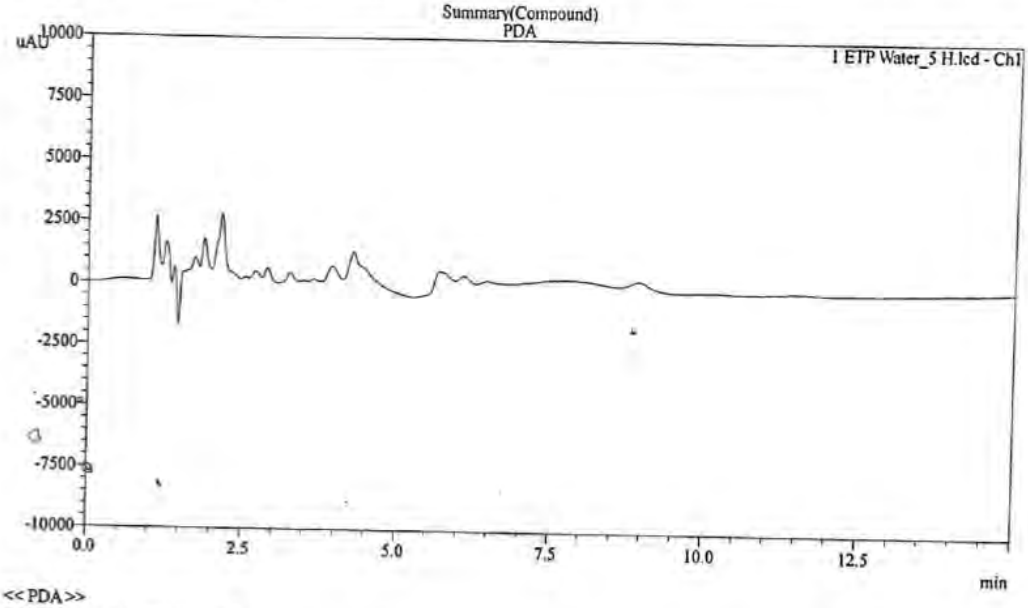
<<PDA>>
ID#1 Compound Name: Cefixime

Title	Sample ID	Ret. Time	Area	Height	Plates/meter(USP)	Tailing Factor	Resolution(USP)
ETP Water_4 H.lcd	ETP Water	0.000	0	0	--	--	--
Average		0.000	0	0	--	--	--
%RSD		0.000	0.000	0.000	0.000	0.000	0.000
Standard Deviation		0.000	0	0	--	--	--

Figure 4.8: Chromatogram of 4hr tested decontaminated and neutralized by CH_3COOH for Cefixime wastewater

System Configuration
<<Instrument>>
Instrument Name : CE-QC-HPL-001
Description : LC-2030C 3D

Sample Information
Acquired by : Ruksana Sultana
Project Name : Cefixime
Sample Name : ETP Water
Sample ID : ETP Water
Method File : Rofixim PFS.lcm
Data File : ETP Water_5 H.lcd
Vial# : 8
Injection Volume : 10
Date Acquired : 28/06/2018 17:20:33
Date Processed : 30/06/2018 08:30:21



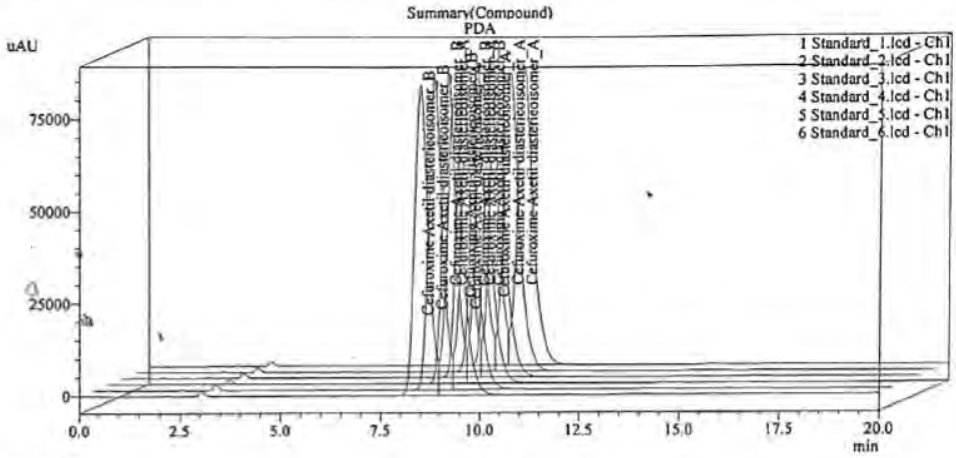
<< PDA >>
ID#1 Compound Name: Cefixime

Title	Sample ID	Ret. Time	Area	Height	Plates/meter(USP)	Tailing Factor	Resolution(USP)
ETP Water_5 H.lcd	ETP Water	0.000	0	0	--	--	--
Average		0.000	0	0	--	--	--
%RSD		0.000	0.000	0.000	0.000	0.000	0.000
Standard Deviation		0.000	0	0	--	--	--

Figure 4.9: Chromatogram of 5hr tested decontaminated and neutralized by CH_3COOH for Cefixime wastewater

System Configuration
<<Instrument>>
Instrument Name : CE-QC-HPL-001
Description : LC-2030C 3D

Sample Information
Acquired by : Raihana Sultana
Project Name : Cefuroxime
Sample Name : Rofurox PFS 70 ml
Sample ID : Standard
Method File : Rofurox PFS 70 ml.lcm
Data File : Standard_1.lcd
Vial# : 2
Injection Volume : 10
Batch File : 14.07.18.lcb
Date Acquired : 14/07/2018 12:49:58
Date Processed : 14/07/2018 16:17:23



<< PDA >>

ID#1 Compound Name: Cefuroxime Axetil diastereoisomer_B

Title	Sample ID	Ret. Time	Area	Plates/meter(USP)	Tailing Factor	Area%
Standard_1.lcd	Standard	8.519	1994909	19586	--	47.424
Standard_2.lcd	Standard	8.514	1997962	19586	--	47.514
Standard_3.lcd	Standard	8.508	2001082	19628	--	47.455
Standard_4.lcd	Standard	8.504	1992952	19634	--	47.356
Standard_5.lcd	Standard	8.497	1998019	19701	--	47.475
Standard_6.lcd	Standard	8.491	1992880	19678	--	47.403
Average		8.505	1996301	19635	--	47.438
%RSD		0.125	0.164	0.240	0.000	0.117
Standard Deviation		0.011	3269	47	--	0.056

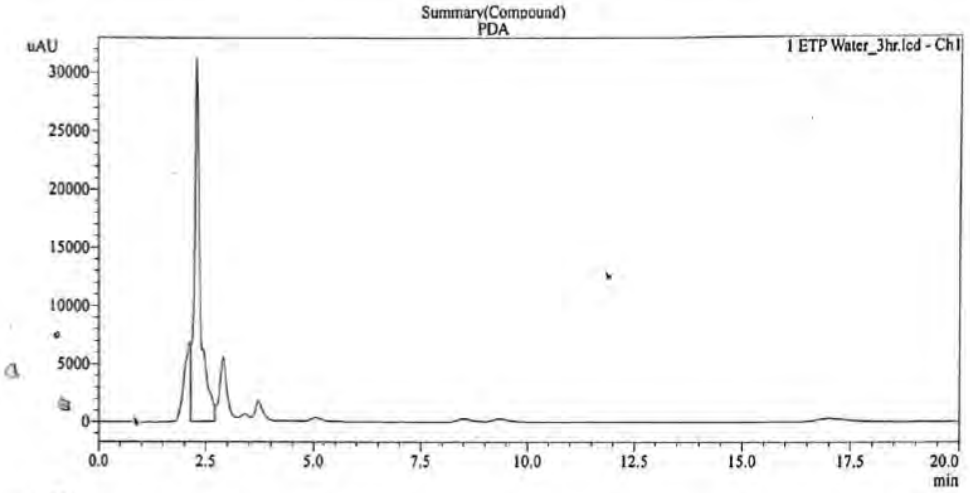
ID#2 Compound Name: Cefuroxime Axetil diastereoisomer_A

Title	Sample ID	Ret. Time	Area	Plates/meter(USP)	Tailing Factor	Area%
Standard_1.lcd	Standard	9.370	2211672	18872	--	52.576
Standard_2.lcd	Standard	9.364	2207057	18919	--	52.486
Standard_3.lcd	Standard	9.356	2215725	18933	--	52.545
Standard_4.lcd	Standard	9.350	2215505	18989	--	52.644
Standard_5.lcd	Standard	9.342	2210596	19025	--	52.525
Standard_6.lcd	Standard	9.336	2211222	18966	--	52.597
Average		9.353	2211963	18951	--	52.562
%RSD		0.138	0.148	0.286	0.000	0.106
Standard Deviation		0.013	3264	54	--	0.056

Figure 4.10: Chromatogram of standard solution of Cefuroxime for NaOH and CH_3COOH treatment

System Configuration
<<Instrument>>
Instrument Name : CE-QC-HPL-001
Description : LC-2030C 3D

Sample Information
Acquired by : Raihana Sultana
Project Name : Cefuroxime
Sample Name : Rofurox PFS 70 ml
Sample ID : ETP Water_3hr
Method File : Rofurox PFS 70 ml.lcm
Data File : ETP Water_3hr.lcd
Vial# : 3
Injection Volume : 10
Batch File : 14.07.18.lcb
Date Acquired : 14/07/2018 14:52:15
Date Processed : 14/07/2018 16:17:49



<< PDA >>

ID#1 Compound Name: Cefuroxime Axetil diasterieoisomer_B

Title	Sample ID	Ret. Time	Area	Plates/meter(USP)	Tailing Factor	Area%
ETP Water_3hr.lcd	ETP Water_3hr	0.000	0	--	--	0.000
Average		0.000	0	--	--	0.000
%RSD		0.000	0.000	0.000	0.000	0.000
Standard Deviation		0.000	0	--	--	0.000

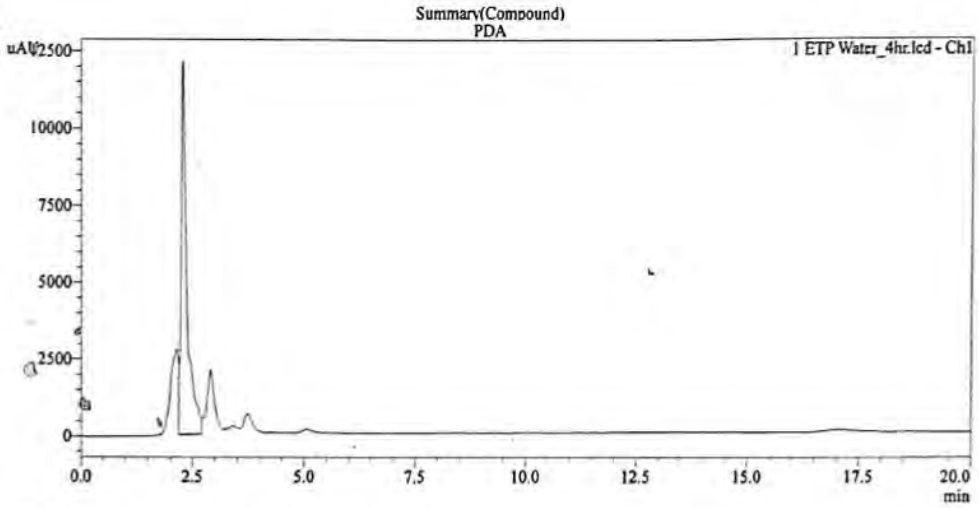
ID#2 Compound Name: Cefuroxime Axetil diasterieoisomer_A

Title	Sample ID	Ret. Time	Area	Plates/meter(USP)	Tailing Factor	Area%
ETP Water_3hr.lcd	ETP Water_3hr	0.000	0	--	--	0.000
Average		0.000	0	--	--	0.000
%RSD		0.000	0.000	0.000	0.000	0.000
Standard Deviation		0.000	0	--	--	0.000

Figure 4.11: Chromatogram of 3hr tested decontaminated and neutralized by CH_3COOH for Cefuroxime wastewater

System Configuration
<<Instrument>>
Instrument Name : CE-QC-HPL-001
Description : LC-2030C 3D

Sample Information
Acquired by : Raihana Sultana
Project Name : Cefuroxime
Sample Name : Rofurox PFS 70 ml
Sample ID : ETP Water_4hr
Method File : Rofurox PFS 70 ml.lcm
Data File : ETP Water_4hr.lcd
Vial# : 4
Injection Volume : 10
Batch File : 14.07.18.lcb
Date Acquired : 14/07/2018 15:12:38
Date Processed : 14/07/2018 16:17:44



<< PDA >>

ID#1 Compound Name: Cefuroxime Axetil diasterieoisomer_B

Title	Sample ID	Ret. Time	Area	Plates/meter(USP)	Tailing Factor	Area%
ETP Water_4hr.lcd	ETP Water_4hr	0.000	0	--	--	0.000
Average		0.000	0	--	--	0.000
%RSD		0.000	0.000	0.000	0.000	0.000
Standard Deviation		0.000	0	--	--	0.000

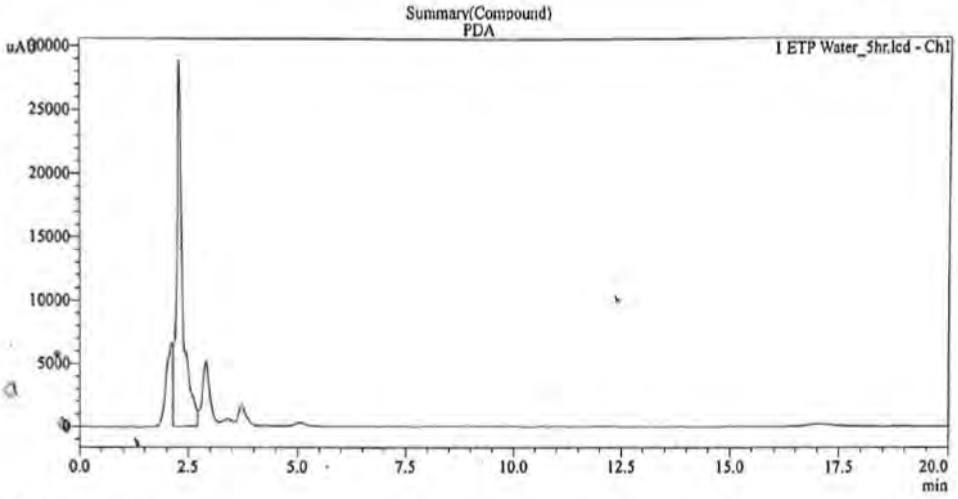
ID#2 Compound Name: Cefuroxime Axetil diasterieoisomer_A

Title	Sample ID	Ret. Time	Area	Plates/meter(USP)	Tailing Factor	Area%
ETP Water_4hr.lcd	ETP Water_4hr	0.000	0	--	--	0.000
Average		0.000	0	--	--	0.000
%RSD		0.000	0.000	0.000	0.000	0.000
Standard Deviation		0.000	0	--	--	0.000

Figure 4.12: Chromatogram of 4hr tested decontaminated and neutralized by CH_3COOH for Cefuroxime wastewater

System Configuration
<<Instrument>>
Instrument Name : CE-QC-HPL-001
Description : LC-2030C 3D

Sample Information
Acquired by : Raihana Sultana
Project Name : Cefuroxime
Sample Name : Rofurox PFS 70 ml
Sample ID : ETP Water_5hr
Method File : Rofurox PFS 70 ml.lcm
Data File : ETP Water_5hr.lcd
Vial# : 5
Injection Volume : 10
Batch File : 14.07.18.lcb
Date Acquired : 14/07/2018 15:33:00
Date Processed : 14/07/2018 16:17:39



<< PDA >>
ID#1 Compound Name: Cefuroxime Axetil diastereoisomer_B

Title	Sample ID	Ret. Time	Area	Plates/meter(USP)	Tailing Factor	Area%
ETP Water_5hr.lcd	ETP Water_5hr	0.000	0	--	--	0.000
Average		0.000	0	--	--	0.000
%RSD		0.000	0.000	0.000	0.000	0.000
Standard Deviation		0.000	0	--	--	0.000

ID#2 Compound Name: Cefuroxime Axetil diastereoisomer_A

Title	Sample ID	Ret. Time	Area	Plates/meter(USP)	Tailing Factor	Area%
ETP Water_5hr.lcd	ETP Water_5hr	0.000	0	--	--	0.000
Average		0.000	0	--	--	0.000
%RSD		0.000	0.000	0.000	0.000	0.000
Standard Deviation		0.000	0	--	--	0.000

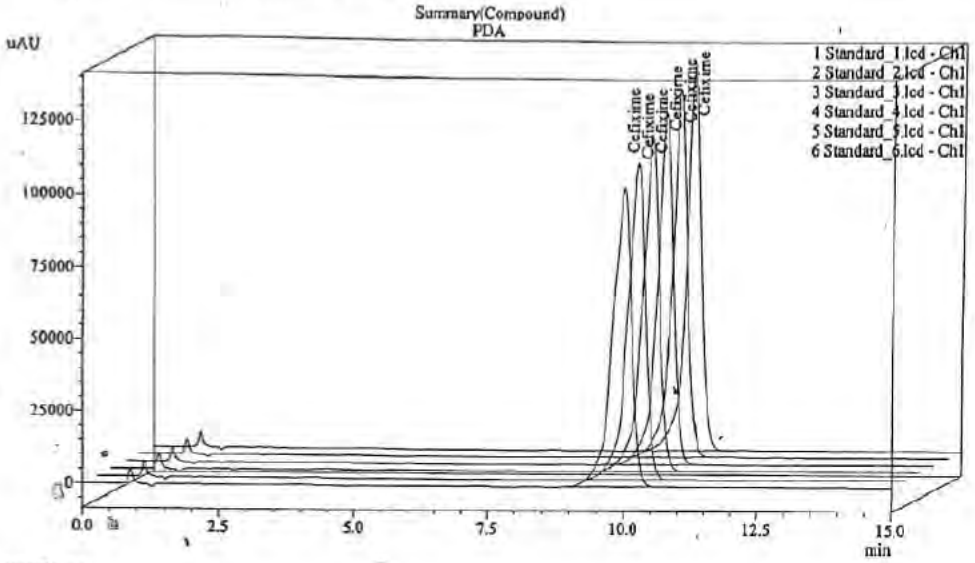
Figure 4.13: Chromatogram of 5hr tested decontaminated and neutralized by CH_3COOH for Cefuroxime wastewater

4.4.1 Effects of NaOH and CH_3COOH in decontamination process

Figure 4.6 shows the chromatogram of standard solution of Cefixime (β-Lactam ring) where retention time of respective compound is 10.676 minutes. Figure 4.7, 4.8 and 4.9 shows the chromatogram of 3 hr, 4 hr and 5 hr decontaminated wastewater where no peak (Retention time 0.0 minutes) has found for Cefixime which is the indication of successful decontamination of wastewater by NaOH followed by CH_3COOH neutralization. Similarly Figure 4.10 shows the standard solution of Cefuroxime Axetil with retention time of 8.505 minutes for Diasterieoisomer B and 9.353 minutes for Diasterieisomer A. Figure 4.11, 4.12 and 4.13 shows the absence of peak (Retention time 0.0 minutes) for Cefuroxime Axetil in respective chromatogram for 3 hr, 4 hr and 5 hr decontaminated wastewater by NaOH followed by neutralization of decontaminated wastewater by CH_3COOH. It seems that both of compounds (Cefixime and Cefuroxime) are successfully decontaminated at 3 hr retention time and since the sample solution chromatogram shows absence of peaks of Cefixime and Cefuroxime Axetil, therefore it can be said that there is no reformation of β-Lactam ring for neutralization by CH_3COOH.

System Configuration
<<Instrument>>
Instrument Name : CB-QC-HPL-001
Description : LC-2030C 3D

Sample Information
Acquired by : Ruksana Sultana
Project Name : Cefixime
Sample Name : Cefixime WS
Sample ID : Standard
Method File : Rofixim PFS.lcm
Data File : Standard_1.lcd
Vial# : 3
Injection Volume : 10
Date Acquired : 05/05/2018 17:17:41
Date Processed : 06/05/2018 08:15:43



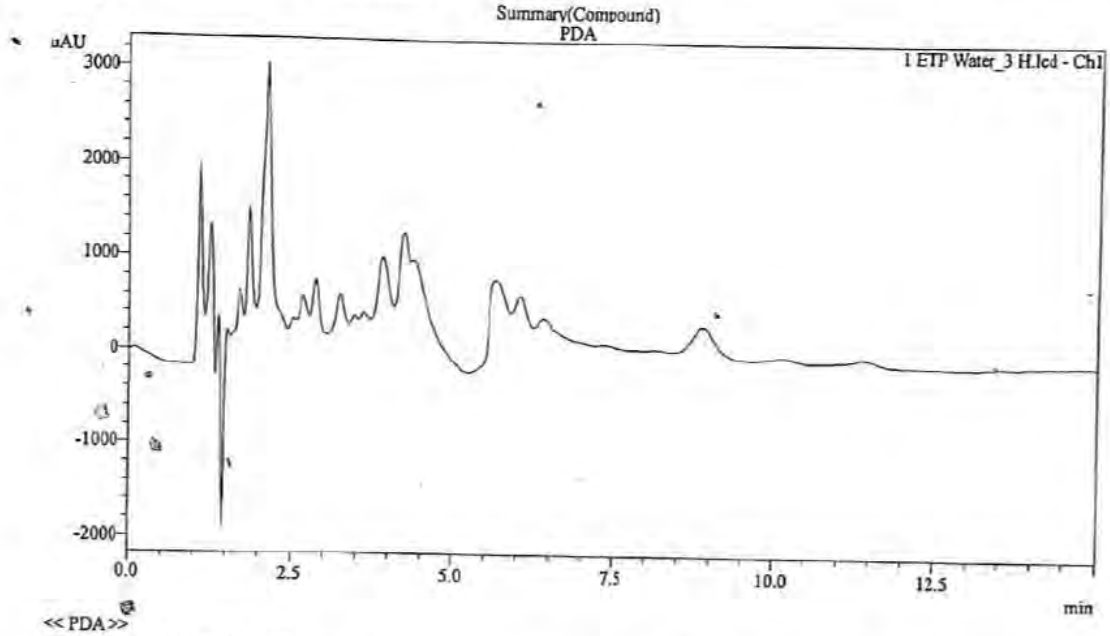
<< PDA >>
ID#1 Compound Name: Cefixime

Title	Sample ID	Ret. Time	Area	Height	Plates/meter(USP)	Tailing Factor	Resolution(USP)
Standard_1.lcd	Standard	9.997	2579025	133747	45699	0.843	-
Standard_2.lcd	Standard	9.997	2578774	127684	41070	0.830	-
Standard_3.lcd	Standard	9.996	2578442	121880	36473	0.818	-
Standard_4.lcd	Standard	9.998	2577983	115978	31658	0.806	-
Standard_5.lcd	Standard	10.000	2578365	110220	26809	0.796	-
Standard_6.lcd	Standard	10.002	2576396	104102	25867	0.785	-
Average		9.998	2578164	118935	34596	0.813	-
%RSD		0.022	0.036	9.282	22.913	2.672	0.000
Standard Deviation		0.002	937	11040	7927	0.022	-

Figure 4.14: Chromatogram of standard solution of Cefixime for NaOH and HCl treatment

System Configuration
<<Instrument>>
Instrument Name : CE-QC-HPL-001
Description : LC-2030C 3D

Sample Information
Acquired by : Ruksana Sultana
Project Name : Cefixime
Sample Name : ETP Water
Sample ID : ETP Water
Method File : Rofixim PFS.lcm
Data File : ETP Water_3 H.lcd
Vial# : 6
Injection Volume : 10
Date Acquired : 05/05/2018 18:49:54
Date Processed : 06/05/2018 08:16:03



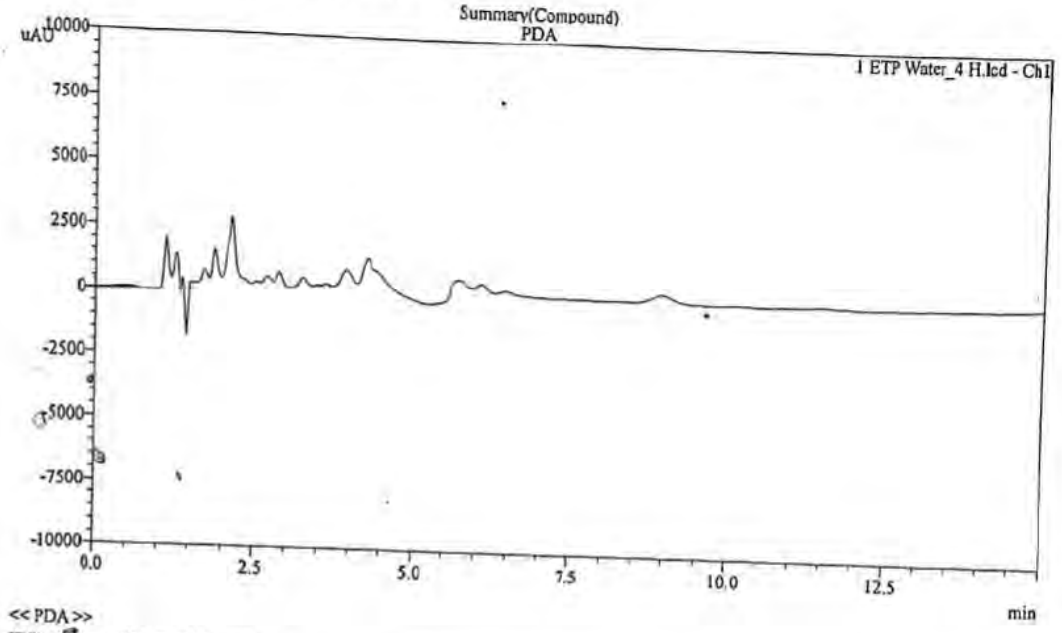
<< PDA >>
ID#1 Compound Name: Cefixime

Title	Sample ID	Ret. Time	Area	Area%	Height	Plates/meter(USP)
ETP Water_3 H.lcd	ETP Water	0.000	0	0.000	0	–
Average		0.000	0	0.000	0	–
%RSD		0.000	0.000	0.000	0.000	0.000
Standard Deviation		0.000	0	0.000	0	–

Figure 4.15: Chromatogram of 3hr tested decontaminated and neutralized by HCl wastewater for Cefixime

System Configuration
<<Instrument>>
Instrument Name : CE-QC-HPL-001
Description : LC-2030C 3D

Sample Information
Acquired by : Ruksana Sultana
Project Name : Cefixime
Sample Name : ETP Water
Sample ID : ETP Water
Method File : Refixim PFS.lcm
Data File : ETP Water_4 H.lcd
Vial# : 7
Injection Volume : 10
Date Acquired : 05/05/2018 19:05:17
Date.Processed : 06/05/2018 08:15:58



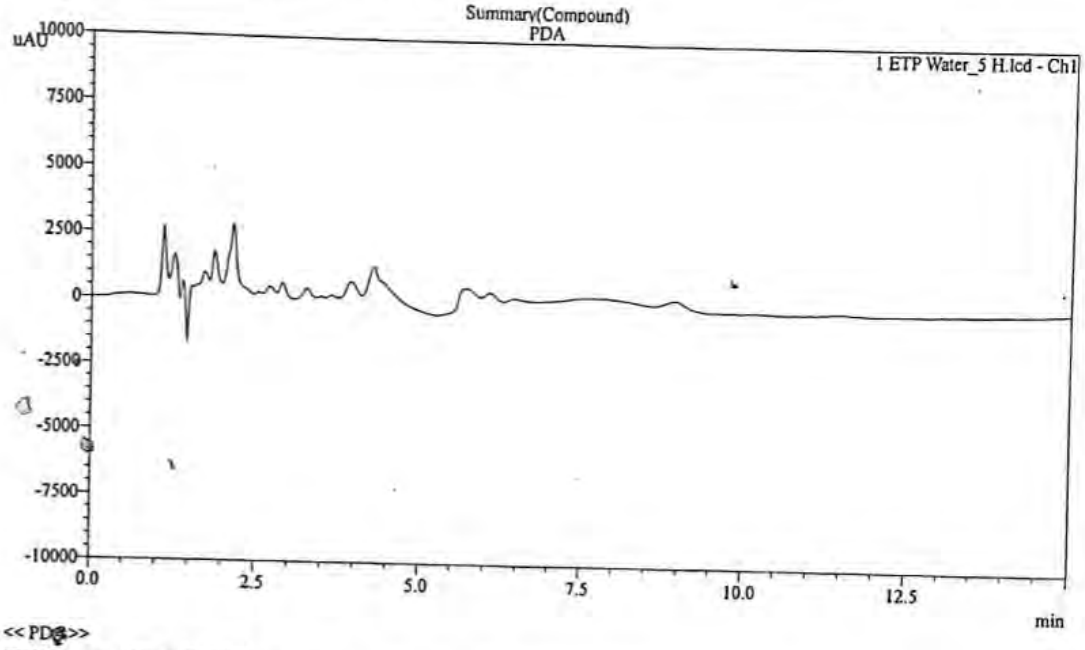
<< PDA >>
ID#1 Compound Name: Cefixime

Title	Sample ID	Ret. Time	Area	Height	Plates/meter(USP)	Tailing Factor	Resolution(USP)
ETP Water_4 H.lcd	ETP Water	0.000	0	0	--	--	--
Average		0.000	0	0	--	--	--
%RSD		0.000	0.000	0.000	0.000	0.000	0.000
Standard Deviation		0.000	0	0	--	--	--

Figure 4.16: Chromatogram of 4hr tested decontaminated and neutralized by HCl wastewater for Cefixime

System Configuration
<<Instrument>>
Instrument Name : CE-QC-HPL-001
Description : LC-2030C 3D

Sample Information
Acquired by : Ruksana Sultana
Project Name : Cefixime
Sample Name : ETP Water
Sample ID : ETP Water
Method File : Rofixim PFS.lcm
Data File : ETP Water_5 H.lcd
Vial# : 8
Injection Volume : 10
Date Acquired : 05/05/2018 20:37:32
Date Processed : 06/05/2018 08:16:37



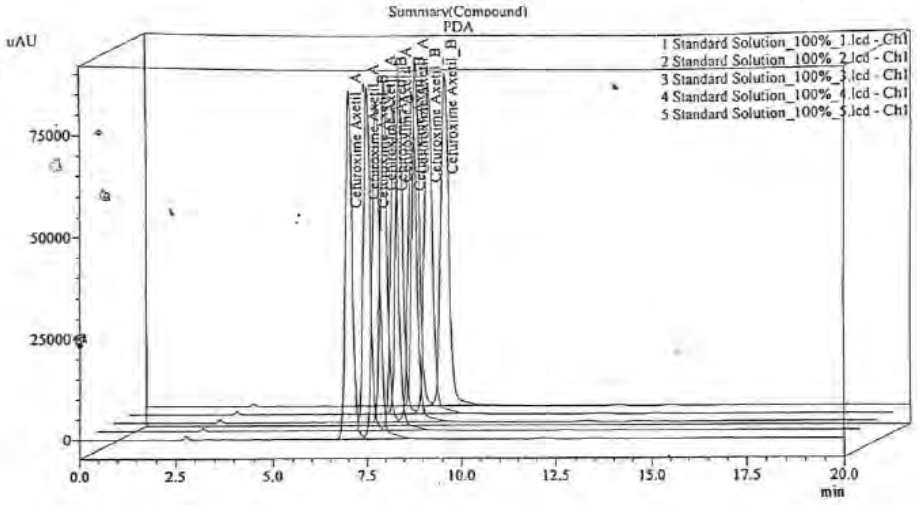
<< PDA >>
ID#1 Compound Name: Cefixime

Title	Sample ID	Ret. Time	Area	Height	Plates/meter(USP)	Tailing Factor	Resolution(USP)
ETP Water_5 H.lcd	ETP Water	0.000	0	0	–	–	–
Average		0.000	0	0	–	–	–
%RSD		0.000	0.000	0.000	0.000	0.000	0.000
Standard Deviation		0.000	0	0	–	–	–

Figure 4.17: Chromatogram of 5hr tested decontaminated and neutralized by HCl wastewater for Cefixime

System Configuration
<<Instrument>>
Instrument Name : CE-QC-HPL-001
Description : LC-2030C 3D

Sample Information
Acquired by : Raiiana Sultana
Project Name : Cefuroxime
Sample Name : Rofurox PFS 70 ml
Sample ID : Standard
Method File : Rofurox PFS 70 ml.lcm
Data File : Standard_1.lcd
Vial# : 2
Injection Volume : 10
Batch File : cal 25.04.18.lcb
Date Acquired : 25/04/2018 13:19:31
Date Processed : 29/04/2018 12:16:44



<< PDA >>

ID#1 Compound Name: Cefuroxime Axetil diastereoisomer_B

Title	Sample ID	Ret. Time	Area	Height	Plates/meter(USP)	Tailing Factor
Standard Solution_100%_1.lc	Standard	7.078	1124863	85427	42587	1.102
Standard Solution_100%_2.lc	Standard	7.079	1123290	85163	42430	1.101
Standard Solution_100%_3.lc	Standard	7.091	1123684	84949	42239	1.102
Standard Solution_100%_4.lc	Standard	7.065	1124208	85758	42677	1.101
Standard Solution_100%_5.lc	Standard	7.003	1125458	86953	43079	1.100
Average		7.063	1124301	85650	42603	1.101
%RSD		0.497	0.078	0.921	0.737	0.062
Standard Deviation		0.035	875	789	314	0.001

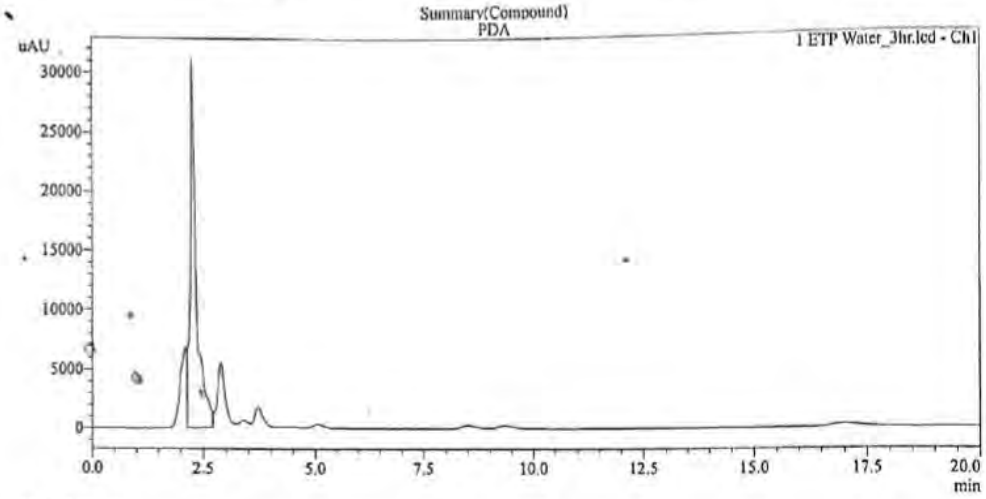
ID#2 Compound Name: Cefuroxime Axetil diastereoisomer_A

Title	Sample ID	Ret. Time	Area	Height	Plates/meter(USP)	Tailing Factor
Standard Solution_100%_1.lc	Standard	7.843	1220984	82775	43731	1.097
Standard Solution_100%_2.lc	Standard	7.843	1218946	82468	43478	1.098
Standard Solution_100%_3.lc	Standard	7.859	1216926	82178	43427	1.096
Standard Solution_100%_4.lc	Standard	7.824	1221725	83017	43805	1.098
Standard Solution_100%_5.lc	Standard	7.748	1222459	84360	44391	1.097
Average		7.823	1220208	82959	43766	1.097
%RSD		0.561	0.185	1.017	0.878	0.067
Standard Deviation		0.044	2255	844	384	0.001

Figure 4.18: Chromatogram of standard solution of Cefuroxime for NaOH and HCl treatment

System Configuration
<<Instrument>>
Instrument Name : CE-QC-HPL-001
Description : LC-2030C 3D

Sample Information
Acquired by : Raihana Sultana
Project Name : Cefuroxime
Sample Name : Rofurox PFS 70 ml
Sample ID : ETP Water_3hr
Method File : Rofurox PFS 70 ml.lcm
Data File : ETP Water_3hr.lcd
Vial# : 3
Injection Volume : 10
Batch File : cal 25.04.18.lcb
Date Acquired : 25/04/2018 15:42:09
Date Processed : 29/04/2018 12:17:17



<< PDA >>

ID#1 Compound Name: Cefuroxime Axetil diasterieoisomer_B

Title	Sample ID	Ret. Time	Area	Plates/meter(USP)	Tailing Factor	Area%
ETP Water_3hr.lcd	ETP Water_3hr	0.000	0	--	--	0.000
Average		0.000	0	--	--	0.000
%RSD		0.000	0.000	0.000	0.000	0.000
Standard Deviation		0.000	0	--	--	0.000

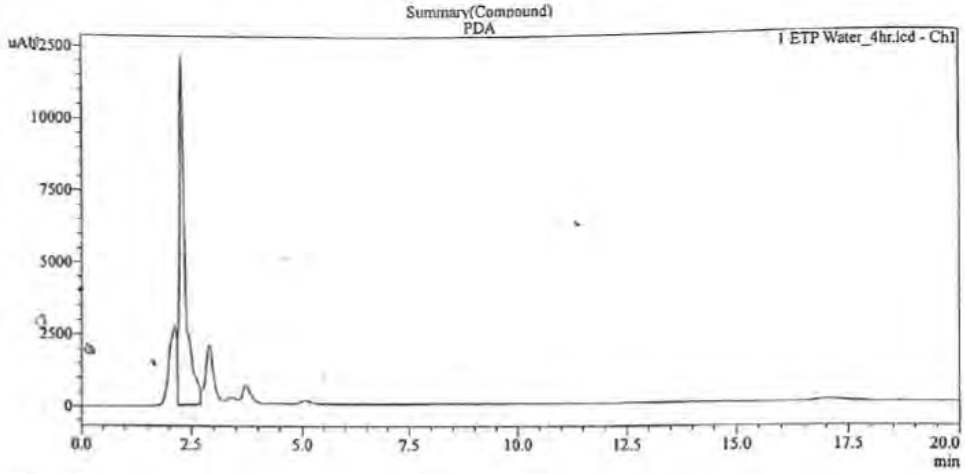
ID#2 Compound Name: Cefuroxime Axetil diasterieoisomer_A

Title	Sample ID	Ret. Time	Area	Plates/meter(USP)	Tailing Factor	Area%
ETP Water_3hr.lcd	ETP Water_3hr	0.000	0	--	--	0.000
Average		0.000	0	--	--	0.000
%RSD		0.000	0.000	0.000	0.000	0.000
Standard Deviation		0.000	0	--	--	0.000

Figure 4.19: Chromatogram of 3hr tested decontaminated and neutralized by HCl wastewater for Cefuroxime

System Configuration
<<Instrument>>
Instrument Name : CE-QC-HPL-001
Description : LC-2030C 3D

Sample Information
Acquired by : Raihana Sultana
Project Name : Cefuroxime
Sample Name : Rofurox PFS 70 ml
Sample ID : ETP Water_4hr
Method File : Rofurox PFS 70 ml.lcm
Data File : ETP Water_4hr.lcd
Vial# : 4
Injection Volume : 10
Batch File : cal 25.04.18.lcb
Date Acquired : 25/04/2018 16:02:31
Date Processed : 29/04/2018 12:17:11



<< PDA >>

ID#1 Compound Name: Cefuroxime Axetil diastereoisomer_B

Title	Sample ID	Ret. Time	Area	Plates/meter(USP)	Tailing Factor	Area%
ETP Water_4hr.lcd	ETP Water_4hr	0.000	0	--	--	0.000
Average		0.000	0	--	--	0.000
%RSD		0.000	0.000	0.000	0.000	0.000
Standard Deviation		0.000	0	--	--	0.000

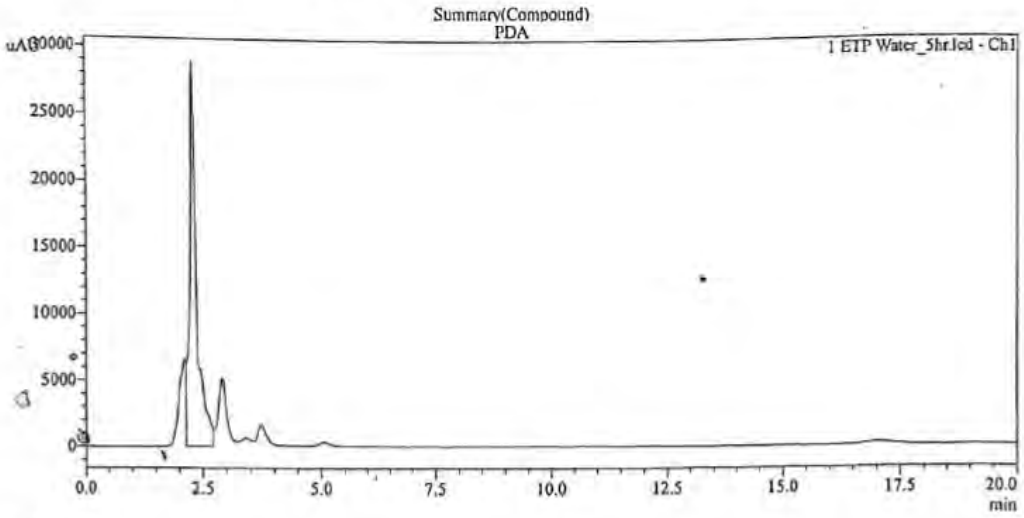
ID#2 Compound Name: Cefuroxime Axetil diastereoisomer_A

Title	Sample ID	Ret. Time	Area	Plates/meter(USP)	Tailing Factor	Area%
ETP Water_4hr.lcd	ETP Water_4hr	0.000	0	--	--	0.000
Average		0.000	0	--	--	0.000
%RSD		0.000	0.000	0.000	0.000	0.000
Standard Deviation		0.000	0	--	--	0.000

Figure 4.20: Chromatogram of 4hr tested decontaminated and neutralized by HCl wastewater for Cefuroxime

System Configuration
<<Instrument>>
Instrument Name : CE-QC-HPL-001
Description : LC-2030C 3D

Sample Information
Acquired by : Raihana Sultana
Project Name : Cefuroxime
Sample Name : Rofurox PFS 70 ml
Sample ID : ETP Water_5hr
Method File : Rofurox PFS 70 ml.lcm
Data File : ETP Water_5hr.lcd
Vial# : 5
Injection Volume : 10
Batch File : cal 25.04.18.lcb
Date Acquired : 25/04/2018 16:43:15
Date Processed : 29/04/2018 12:17:01



<< PDA >>

ID#1 Compound Name: Cefuroxime Axetil diastereoisomer_B

Title	Sample ID	Ret. Time	Area	Plates/meter(USP)	Tailing Factor	Area%
ETP Water_5hr.lcd	ETP Water_5hr	0.000	0	--	--	0.000
Average		0.000	0	--	--	0.000
%RSD		0.000	0.000	0.000	0.000	0.000
Standard Deviation		0.000	0	--	--	0.000

ID#2 Compound Name: Cefuroxime Axetil diastereoisomer_A

Title	Sample ID	Ret. Time	Area	Plates/meter(USP)	Tailing Factor	Area%
ETP Water_5hr.lcd	ETP Water_5hr	0.000	0	--	--	0.000
Average		0.000	0	--	--	0.000
%RSD		0.000	0.000	0.000	0.000	0.000
Standard Deviation		0.000	0	--	--	0.000

Figure 4.21: Chromatogram of 5hr tested decontaminated and neutralized by HCl wastewater for Cefuroxime

4.4.2 Effect of NaOH and HCl in Decontamination process

Figure 4.14 shows the chromatogram of standard solution of Cefixime (β-Lactam ring) where retention time of respective compound is 9.998 minutes. Figure 4.15, 4.16 and 4.17 shows the 3 hr, 4 hr and 5 hr decontaminated wastewater chromatogram where no peak (Retention time 0.0 minutes) has found for Cefixime which is the indication of successful decontamination of wastewater by NaOH followed by neutralization of decontaminated wastewater by HCl. Figure 4.18 shows the standard solution of Cefuroxime Axetil with retention time of 7.063 minutes for Diasterieoisomer B and 7.823 minutes for Diasterieisomer A. Figure 4.19, 4.20 and 4.21 shows the absence of peak (Retention time 0.0 minutes) for Cefuroxime Axetil (β-Lactam ring) in respective chromatogram for 3 hr, 4 hr and 5 hr decontaminated wastewater by NaOH followed by neutralization of decontaminated wastewater by HCl. As two β-Lactam ring (Cefixime and Cefuroxime) are absent in sample solution chromatogram of 3 hr, 4 hr and 5 hr decontaminated wastewater it can be said that the sample wastewater has successfully been decontaminated in 3 hr retention time and no reformation of β-Lactam ring for neutralization by HCl.

4.4.3 Effect of Use of Acids in Decontamination on Microorganism in Biological ETP

Microbiological analysis (Aerobic bacterial count test) of wastewater sample collected from Aeration tank was carried out separately in industry's quality control laboratory to study the effects of acids used (HCl and CH_3COOH) in decontamination tank on bacteriological quality of water which may have some effects on biological unit in ETP. Table 4.3 presents the test result. It shows that the bacterial count remains the same for both cases and therefore, it can be said that there is no significant effect on microorgnism due to using two different acids.

Table 4.3: Microbiological test result of wastewater sample collected from Aeration tank.

Sample	Total Aerobic Microbial Count (cfu/ml)
Wastewater sample from decontamination tank treated with NaOH and HCl (pH-around 7.0)	390
Wastewater sample from decontamination tank treated with NaOH and CH_3COOH (pH-around 7.0)	400

4.5 Performance Evaluation of ETP Units

Wastewater samples collected from different units of Common ETP (Samples S_1, S_2, S_3, S_4, S_5, S_6) were analysed in the laboratory and the treatment effciciency has been evaluated for each units (Decontamination tank, Combined tank, Aeration tank, Clarifier tank, DO increase tank, Filtration) for the selected parametrs. The performance of different units in CETP in treating the selected parametrs are described below.

pH: Influent wastewater is slightly acidic ranging from 6.05-6.88. As influent enters into the Aeration Tank, pH is maintained around 7.5 by auto dosing of HCl or NaOH because at low pH metal remains in soluble state and at high pH metal becomes insoluble and accumulates on the surface of sludge which is hazardous for environment. After that, pH remains quite same in rest of the units which meet the national wastewater discharge standard of pH 6.5-8.5 (ECR, 1997). It has been found from Figure 4.22, that pH of the effluent in different units of ETP is ranging from 7.46 to 7.11 which meet the ECR 1997 standard limit (6.5-8.5).

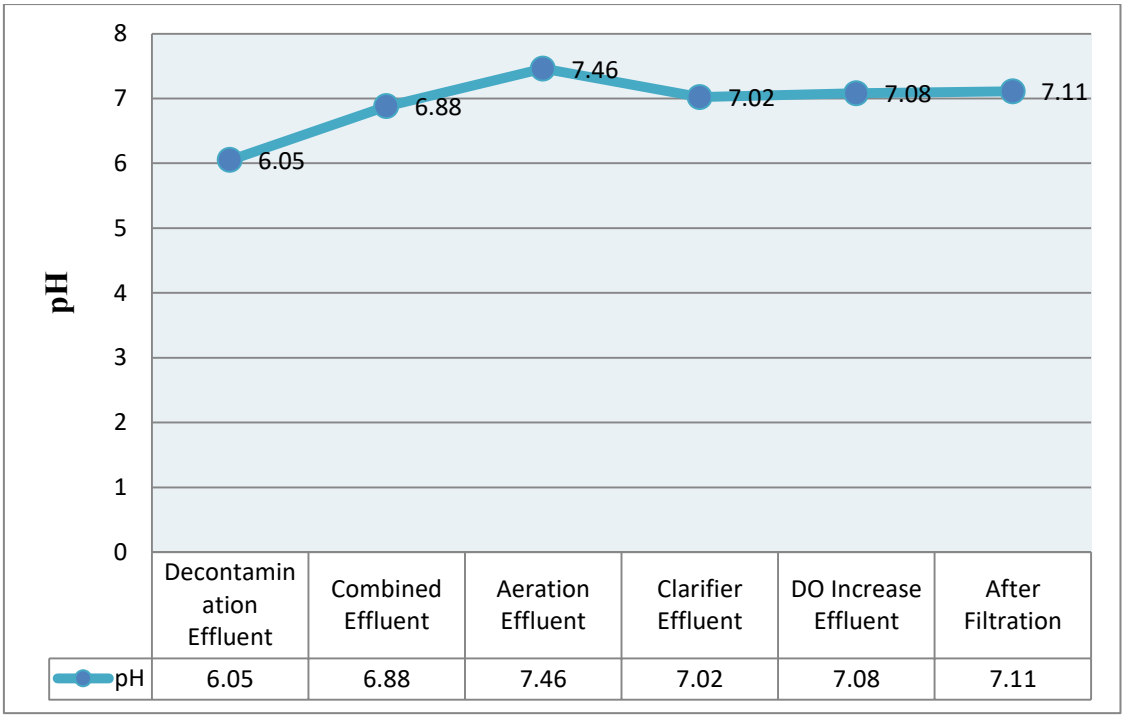


Figure 4.22: Variation of pH in different units of ETP

Electrical Conductivity (EC): Figure 4.23 shows the gradual increase of EC from initial stage (S_1- 941 µS/cm) to final stage of ETP (S_6-858 µS/cm); the reason behind this is the addition of Urea and TSP for bacterial feed and HCl for pH adjustment in aeration unit. The test result shows that the final EC at the outlet (858 µS/cm) is within the limit of ECR, 1997 (1200 µS/cm).

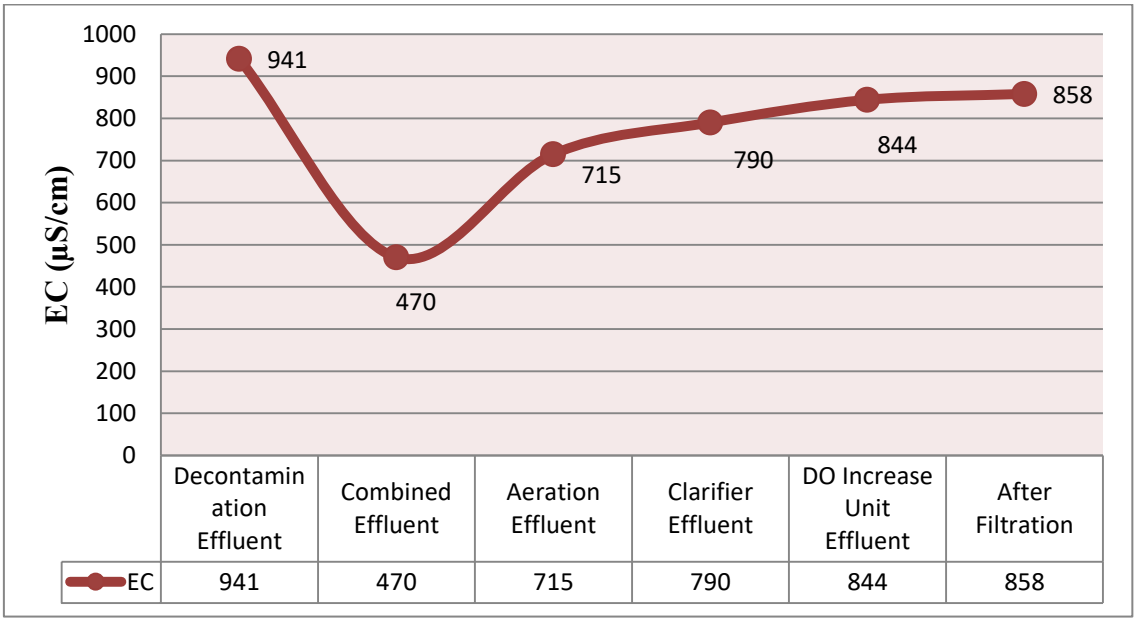


Figure 4.23: Variation of EC (µS/cm) in different units of ETP

Turbidity: Figure 4.24 shows the lower turbidity at decontamination tank, S_1 (25.5 NTU) and then slight increase when wastewater is mixed from two sources (decontamination tank and solid dosage units) in combined tank, S_2 (53.2 NTU). After Clarifier, the turbidity increases from 20 NTU to 93.3 NTU at the final disposal point. Presence of sludge in the treated effluent might be the cause of increasing turbidity.

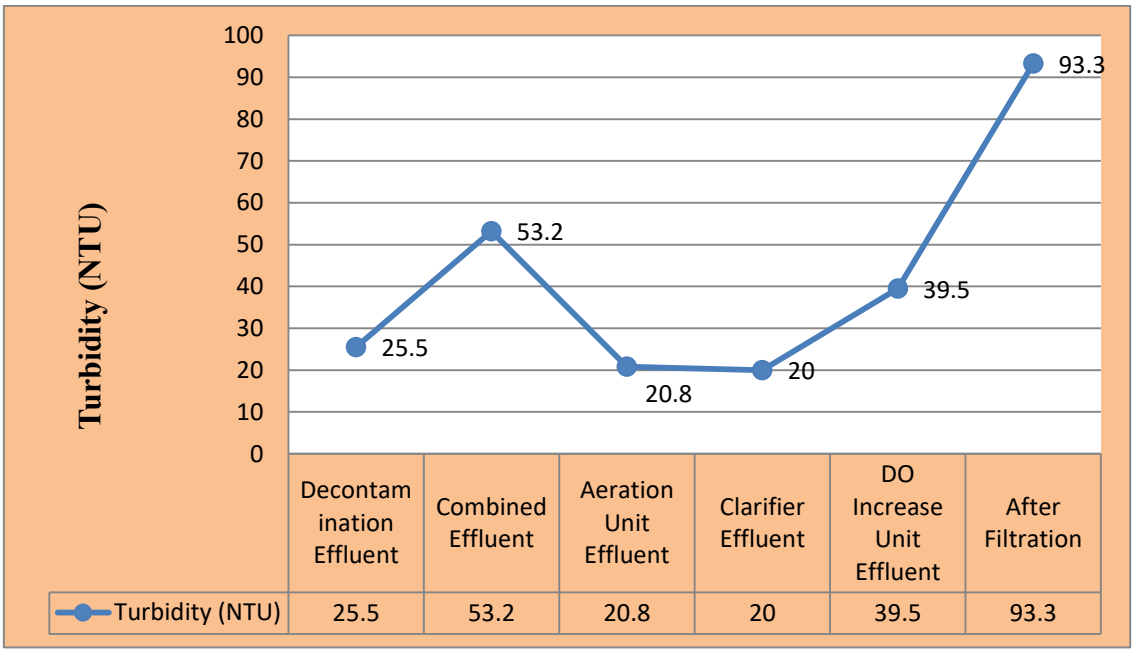


Figure 4.24: Turbidity Variation at different stages of treatment.

TDS and TSS: Although decontamination tank (S_1) contain high TSS 2621 mg/L but its concentration decreases to a significant level S_2 (49 mg/L) at Combined tank due to high volume of relatively low concentration of TDS and TSS containing wastewater discharged from Solid Dosage Unit. Figure 4.25 shows the final concentration of TDS and TSS in each unit of CETP. This figure shows that amount of TDS and TSS increases gradually from Common Tank to final discharge after filtration. Therefore, it can be said that none of the units are effective in decreasing TDS and TSS, rather, they contribute to increase in concentration, and only the dilution from solid dosage unit is effective in reducing TDS and TSS in the CETP. However, since the concentration of TDS (330 mg/L) and TSS (49 mg/L) in Common Tank is well below the allowable limit of discharge, therefore, the treated effluent having 508 mg/L TDS and 125 mg/L TSS also comply with the Standard limit of ECR, 1997.

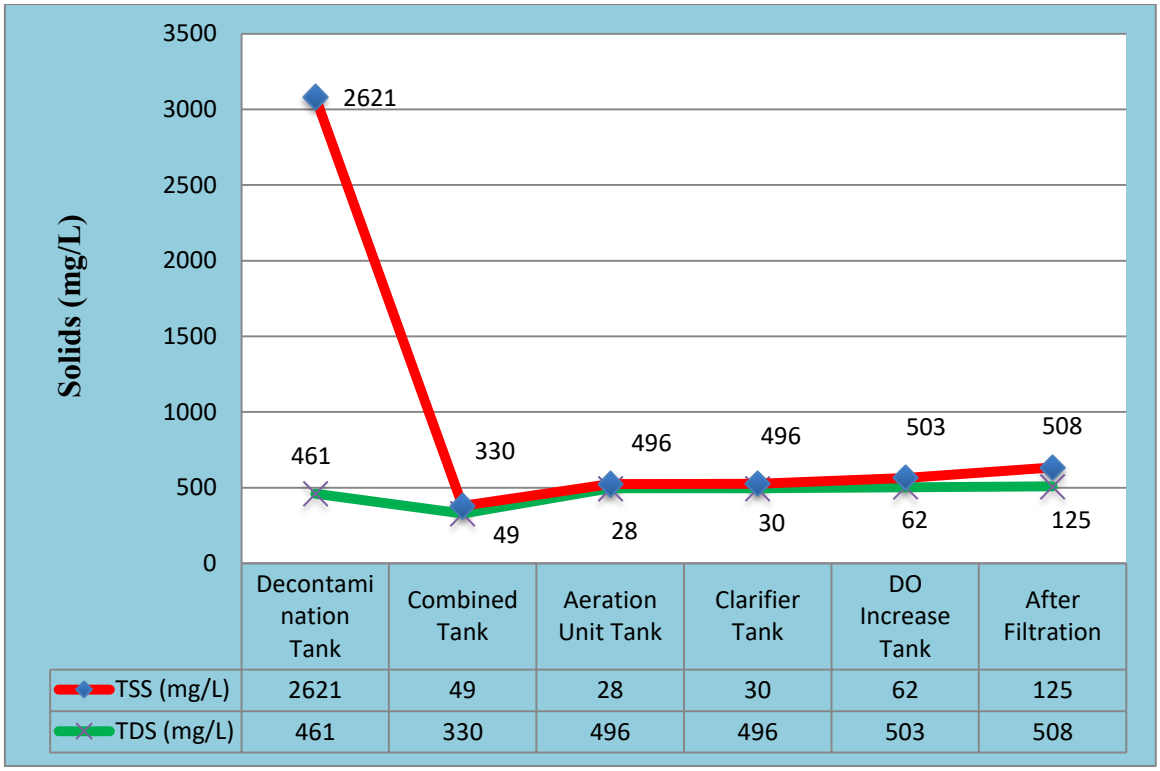


Figure 4.25: Remaining TDS and TSS concentration of wastewater at different units of ETP

Color: The concentration of Color of collected wastewater samples is shown in Figure 4.26. From the figure, it is observed that most of the color removal (61%) happened in Aeration tank of CETP. Since there is no National Standard limit of Color for Wastewater Discharge, therefore, it could not be compared.

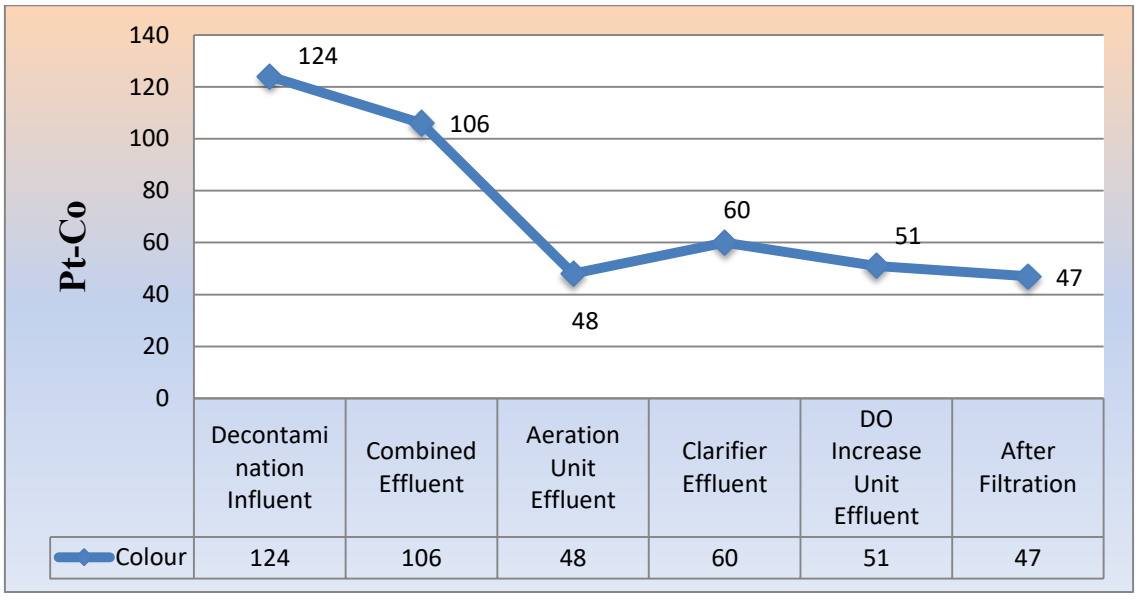


Figure 4.26: Color Variation at different units of ETP

DO: Figure 4.27 presents the DO concentration in wastewater at different units of CETP. It is clear that raw wastewater from Decontamination tank S_1 and combined wastewater, S_2 shows the minimum value of DO 0.29 mg/L and 0.16 mg/L respectively. After that DO slightly increase in aeration tank, S_3 (2.92 mg/L) and shows the maximum value (5.06 mg/L) in DO increase tank by means of root blower. DO value decreases after passing through Sand and Activated Carbon Filtration unit and final DO (2.28 mg/L) does not comply with the ECR, 97 limits (4.5-8 mg/L).

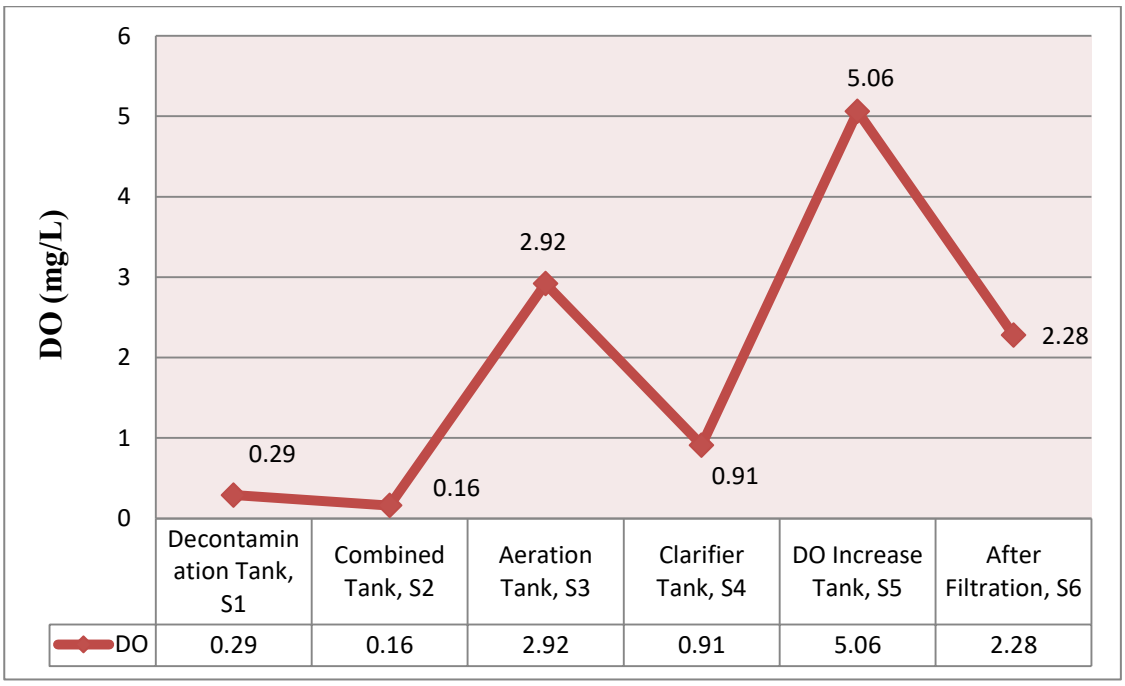


Figure 4.27: DO variation at different units of ETP

COD and BOD_5: Initial BOD_5 and COD of raw wastewater in decontamination tank are 200 mg/L and 504 mg/L respectively. It reduces to 76 mg/L and 162 mg/L respectively in the Combined Effluent Tank. The decreased concentration is due to the dilution effect of high volume of relatively low concentration of BOD_5, COD containing wastewater coming from Solid dosage unit. Figure 4.28 shows the remaining concentration of BOD_5 and COD in different units of CETP. The overall efficiency of BOD_5 and COD removal of CETP is around 71% and 36% respectively (Figure 4.28). From this Figure, it is evident that most of the BOD_5 and COD removal occurred in Aeration tank (removal efficiency 66% and 46% respectively). After aeration unit, BOD_5 concentration remains almost same (around 20-22 mg/L) in the following units (Clarifier, DO Increase Unit and Filtration). Regarding COD

concentration, the situation is slightly different. In aeration unit, remaining COD value is 88 mg/L and the concentration was found increasing in the subsequent units (Clarifier-95 mg/L, DO Increase Unit-98 mg/L and Filtration-103 mg/L). The probable reason might be the presence of sludge in the wastewater and carry over to the next unit. However this could not be confirmed in the scope of the present study. Although the removal of BOD_5 and COD are not high, the final treated effluent satisfies the DoE standard for Inland Surface Water Discharge limit (BOD_5 50mg/L) and (COD 200 mg/L) (ECR, 1997) since the influent concentration in Common Tank is relatively low.

It should be noted here that the overall COD removal obtained in the present study is much less than the findings obtained by Islam (2012) (95%). The reason for high removal (Islam, 2012) is that the ETP has Coagulation unit along with Biological unit.

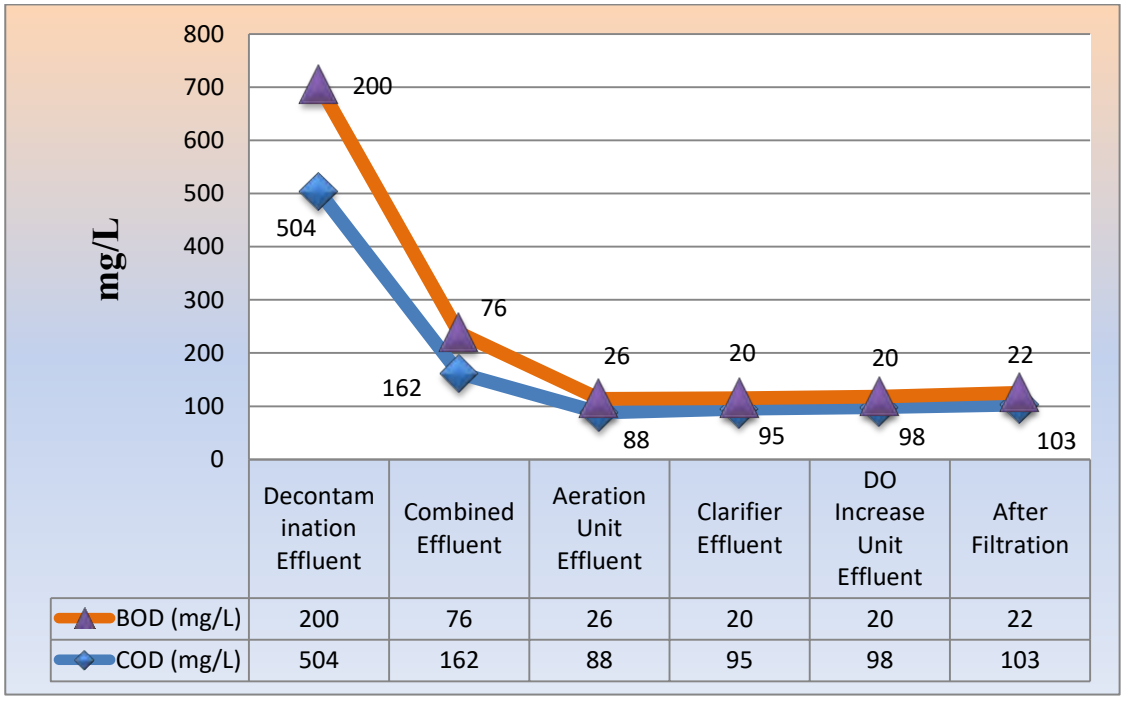


Figure 4.28: Remaining Concentration of COD and BOD_5 Variation at different units of ETP

Heavy Metal: Laboratory results (Table 4.4) showed that the wastewater collected from different units of CETP do not have significant amount of Cu, Cd, Cr and Zn and the concentrations are well below the limit (ECR, 1997). From literature, it is also found that pharmaceutical water usually does not contain much heavy metal (Ramolla and Singh, 2013, Vanerkar, et. al., 2013). The concentration of Zn in Aeration unit is found higher (0.56 mg/L) than in the Common Tank (0.312 mg/L). The reason for

increase in Zn content from combined effluent to aeration tank might be due to the return sludge but it needs further clarification.

Table 4.4: Heavy Metal content at different units of ETP

Metal (ppm)	Decontamination Effluent	Combined Tank Effluent	Aeration Tank Effluent	Clarifier Effluent	DO Increase Tank Effluent	After Filtration
Cd	0.005	0	0.013	0.004	0.005	0.002
Cr	0.006	0.003	0.045	0.003	0.003	0.002
Cu	0.018	0.005	0.003	0.004	0.003	0.004
Zn	2.057	0.312	0.555	0.561	0.524	0.530

Chloride: Figure 4.29 shows the gradual increase of Chloride content (96 mg/L – 154 mg/L) in the subsequent units of ETP. Though the chloride may impact fresh water organisms and plants by altering reproduction rates, increasing species mortality and changing the characteristics of the entire local ecosystem but the chloride content in experimental ETP meets within the favorable limit of DoE standard 600 mg/L (ECR, 1997).

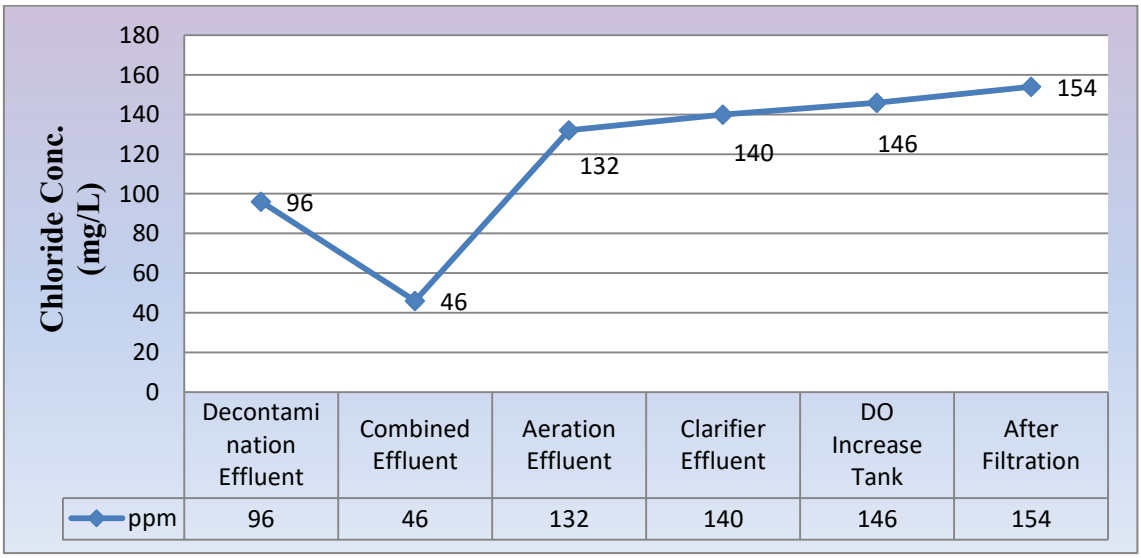
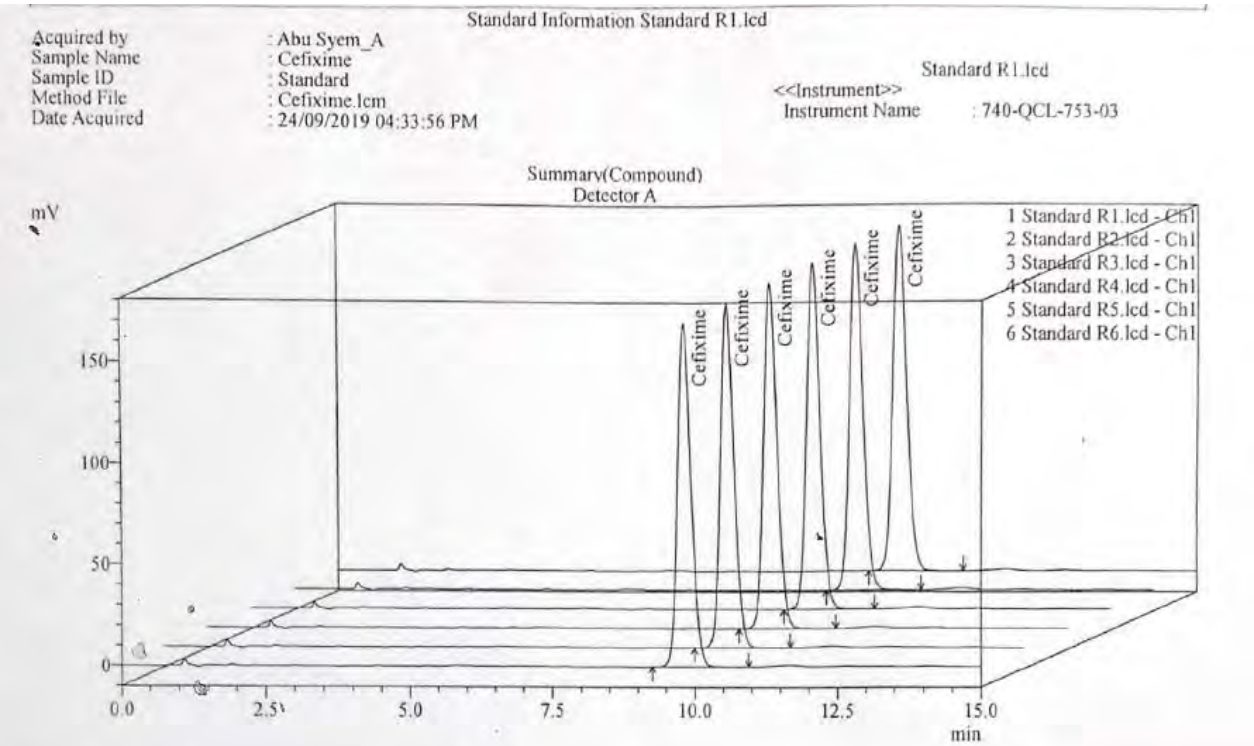


Figure 4.29: Chloride content variation at different units of ETP

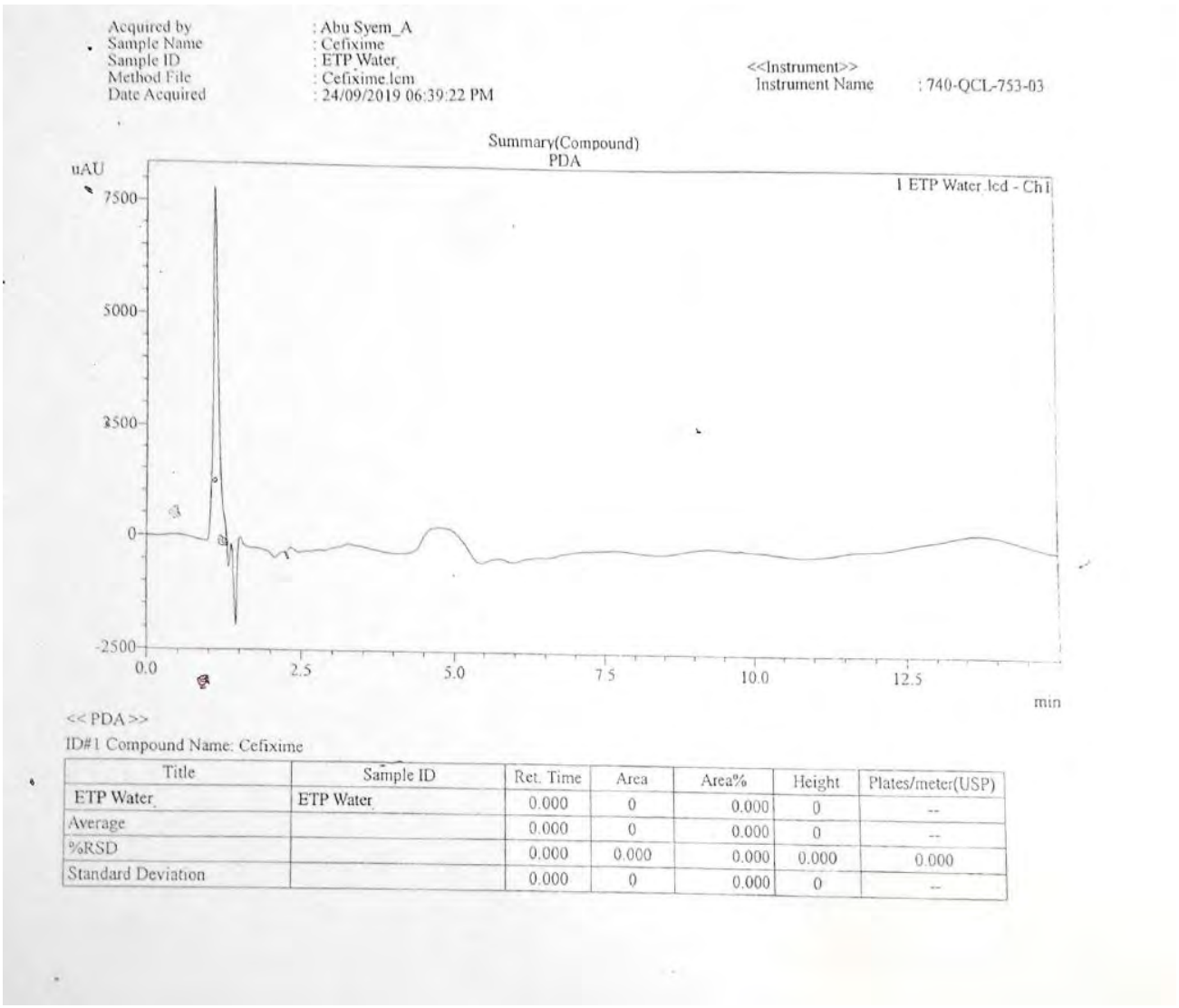
β-Lactam Ring: Although theoretically there is no possibility of reformation of β-Lactam ring in wastewater after decontamination in CETP, to confirm it, the treated effluent just before its final discharge into the surface water, has been again analyzed in HPLC to test any presence of β-Lactam ring for Cefixime and Cefuroxime Axetil separately. Figure 4.30 and Figure 4.31 shows the chromatogram of standard and final treated ETP effluent respectively for analysis of Cefixime. Figure 4.32 and Figure 4.33 show the chromatogram of standard and final treated ETP effluent respectively for analysis of Cefuroxime Axetil.



<< Detector A >>
ID#1 Compound Name: Cefixime

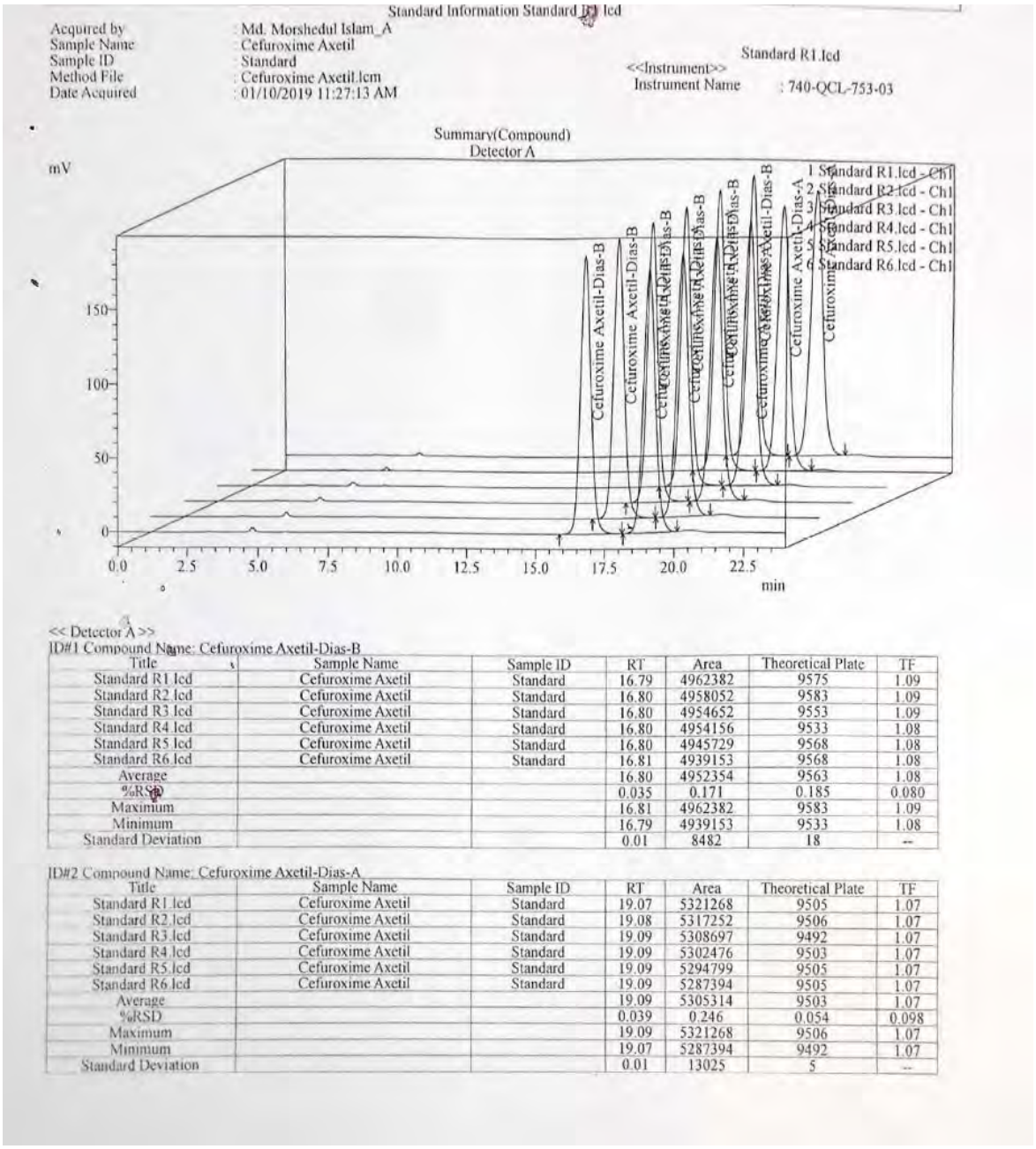
Title	Sample Name	Sample ID	RT	Area	Theoretical Plate	TF
Standard R1.lcd	Cefixime	Standard	9.82	2792783	8002	1.23
Standard R2.lcd	Cefixime	Standard	9.81	2800329	7999	1.23
Standard R3.lcd	Cefixime	Standard	9.81	2804545	7993	1.23
Standard R4.lcd	Cefixime	Standard	9.81	2798881	7993	1.23
Standard R5.lcd	Cefixime	Standard	9.81	2797785	7984	1.23
Standard R6.lcd	Cefixime	Standard	9.80	2798330	7974	1.23
Average			9.81	2798775	7991	1.23
%RSD			0.063	0.136	0.127	0.037
Maximum			9.82	2804545	8002	1.23
Minimum			9.80	2792783	7974	1.23
Standard Deviation			0.01	3816	10	--

Figure 4.30: Chromatogram of standard Solution of Cefixime



Title	Sample ID	Ret. Time	Area	Area%	Height	Plates/meter(USP)
ETP Water	ETP Water	0.000	0	0.000	0	--
Average		0.000	0	0.000	0	--
%RSD		0.000	0.000	0.000	0.000	0.000
Standard Deviation		0.000	0	0.000	0	--

Figure 4.31: Chromatogram of ETP effluent for the analysis of Cefixime



<< Detector A >>

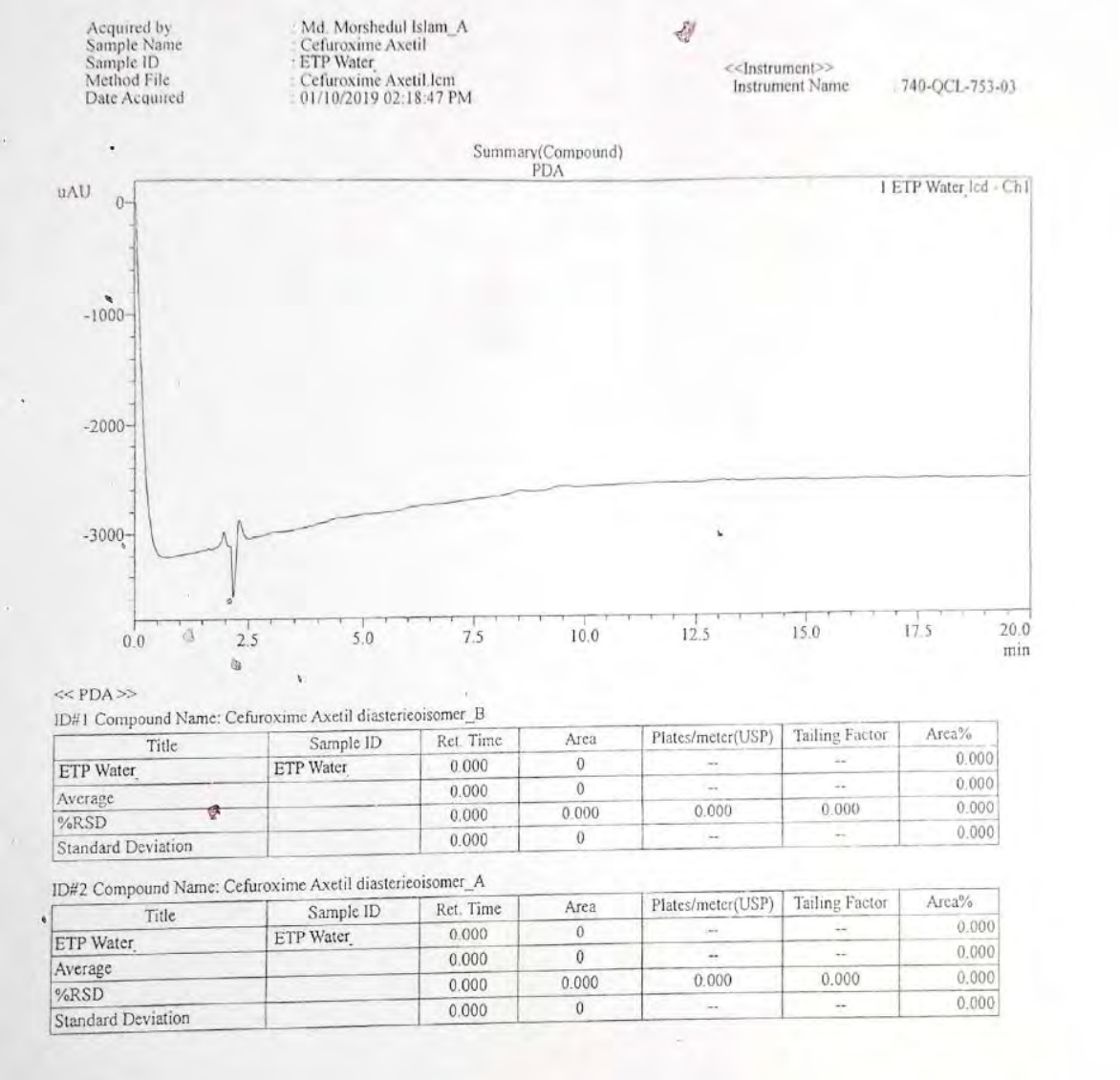
ID#1 Compound Name: Cefuroxime Axetil-Dias-B

Title	Sample Name	Sample ID	RT	Area	Theoretical Plate	TF
Standard R1.lcd	Cefuroxime Axetil	Standard	16.79	4962382	9575	1.09
Standard R2.lcd	Cefuroxime Axetil	Standard	16.80	4958052	9583	1.09
Standard R3.lcd	Cefuroxime Axetil	Standard	16.80	4954652	9553	1.09
Standard R4.lcd	Cefuroxime Axetil	Standard	16.80	4954156	9533	1.08
Standard R5.lcd	Cefuroxime Axetil	Standard	16.80	4945729	9568	1.08
Standard R6.lcd	Cefuroxime Axetil	Standard	16.81	4939153	9568	1.08
Average			16.80	4952354	9563	1.08
%RSD			0.035	0.171	0.185	0.080
Maximum			16.81	4962382	9583	1.09
Minimum			16.79	4939153	9533	1.08
Standard Deviation			0.01	8482	18	---

ID#2 Compound Name: Cefuroxime Axetil-Dias-A

Title	Sample Name	Sample ID	RT	Area	Theoretical Plate	TF
Standard R1.lcd	Cefuroxime Axetil	Standard	19.07	5321268	9505	1.07
Standard R2.lcd	Cefuroxime Axetil	Standard	19.08	5317252	9506	1.07
Standard R3.lcd	Cefuroxime Axetil	Standard	19.09	5308697	9492	1.07
Standard R4.lcd	Cefuroxime Axetil	Standard	19.09	5302476	9503	1.07
Standard R5.lcd	Cefuroxime Axetil	Standard	19.09	5294799	9505	1.07
Standard R6.lcd	Cefuroxime Axetil	Standard	19.09	5287394	9505	1.07
Average			19.09	5305314	9503	1.07
%RSD			0.039	0.246	0.054	0.098
Maximum			19.09	5321268	9506	1.07
Minimum			19.07	5287394	9492	1.07
Standard Deviation			0.01	13025	5	---

Figure 4.32: Chromatogram for the standard solution of Cefuroxime Axetil



<< PDA >>

ID#1 Compound Name: Cefuroxime Axetil diastereoisomer_B

Title	Sample ID	Ret. Time	Area	Plates/meter(USP)	Tailing Factor	Area%
ETP Water_	ETP Water_	0.000	0	--	--	0.000
Average		0.000	0	--	--	0.000
%RSD		0.000	0.000	0.000	0.000	0.000
Standard Deviation		0.000	0	--	--	0.000

ID#2 Compound Name: Cefuroxime Axetil diastereoisomer_A

Title	Sample ID	Ret. Time	Area	Plates/meter(USP)	Tailing Factor	Area%
ETP Water_	ETP Water_	0.000	0	--	--	0.000
Average		0.000	0	--	--	0.000
%RSD		0.000	0.000	0.000	0.000	0.000
Standard Deviation		0.000	0	--	--	0.000

Figure 4.33: Chromatogram of ETP effluent for the analysis of Cefuroxime Axetil

In Figure 4.30 retention time of standard Cefixime is 9.81 minutes, whereas no peaks (Retention time- 0.0 minutes) for cefixime in ETP effluent (Figure 4.31). Figure 4.32 show the chromatogram of standard Cefuroxime Axetil with retention time of 16.80 minutes for diastereoisomer B and 19.09 minutes for diastereoisomer A. But Figure 4.33 shows the absence of peaks for Cefuroxime Axetil (Retention time- 0.0 minutes) in ETP sample water. Therefore, from Figure 4.30, 4.31, 4.32 and 4.33 it can be concluded that there

exists no β-Lactam ring (antibiotic) in CETP treated effluent discharged into the surface water.

4.6 Cost Comparison between HCl and CH_3COOH in Decontamination

A cost comparison of using these two types of acids has been made considering the selection of acid from economic point of view. From the laboratory experiment, it has been found that 0.1 ml HCl and 0.3 ml CH_3COOH were required to decontaminate 50 ml of wastewater sample. Therefore, the amount of acid required per month to decontaminate the total amount of wastewater in decontamination tank (Flow rate 3.5 m^3/day) is determined and presented in Table 4.5. The cost involved in using both the acids is also shown in Table 4.5.

Table 4.5: Amount and Cost of Acids used in Decontamination Process.

Type of Acid	**Amount of Total Chemicals Consumption Litre/month**	**Unit Cost (BDT/Litre)**	**Cost (BDT/month)**
HCl	168	10/-	1,680/-
CH_3COOH	630	125/-	78,750/-

From Table 4.5 it is evident that, monthly cost of usingCH_3COOH is much higher than that of HCl. It should also be mentioned here that due to high amount of CH_3COOH requirement in the process, it took more time than HCL for decontamination process of wastewater.

Chapter 5
CONCLUSION

5.1 Discussion

Pharmaceutical industry is one of the most technically developed sectors in Bangladesh. It produces a wide range of medicines for local market as well as for global market. Along with its products, it also generates wastewater which is a concern from environmental point of view. Unfortunately, like textile wastewater, treatment of pharmaceutical wastewater is not given much importance in our county. The treatment of pharmaceutical wastewater before discharging into the environment should be given more care since it often contains antibiotics and if any residue of antibiotic remains in discharged water, it will contaminate water and soil and eventually affect human health.

The present study looks into the decontamination of β-Lactam antibiotic residues (Cephalosporin) containing wastewater and also the effectiveness of the Effluent Treatment Plant in treating the wastewater. For decontamination, a trial was made in the present study to use organic CH_3COOH with NaOH in order to reduce the effects on microorganism in treating wastewater by biological treatment process. The effectiveness of this decontamination process has been compared with the commonly used inorganic HCl with NaOH. The study result shows that both the combination of chemicals are effective in decontaminating β-Lactam ring but the amount of CH_3COOH acid required in this process is higher than HCl for a specific time. Again cost of CH_3COOH is higher than that of HCl. Regarding the effect of two acids on microorganism in biological treatment, study findings show that the microbial count remains the same in Aeration tank for both the cases. Since there is a dearth of researches related to the treatment of antibiotic containing pharmaceutical wastewater, especially in the context of Bangladesh, therefore, the present study findings could not be compared with the previous study results. According to Fukustu,et. al., (2006), β-Lactam antibiotics were found better degraded by Hydroxylamine compared to NaOH. Since Hydroxylamine is a costly chemical, therefore the present study did not consider its use as a cost effective chemical for Bangladesh.

The performance of different units of a Biological (Activated Sludge Process) CETP has also been studied in the present study. From this research, it is observed that the β-Lactam ring is completely absent i.e. no reformation of β-Lactam ring in final discharge of CETP and contaminant in antibiotic containing wastewater is significantly reduced

(reduction of BOD_5 62%, COD 68%, TSS 98% and TDS 28%) in Combined Tank with the mixing of relatively high volume and low concentration of wastewater from Sold Dosage Unit. Therefore, the dominant treatment process here is the Dilution. After then, Aeration tank plays a major role in treating the contaminant. The removal of BOD_5 and COD mostly occurs in Aeration Tankof CETP (66%, 46% respectively). It has been observed from the laboratory analysis that COD, BOD_5, TDS, TSS and Turbidity of wastewater in Clarifier, DO Increase Tank and Filtration unit, has not been removed much. Contrary to their removal, the concentrations of those parameters were found increasing in those units. Therefore it can be said that none of these units are effective in removing these contaminants. It was observed while sampling from the clarifier that the sludge was not properly settled down rather than carried away with the treated water to the following units. This might be the cause of increased amount of COD, TDS, EC, TSS and Turbidity in those units. Having relatively less initial concentration of contaminants in combined tank, the effluent discharged from the final point of CETP complies with the ECR, 1997 Standard limit regarding pH, TDS, TSS, BOD_5 and COD values, except DO concentration. DO value was increased (5.06 mg/L) in DO increase tank but then reduced to an amount of 2.28 mg/L after passing through the Filtration unit.

Therefore, regarding the performance of CETP in the studied industry, it can be said that the Decontamination Tank, Combined Tank and Aeration Tank are efficiently take part in the treatment of the industry's wastewater, other units role in treatments (Clarifier, Filtration unit) is insignificant.

5.2 Conclusion

The major conclusions drawn from the present study are as follows:

a. Both HCl and CH_3COOH are effective in decontamination of β-Lactam ring residues in wastewater discharged from antibiotic production unit.
b. Although 6 hr reaction time is practiced in studied industry, but 3 hr reaction time is found effective for decontamination process using HCl/CH_3COOH acid with NaOH.
c. As the chromatogram of sample solution represents the absence of peaks for Cefixime and Cefuroxime Axetil, so it can be said that there is no reformation of β-Lactam ring after neutralization by HCl or CH_3COOH.

d. Amount of HCl required in decontamination process is less than that of CH_3COOH
e. Cost of using CH_3COOH is higher (around 98%) than HCl in decontaminating β-Lactam residues
f. Use of organic Acetic Acid has no effect on bacteriological treatment process in CETP as the number of bacterial count remained almost same as with inorganic HCl acid.
g. No reformation or presence of β-Lactam ring in the final effluent at the discharge point from CETP was observed in the present study.
h. Less amount of wastewater (average 3.07 m^3/day) discharged from antibiotic production unit can been characterized as acidic with low DO content and high BOD_5, COD and TSS values exceeding ECR, 1997 limit.
i. The BOD_5, COD and DO concentration of wastewater in Common Tank are higher than that of ECR,1997 limit whereas and TSS are less than ECR, 1997 TDS limit.
j. Dilution process in Combined tank (mixed wastewater from antibiotic production unit and solid dosage unit) mainly reduces the contaminants in industry'swastewater.
k. COD and BOD_5 removal (46% and 66% respectively) is mostly occurred in aeration tank of CETP and the other units (Clarifier, Filtration) are not effective in reducing COD, BOD_5, TDS and TSS
l. Heavy metal such as Cu, Cd, Cr and Zn concentration in both influent and treated effluent are not significant and are well below the ECR, 1997 limit.

5.3 Limitations of the Research

a. 1 hr and 2 hr decontamination retention time evaluation is necessary for comparative discussion of decontamination performance with 3 hr retention time.
b. Individual ETP performance using HCl and CH_3COOH separately as neutralization of decontaminated wastewater could not be conducted due to the complex official constrain from the industry.
c. Microbial count test in field scale rather than lab scale is more preferable for counting microbial community.

d. Due to lack of related research the study findings could not be compared with others

5.4 Recommendation

Efficient and cost effective method of decontamination of Pharmaceutical liquid waste will encourage the industry people to have and run their ETP regularly and thus help in reducing the threat to the environment and public health to a great extent. The following recommendations are made from the present study which will enhance the findings of this study further.

a. In this research work decontamination process was performed only for two compounds i.e. Cefixime and Cefuroxime Axetil which was formulated in Cephalosporin Antibiotic production unit. But there are other several compounds containing β-Lactam ring such as Cefpodoxime, Ceftazidime, Ceftriaxone and Meropenem which should be taken into consideration for better understanding of Decontamination.
b. The cause of ineffectiveness of Clarifier and Filtration unit of CETP should be investigated and attempt should be undertaken to improve their efficiency in treating the wastewater.
c. More research works should be performed to understand and justify the cause of increasing value of COD, TSS, EC, TDS, Turbidity and Zn in the wastewater in Clarifier and Filtration units of ETP.
d. Placement of DO Increase Tank after the Filtration unit instead of placing before it (existing situation) may increase DO content in the final treated effluent and may comply with the ECR, 97 value. This can be studied in a further study.
e. Sludge produced in ETP may contain heavy metals that make sludge ineligible to the environment. A study on the removal of heavy metal from the sludge produced from the ETP can be considered. Also excess sludge which is dried in sludge drying bed should be tested for any presence of β -Lactam residue.

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