REMOVAL OF TETRACYCLINE GROUP OF ANTIBIOTICS BY NANO COMPOSITE OF TITANIUM

By

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A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

In

Environmental Science

INTERNATIONAL ISLAMIC UNIVERSITY, ISLAMABAD

Faculty of Basic and Applied Sciences

Department of Environmental Science

2016

Tetracycline

Final Approval

It is certified that the contents and forms of the thesis entitled

REMOVAL OF TETRACYCLINE GROUP OF MEDICINE BY NANO COMPOSITE OF TITANIUM

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This thesis is dedicated to my parents for their undivided love and support

ACKNOWLEDGEMENTS

This dissertation would not have been possible without the guidance and the help of several individuals who in one way or another contributed and extended their valuable assistance in planning, executing and finally, presentation, of this study.

First and foremost, I would like to express my sincere gratitude to my thesis supervisor **Dr. Maliha Asma** for her continuous support throughout my stay at DES, for her patience, motivation, enthusiasm, and immense knowledge. Her guidance helped me through my research and writing of this thesis. I could not have imagined having a better advisor and mentor for my MS studies.

Many thanks also go to **Mr. Nouman Rafique** (Department of Physics,IIUI) for his guidance and unfailing moral support.

Last but not least, I would like to express my gratitude to all my friends, for their support during this arduous academic odyssey.

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LIST OF ABBREVIATIONS

AC	Activated Carbon
AOPs	Advanced Oxidation Processes
APIs	Active Pharmaceutical Ingredients
CB	Conduction Band
COX	Cyclo-oxygenase
DWSs	Drinking water sources
e^-	Electron
EDCs	Endocrine Disrupting Chemicals
EEA	European Environmental Agency
EPA	Environmental Protection Agency
ERA	Environmental Risk Assessment
eV	Electron Volt
FIMEA	Finnish Medicines Agency
FTIR	Fourier Transform Infrared Spectroscopy
FWHM	Full width of a diffraction line at half of maximum Intensity
GW	Ground Water
h^+	Hole
IBP	Ibuprofen
NRDC	National Research and Development Center
NSAIDs	Non Steroidal Anti- Inflammatory Drugs
$\bullet O^{-2}$	Oxygen Radical
•OH	Hydroxyl Radical
OH^-	Hydroxyl Ion
OTC	Over the Counter
PC	Parent Compound
PhACs	Pharmaceutically Active Compounds
PPCPs	Pharmaceuticals Personal Care Products
ppm	Parts Per Million

SEM	Scanning Electron Microscopy
STPs	Sewage Treatment Plants
SW	Surface Water
TC	Tetracycline
TiO_2	Titanium Dioxide
TNPs	Titania Nano Particles
TP	Transformation Product
UV	Ultra Violet
VB	Valance Band
WHO	World Health Organization
WW	Waste Water
WWTP	Waste Water Treatment Plant
XRD	X - Ray Diffraction
λ	Wavelength

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ABSTRACT

Water is essential for our economy and environment. The harsh reality is that water is limited and depleting at a fast rate. Pakistan ranks at number **80** among **122** nations regarding drinking water quality. Human activities like improper disposal of pharmaceutical waste, municipal and industrial effluents are the main causes for this deterioration. It is now imperative to conserve, treat, reuse and protect our water resources.

Pharmaceuticals are newly identified persistent pollutants and amongst them antibiotics are most contaminated ones. Innovative method of nanotechnology was applied to achieve the removal of pharmaceutical waste (Tetracycline) from water using the nano composite of TiO_2-AC. Such nano composite reduce the energy demand, cost and use of chemicals for medicine removal.

The aim of this study was to develop and ensure the effective degradation of Tetracycline (TC) by successfully developing TiO_2-AC (1%, 2%, and 3% w/w) nano composites. Liquid Impregnation method (LIM) was adopted to achieve required composite.

Tetracycline degradation studies were carried out onto a designed reaction chamber under UV-lamp at different concentrations of medicine, different molar ratio of catalyst (1%, 2%, and 3% w/w TiO_2-AC) and different pH's. The TiO_2-AC2 composite showed significant TC removal efficiency of 96.4%. This composite showed best results for such concentration of the pollutant and could be efficient for the removal of other pharmaceuticals as well.

Introduction

INTRODUCTION

1.1. Background

To meet the demands of fast growing world population for drinking water, there is an increasing pressure on both surface and ground water resources. Contamination of both surface and groundwater resources has severely affected both quality and quantity of water. Only one fourth of the population has access to safe, drinkable water (Farooq *et al.*, 2008). With only 8% waste water being treated and annual loss to Arabian Sea, annual per capita water availability has decreased from 5000 in 1940 to just 1000 litres today (world bank, 2005).

Water degradation has threatened fresh water to become endangered. A commodity which was once free has now become priceless that few can afford. As Pakistan is a water stressed country, this gives rise to many issues and challenges which Pakistan can't ignore (State of Environment Report, 2005). Estimated Annual Economic loss of 0.6-1.44% in Gross Domestic Product is faced by Pakistan because of water related diseases and its treatment (Hashmi *et al.*, 2009).

There are certain pollutants which are persistent in nature and cannot be easily degraded. Pharmaceuticals, pesticides and dyes are considered to constitute this new class of world's pollution. The increase of world population, the origin and spread of new of diseases, and the growth of pharmaceutical industry and day care centers have caused excessive use of pharmaceuticals in human and veterinary, aquaculture and agricultural practices that has led to continuous discharge of a wide array of pharmaceuticals into the environment (WHO, 2011).

Other than prescribed drugs, the use of over the counter drugs has been increased. According to US Food and Drug Administration survey, 69% of respondents were using OTCs from last six months (FDA, 2011).

Discovery of antibiotics has always been considered as the wonder of the 20th century. There is no doubt about it but in the present scenario, the problem being faced is the environmental contamination because of antibiotic resistance in communities and hospitals due to their overuse (Davies and Davies, 2010).

Antibiotics are particularly significant drugs used to treat most common infectious diseases since 1928 with the first ever discovered penicillin, termed as antibiotic in 1940 (Mahyew, 2012). Tetracyclines (TC's) are the second most widely produced and used antibiotics that has been used commonly for the treatment of a wide class of bacterial infections of human and animals. They have been used as growth promoters in agriculture and aquaculture from several decades (Shi *et al.*, 2016) (Chopra and Robert, 2001).

The chemical structures of most commonly used tetracyclines are given in Figure 1.

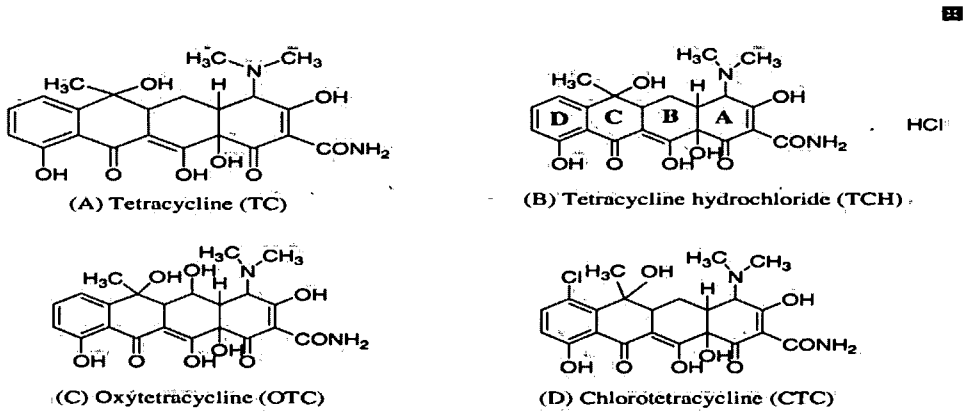


Figure 1.1: Chemical structures of TC group of antibiotics (Mahamalik *et al.*, 2015).

Broad spectrum action of TCs, against many bacterial strains, makes them most frequently used antibiotic. Less market prices and minimal side effects proved them as most abundantly used antibiotics. But instead of its essential roles in both human and veterinary medical world, the emergence of pollution has made it a considering agent (Chopra and Robert, 2001). The adverse effects of TC on living beings are shown in Table 1.

Table: 1.1 Adverse effects of released TC on living beings

Effect On	**Adverse effect**
Human	Damage kidneys and central nervous system.
	Mutagenic (causing mutation in genes)
	Carcinogenic (have potential to cause cancer)
	Affects the process of spermatogenesis (Mahamalik *et al.*, 2015).

	Cause "Immunomodulation" the process of change in the cells of immune system (Romero *et al.*, 2012).
Aquatic species	When directly encounters cause acute and chronic toxicity
	Impact on aquatic photosynthetic organisms.
	Disruption of indigenous microbial populations (Mayhew, 2012)
Microbes	Formation of antibiotic resistant (Zhang *et al.*, 2015)
	Disrupts microbial soil respiration
	Disturbance of; nitrification iron reduction phosphatase activities (Gao *et al.*, 2012).

1.2 Tetracycline residues in the Environment

Antibiotics are primarily poorly absorbed by humans and animals. Large concentrations about 50-80% of TC are discharged from consumers as unmodified compound or parental compound and the other main source is pharmaceutical industry effluent (Guler and Sarioglu, 2014). Human and animal, being the two major users of tetracycline, discharges this antibiotic into the ground and surface water through several ways.

- Human ways of discharge
    - Un-metabolized or partially metabolized tetracycline is discharged in urine and enters the sewage system.
    - Disposal of extra or unnecessary drugs into sewer system.
- Animals ways of discharge
    - Tetracycline present in the animals for antibiotic treatment is transferred into human when they consume meat and enter into sewer system.
    - Animal manure is used as fertilizer on fields.
        - Runoff makes the direct pollution of surface water.
        - Absorption into the soil, contaminates ground water (Jorgensen, 2004).

Various routes of pharmaceuticals entering into our environment are given in Figure 2.

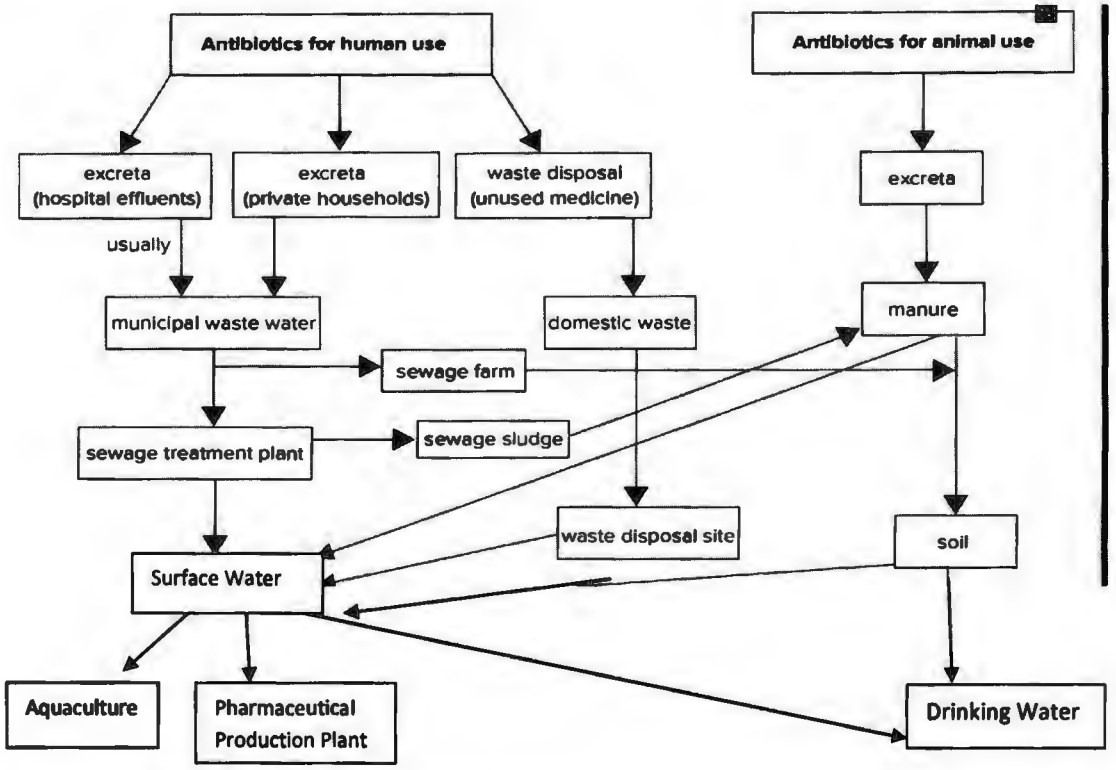


Figure 1.2: Sources and pathways for antibiotic contamination of water and soil (Gelband *et al.*, 2015)

1.3 Treatment Technologies

Over the last decade, many substances have been explored for the degradation of TC and other antibiotics from wastewater, such as Rectorite, mntmorillonite, smectite clay, coal humic acid, palygorskite, aluminum oxide, activated carbon, single walled and multi walled carbon nanotubes, chitosan particles, etc. But still there is a need for cost-effective and efficient latest technologies for degradation of stable pollutants (Gao *et al.*, 2012).

The toxic environmental pollutants, those offer resistance to conventional water treatment methods, can be effectively degraded by Advanced Oxidation Processes (AOPs). Among AOPs, heterogeneous photocatalysis has been engaged to remove a number of environmental pollutants like phenols, toluene, textile dyes, pharmaceuticals, pesticides etc. (Gaya *et al.*, 2008). Photocatalytic oxidation completely oxidizes chemicals to CO_2, H_2O and mineral acids (Velmurugan *et al.*, 2012). Among various catalysts used in AOPs, TiO_2 has been proved to be most promising way to destroy these stable organic pollutants (Zhao *et al.*, 2014) under

Ultraviolet irradiation in wastewater and in air owing to its photochemical stability, non-toxic nature and relatively low cost.

However, research work showed certain limitations of TiO_2 use particularly;
(a) TiO_2 is difficult to separate from the solution after use,
(b) Accumulation of particles in solution, while massive contaminants
(c) Application is difficult in continuous flow systems (Leary and Westwood, 2011).
In solution of these problems, the combination of TiO_2 with some strong adsorbant was suggested like TiO_2/zeolite, TiO_2/activated carbon, TiO_2/Al_2O_3 and TiO_2/SiO_2. Adsorbants prolong the contact time between TiO_2 and contaminant, other than physical absorbing properties (Muthirulan *et al.*, 2013).

The present study designated the validity of AC on TiO_2 catalyste for photocatalysis and removal of the antibiotic Tetracycline with changing physico-chemical conditions. The present aim is to establish a composite, where the massive pollutant is removed by adsorption and the resultant low concentration water then being polished by photocatalysis.

1.4 Objectives

Main objectives of this proposed research are summarized in following points:

- To synthesis nano TiO_2 and TiO_2-AC composite by liquid impregnation method
- To characterize the nano TiO_2 and composite of TiO_2-AC by
    - X-ray diffraction Analysis (XRD)
    - Scanning Electron microscopic (SEM)
    - Fourier transform infrared spectroscopy (FTIR)
- To study the degradation of tetracycline in UV irradiation and dark conditions by
    - UV–Vis spectrophotometer

Literature Review

LITERATURE REVIEW

Water pollution is one of the major threats to public health in Pakistan. Our country is ranked 80 among 122 nations with respect to the drinking water quality. Surface and groundwater water is frequently contaminated with microorganisms, metals, organic and inorganic contaminants, pesticides and pharmaceuticals. Lack of proper disposal of domestic, agricultural and industrial wastes is the main element that contributes to the degradation of water quality (Azizulah *et al.*, 2011).

Nowadays, the depollution of water from pharmaceutical compounds is of high interest, due to evidently increase in its concentrations in drinking and wastewaters (Scheurer *et al.*, 2012). There are 4000-7000 compounds which are used as drugs. Each year a slight increase in number of hazardous compounds have been observed. Therefore, the removal of personal care products (PPCPs), pharmaceuticals and endocrine disrupting chemicals (EDCs) are now a very genuine and demanding task (Choina *et al.*, 2015).

2.1. Pharmaceutical Sources and Occurrences

Pharmaceuticals are chemicals that could be natural or manmade, used in the form of prescribed drugs, over-the-counter (OTC) drugs and veterinary medicines. The active ingredients of these drugs cause pharmacological effects and significant benefits to users. The increase of world population, the origin and spread of new of diseases, and the growth of pharmaceutical industry and day care centers have caused excessive use of pharmaceuticals in human and veterinary, aquaculture and agricultural practices that has led to continuous discharge of a wide array of pharmaceuticals into the environment (WHO, 2011).

Table 2.1: Measured concentrations of selected pharmaceuticals in the aquatic environment (WHO, 2012)

Compound	Median (Maximum) Concentration (ngL^{-1})		References
	Sewage treatment work effluent	Stream or river water	
Diclofenac	424 (2349) 289 (598) —	< LOQ (568) < LOQ < LOQ (195)	Ashton, Hilton & Thomas (2004) Roberts & Thomas

			(2006) Thomas & Hilton (2004)
Tetracycline	—	~1000	Watts *et al.* (1983)
Erythromycin	— < LOQ (1842)	< LOQ (80) < LOQ (1022)	Ashton, Hilton & Thomas (2004)
Paracetamol	< 20 —	— 555	Roberts & Thomas (2006) Bound & Voulvoulis (2006)

More than 30 years ago, PPCPs were first detected in the environment; in 1970s studies conducted in USA confirmed the presence of birth control, heart and pain killer's medicines in wastewater. Most of the work was done by United States Geological Survey and most cited reference in the literature on the presence of pharmaceuticals in water was provided by the same organization, in which 139 streams across 30 states in USA were investigated during 1999 and more than 50 pharmaceutical chemicals were found (WHO, 2011).

Now, pharmaceuticals originate a new class of world pollutant which is of great concern in present years. There are various routes of pharmaceuticals entering into our environment including human or animal discharge, effluents from treatment plants, treated sewage sludge, industrial runoff, medical waste from hospitals, landfill leachate and biosolids (WHO, 2011).

Following are different sources of pharmaceuticals discharges into the water.

- Municipal wastewater treatment plants
- Pharmaceutical industry effluent
- Agricultural runoff
- Animal and Fish Farms
- Hospital and Household
- Urine and feces

Excretion rates of PPCPs through urine and faeces depend on the pharmaceutical substance, the way of intake (orally or intravenously for humans), the age of the patient and the time of administration (Kümmerer *et al.*, 2000; Pérez and Barceló, 2007; Kemper, 2008).

PPCPs once ingested and metabolized are excreted as urine and feces and take the route of sewer flow network as urban wastewater until reaches to wastewater treatment plant (WWTP) or, for households directly discharged into septic tanks (Carrara *et al.*, 2008). Veterinary pharmaceuticals are released into the water, directly and indirectly in many ways, like through direct application in aquaculture (i.e. fish farming) and animal treatment and mostly through leaching from fields of manure spreading on agricultural fields, run-off and livestock waste (Sanderson *et al.*, 2007; Boxall *et al.*, 2004; Boxall, 2008; Khan *et al.*, 2007; Sarmah *et al.*, 2006; Kemper, 2008).

Depending on their biodegradability, temperature and hydrophobicity pharmaceuticals and their metabolites undergo natural depletion by dilution, degradation or adsorption in the environment. Therefore, pharmaceuticals in drinking water and water sources are often found at trace concentrations, as these compounds would have undergone through natural processes like metabolism and removal, if appropriate wastewater treatment processes are applied (WHO, 2012).

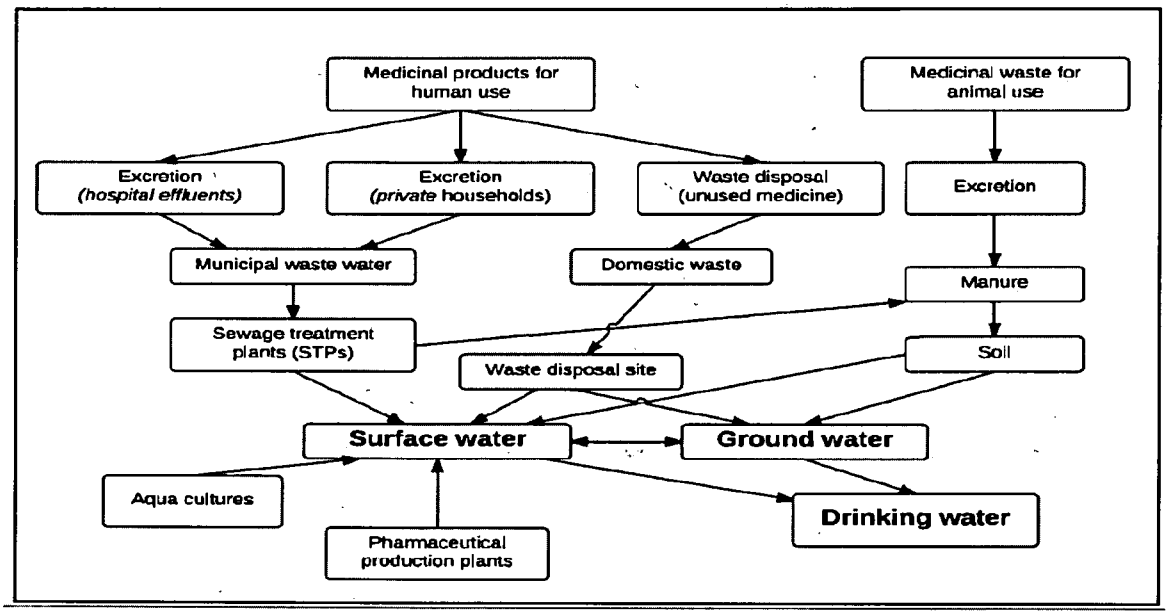


Figure 2.1: Possible pathways for antibiotic exposure to environment (Heberer, 2002)

In 2010 a report published by the European Environmental Agency (EEA) indicated that the status of environmental risks posed by pharmaceuticals "looks worse" than a decade earlier (European Environment Agency, 2010). Every year hundreds of tones of pharmaceuticals are dispensed in communities and because of nonstop entry and combined effects through parallel action make the low persistent compounds to cause unwanted effects in the environment (Fent *et al.*, 2006).

According to the report of NRDC (2009), pharmaceuticals include medical agents such as chemotherapeutic drugs, human and veterinary medicines (both prescription and over-the-counter), and x-ray media. These materials are ending up in the environment through improper disposal. Circumlocutory routes of entry into the water are also problematic. For example in the arid United States, as water resources are depleted, reclaimed wastewater is becoming an increasingly important source for irrigation. This contaminated wastewater used for irrigation is causing pharmaceuticals to enter the soil and potentially leach into the groundwater. Perhaps human use is one of the most common route through which pharmaceuticals enter the water, followed by excretion to a sewage treatment plant and release to surface water as wastewater effluent. Other significant contribution to this problem includes veterinary use, both in farming operations and in aquaculture.

Another potential source of entry into the water is excretion of insufficiently treated wastewater effluent, washed off human body or directly disposed to the sewerage system. In surface waters several groups of pharmaceuticals were studied for their presence. Several parameters identify their presence, quantity and fate in the environment, such as:

- effectiveness of wastewater treatment
- geographical position
- meteorological conditions (mainly rainfall)
- presence in wastewater plants (Hordern *et al.*, 2008).

PPCPs are spatially, temporally, qualitatively and quantitatively shared out depending on whether patients are located in hospitals and other places e.g. schools and private households. Consumers at medical facilities are designated to be treated with more pharmaceuticals than at household (Kümmerer, 2001).

Researchers confirmed the existence of pharmaceuticals in the environment more than last decades, by comparing the detected amount of heart medicines, pain killers and birth control

medicines in the last 30 years to the present concentrations (WHO, 2011). With an increase in the use of prescribed drugs, the use of over-the-counter (OTC) medicines is also increasing. US Food and Drug Administration (FDA) survey to check the status of over-the-counter (OTC) drugs in America, and found out that 69% of the respondents were using OTC pain killer from last 6 months (FDA, 2011).

That was the situation of a well developed country with literate people, now what could be the status of less developed countries, where health facilities are beyond one's budget. Concerned problem is that the use of both prescribed and OTC's has been increased and ending up into our water resources. Actually our typical water treatment plants are not capable to eliminate pharmaceuticals from water, so effluents of such treatment plants enters into water bodies that can end up in drinking water supplies (Connors *et al.*, 2013).

Surface water and ground water are physically closely connected, and therefore, these mediums can contaminate one another, ground water is polluted from treated wastewater reuse, artificial recharge of polluted surface water and of exfiltration from ground water to connected surface water (Jux *et al.*, 2001).

2.1.1 Antibiotic Residues in the Environment

The group of PPCPs comprises of antibiotics, pain killers and impotence drugs. Antibiotics are particularly significant in medicines; they have decreased the common infectious illness and become vital for many medical considerations. Antibiotic is a killing agent for bacteria or it slows down its development. Antibiotics are just one class of antimicrobials, which is an extensive group including many others working against other harmful microbes like anti-viral, anti-parasitic, anti-fungal drugs. Antibiotics are chemical substances formed by microbes (e.g. germs such as fungi and bacteria). In 1928 Alexander Fleming made the advancement in medical history by discovering first antibiotic.

Antibiotics are of two types based on the way of action. One class of antibiotic is 'bactericidal', they kill bacteria. Second class of antibiotics is 'bacteriostatic', they inhibit the growth of bacteria. Some antibiotics are 'broad-spectrum' antibiotics as they can treat a wide range of infectious diseases. Others are 'narrow-spectrum' antibiotics that can only treat just a few types of infections (Bayarski).

Antibiotic residues introduce into the environment from consumer's (human and animal) waste and from industrial runoff (as discussed above). When medication is taken by consumers, it is released into the environment through urine and feces (Daghrir and Drogui, 2013). Unused antibiotics are also flushed down by consumers, improper dispose of medical wastes by hospitals, and residues leakage from damaged pipeline systems into soil and groundwater. Once released into the environment, very little amount is degraded, while most of antibiotics survive the treatment in the waste water treatment plants (Michael *et al.,* 2013), and residues of antibiotics are found in rivers, sediments, and soils.

Soil is polluted with antibiotics by direct seepage of animal feed or pass through animals and deposit there as waste. Researchers showed that high concentration of antibiotics can enter into the environment through animals: about 90 % of administered dose can be excreted in urine and about 75 % in feces (Sarmah *et al.,* 2006). At the end, antibiotics seep into the ground water and enter into the environment. In aquaculture, antibiotics dissolve and spread into the water and deposit in sediments (Gelband *et al.,* 2015).

In a study of China, high percentage of antibiotics have been found in sediments and water samples, and responsible individuals were suggested to use fish ponds as reservoirs of antibiotic residues (Xiong *et al.,* 2015). Antibiotic industrial runoff adds a high percentage of antibiotics and other medicines into the local environment. In India, an area of extensive pharmaceutical industries like Hyderabad has up to 100 facilities that export drugs to the United States, Europe and other world. The waste of these industries is processed at one single treatment facility. In 2008, the treated water of this facility was accessed from 2 adjacent lakes and 6 nearby wells (Fick *et al.,* 2009). Results showed extreme contamination in that water. One lake had high levels of cetirizine and ciprofloxacin antibiotics that "exceeded human therapeutic blood plasma concentrations" (Gelband *et al.,* 2015).

Table 2.2: Frequency detection of pharmaceuticals, hormones and other organic wastewater contaminants in U.S. streams in years 1999–2000 (Choina *et al.,* 2013).

Sr.No	Category of contaminants	Percentage of streams with contaminants
1	Steroids	89
2	Nonprescription drugs	81

3	Insect repellent	74
4	Disinfectants Phenol	66
5	Antibiotics	48
6	Reproductive hormones	37
7	Other prescription drugs	32

2.1.2 Tetracycline group of Antibiotics

With the discovery of penicillin in 1928, the term antibiotic was first used in 1945 by Waksman as, "a chemical substance, originated by microbes that have antibiotic powers" (Mayhew, 2012). With development, now various kinds of antibiotics are there and taken by patients depending on the kind of infection patients suffer. The major classes of antibiotics are:

- Aminoglycosides
- Fluoroquinolones
- Penicillins
- Macrolides
- Cephalosporins
- Tetracyclines (Bayarski, 2012)

Tetracyclines (TC's), discovered in 1940s, are the second most widely produced and used antibiotics that has been used commonly to treat a wide class of bacterial infections for several decades (Shi *et al.*, 2016). The mechanism of action of tetracyclines is restraining the protein synthesis by avoiding the attachment of aminoacyl-tRNA to the ribosomal acceptor (A) site (Chopra and Robert, 2001). Tetracyclines are being used to treat various diseases including mild acne, upper respiratory tract infections, Rocky Mountain spotted fever, sexually transmitted diseases, Lyme disease, urinary tract infections, typhus (*Bayarski)*. They are also used as preventive treatment of malaria caused by mefloquine-resistant *Plasmodium falciparum*. The tetracyclines have been applied to cure the infections in poultry, cattle, sheep, and swine. In some countries, including the US, little amount of tetracyclines are added to animal forage to work as growth booster (Chopra and Robert, 2001).

Antibiotic	2006 (million pounds)	2007 (million pounds)
Ionophores, arsenicals, bambermycin, carbadox, and tiamulin	11.1	10.6
Tetracyclines	9.2	10.7
Cephalosporins, macrolides, lincosamides, polypeptides, streptogramins, fluoroquinolones, and other minor classes of antibiotics	4.4	4.5
Sulfonamides and penicillins	1.2	1.8
Aminoglycosides	0.33	0.20

Figure 2.2: Major Antibiotics sold in 2006 and 2007 (NRCD, 2009)

Instead of human and veterinary medicines tetracyclines are used in agriculture to treat bacterial infections and aquaculture to cure infections as infections in salmon, catfish, and lobsters. They also inhibit the infection caused by *Erwinia amylovara* on fruit plants and other trees. Tetracyclines are injected into palm trees for the treatment of mycoplasma infections (lethal yellow), and to control *Xanthomonas campestis* (black rot) infection on seeds. They are also used to treat insects of commercial value; i.e, oxytetracycline is used to treat the illness of the honeybee called foulbrood, caused either by *Bacillus larvae* or *Streptococcus pluton* (Chopra and Robert, 2001). Following figure is showing the comparative demand of various antibiotics for agricultural use.

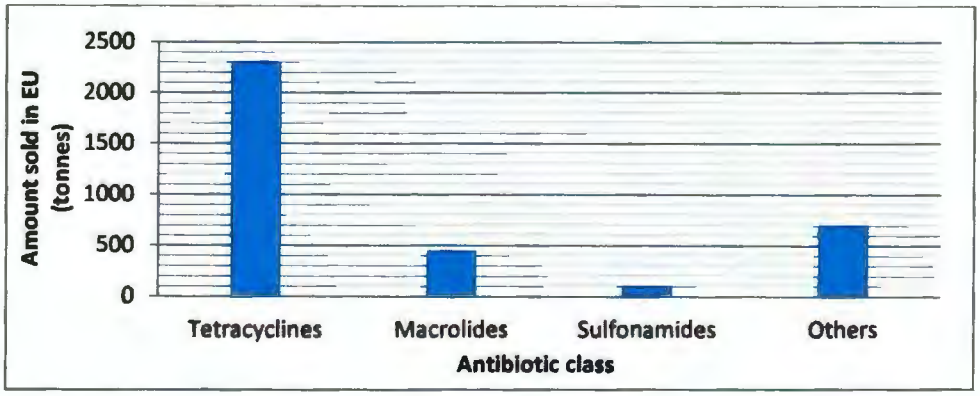


Figure 2.3: Quantity of Antibiotics sold for Agricultural uses. (Mayhew, 2012)

Table: 2.3 commonly prescribed tetracyclines (Domenico and Fuoco, 2012).

Compounds	% Enteric absorption	%Serum protein binding	Renal clearance (ml/min)	Half-Life (hours)
Oxytetracycline	58	30	90	10
Tetracycline	80	60	65	9
Doxycycline	93	85	16	15
Demeclocycline	66	75	31	13
Chlorotetracycline	30	55	35	6
Meclocycline	-	-	-	-
Minocycline	95	90	10	20
Rolitetracycline	-	-	-	-
Tigecycline	-	90	-	32
Doxorububicine	-	70	-	55

Among all, Oxytetracycline (OTC) is the most abundantly found tetracyclines in water bodies and sediments all over the world. Several studies have proved the occurrence of OTC in surface water at lg/L and even mg/L. Sewage of the wastewater treatment plant of a factory in China was investigated by Li *et al.* 2008 and extreme concentrations of OTC (20–800 mg/L) have been detected that was polluting the surface water with OTC concentration. Concentration of antibiotic OTC in surface water was studied at UK and found out to be 4.49-32.00 lg/L (Milic *et al.*, 2013).

2.2 Adverse effects of Tetracycline antibiotic waste on Environment

Now a day antibiotics have become the most regularly prescribed medicines. During the last decade, consumption of antibiotic drugs increased by 36% (54 083 964 813 standard units in 2000 & 73 620 748 816 standard units in 2010) and in terms of production and usage TCs are second most widely used antibiotics in the world (Boeckel *et al.*, 2014). This excessive use of antibiotics is causing adverse effects on biotic as well as abiotic environment.

Antibiotics are primarily poorly absorbed by humans and animals. Large concentrations about 50-80% of antibiotics including TC are discharged through urine and feces of consumers

as unmodified compound or parental compound, the other main source is pharmaceutical industry effluent. Fractions of TC are frequently found in soil and various bodies of water influenced by raw or treated waste water such as surface and ground water and many of which are used as drinking water resources (Guler and Sarioglu, 2014). The increased amount of TCs in the soil and water is raising the hazards for the bacteria and non target organisms because these can promote the growth of resistant bacteria and resistant genes in long term as well as damage to ecosystem (Zhang *et al.*, 2015).

These antibiotic resistant bacteria are not killed by commonly available antibiotics (Bayarski, 2012). Thus pharmacists are inventing new drugs that would be new to bacteria but high prices of those drugs made them unaffordable and restricts their use in developing and underdeveloped countries (Boeckel *et al.*, 2014). Secondly, the adverse effects of previous antibiotics would remain constant or either gets worse.

This new class of pollutant can cause biological effects on living organisms in non-predictable way and proving extremely hazardous at each level of hierarchal system, from cells to human to ecosystem (Choina *et al*,. 2015). It directly encounters with aquatic species and cause possible hazardous effects, both acute and chronic toxicity, disturbance in indigenous microbial populations and impact on aquatic photosynthetic organisms. Tetracycline has also revealed to disturb the microbial soil respiration, nitrification, iron decrease and phosphatase activities (Gao *et al.*, 2012).

A greenhouse study was conducted to observe the impact of antibiotic manure on plants in the soil. Corn, green onion and cabbage absorbed chlortetracycline, although concentrations were low but increased with increase amount of antibiotic applied in manure. The study concluded the potential health hazards related to food consumption. Other studies have shown that high level of antibiotics in swine production can also produce antibiotic resistance in airborne microbes. Thus antibiotics are able to impact not only water, but also soil and air quality (Mayhew, 2012).

In human body kidney and central nervous system is damaged. TC is mutagenic (causing mutation in genes) and carcinogenic have potential to cause cancer). It also affects the process of

spermatogenesis. This way it is causing imbalance in ecosystem equilibrium, and thus it is recognized as threat to world's health (Mahamalik *et al.*, 2015).

Immunomodulation is a process of change in the cells of immune system. This change could be quantitative and/or in function of cells. A number of drugs and environmental chemicals are believed to cause immunomodulation, including antibiotics. Presently reports have been presented on the effects of oxytetracycline on fish immune system. Results showed that oxytetracycline can suppress immune functions of carp, turbot and Atlantic cod. Oxytetracycline was found in low concentrations in sediments, but it is persistent even in low amounts. The half-life of oxytetracycline was measured to be 125, 144 and 87 days under each of the cages in the sediments. At the end of the experiment, all three sediments contained 100% oxytetracycline resistant bacteria. Figure 2.4 is showing the studies of Romero *et al.*, 2012.

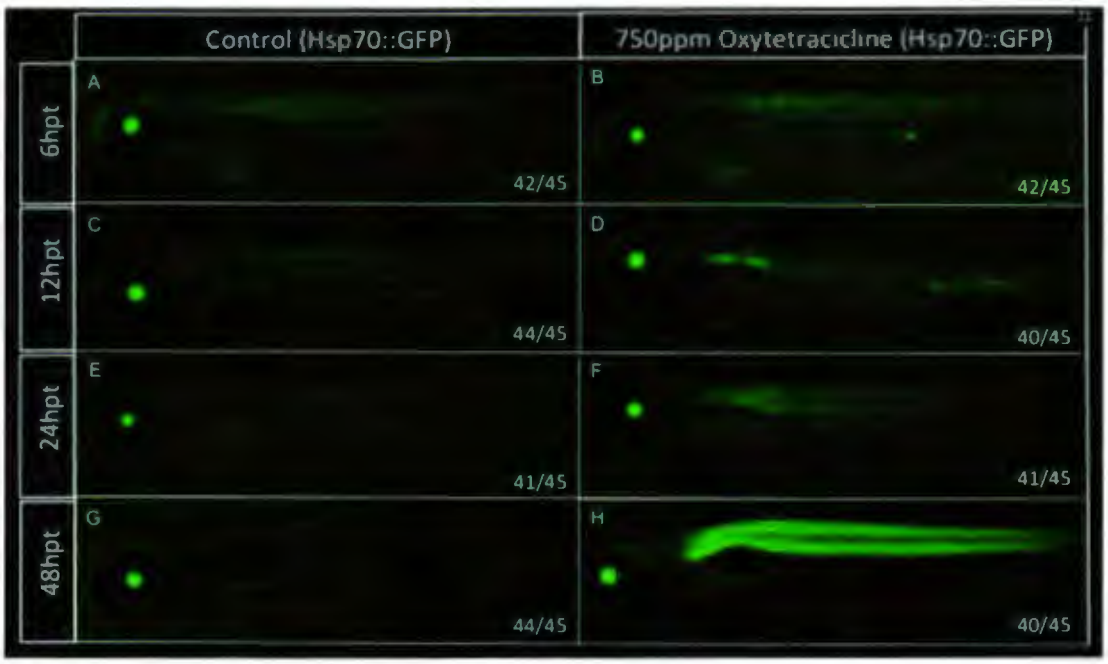


Figure: 2.4 Oxytetracycline effects on stress levels.

Lateral view of Hsp70::GFP transgenic larva incubated in 750 ppm oxytetracycline or control medium for 6 hrs (A, B), 12 hrs (C, D), 24 hrs (E, F) and 48 hrs (G, H) (hpt, hours post treatment) (Romero *et al.*, 2012).

2.3. Treatment of Tetracycline (past studies)

TCs are stable compounds and normally resistant to biodegradation. Biological degradation, if occur, has a much slower rate or negligible (Mahamalik *et al.*, 2015). Traditional treatment technologies for water and wastewater are also insufficient for the removal of these substances. Scientists researched on many different substances for the removal of TC including; Rectorite, mntmorillonite, aluminum oxide, smectite clay, coal humic acid, palygorskite, carbon (activated), chitosan particles, single and multi walled carbon nanotubes. But still there is need for proficient and commercially reasonable latest methodologies for degradation of such stable pollutants (Gao *et al.*, 2012).

Presently, Advanced Oxidation Processes (AOPs) are latest technologies, gaining attention of every researcher. AOPs are a group of chemical processes for the degradation of impurities in water and wastewater by oxidation process. These techniques are efficient for the removal of non-biodegradable contaminants especially for pesticides and pharmaceuticals in water (Mahamalik *et al.*, 2015).

Most commonly used AOPs (Mahamalik *et al.*, 2015) for antibiotics are following:

* Photolysis
* Adsorption
* Ozonation
* Fenton oxidation
* Ozonation in combination of ultrasound irradiation.

All AOP technologies are based on the generation of highly reactive free radicals like hydroxyl radical, to act as an initiator that converts the pollutants into harmless compounds (Tan *et al.*, 2011).

The interest in Photocatalytic reactions was initiated, almost three decades ago, when researchers suggested it as a potential way to promote the dissociation of water to produce molecular hydrogen and molecular oxygen to degrade organic pollutants using solar illumination. Photocatalysis mainly consists of the combination of photochemistry and catalysis. Catalysis is the process where a substance called catalyst triggers the rate of a chemical reacion without being altered or consumed in the end. In photo catalysis reaction light is used to activate

a substance which accelerates the rate of chemical reaction exactly like in catalysis. And the substance used as modifier in light dependent process is called as photo catalyst (Othani, 2011).

Among these, "photocatalysis" is the most discussed method in the destruction of harmful pollutants. The photocatalytic process starts with absorbtion of photon by semiconductor and at the ends, water and carbon dioxides are released as by products.

Subsequently, additional applications of photocatalysis were also discovered and these applications could be classified into following areas as shown in Fig. 2.3.

There are several advantages associated with photocatalysis process (Matthews, 1992) which make it a good alternative to other treatment technologies like;

- The photocatalyst is non-hazardous
- Avoidnence of hazardous and expensive oxidizing chemicals
- Complete breakdown of majority of pollutants, and
- The effluent is environmentally benign.

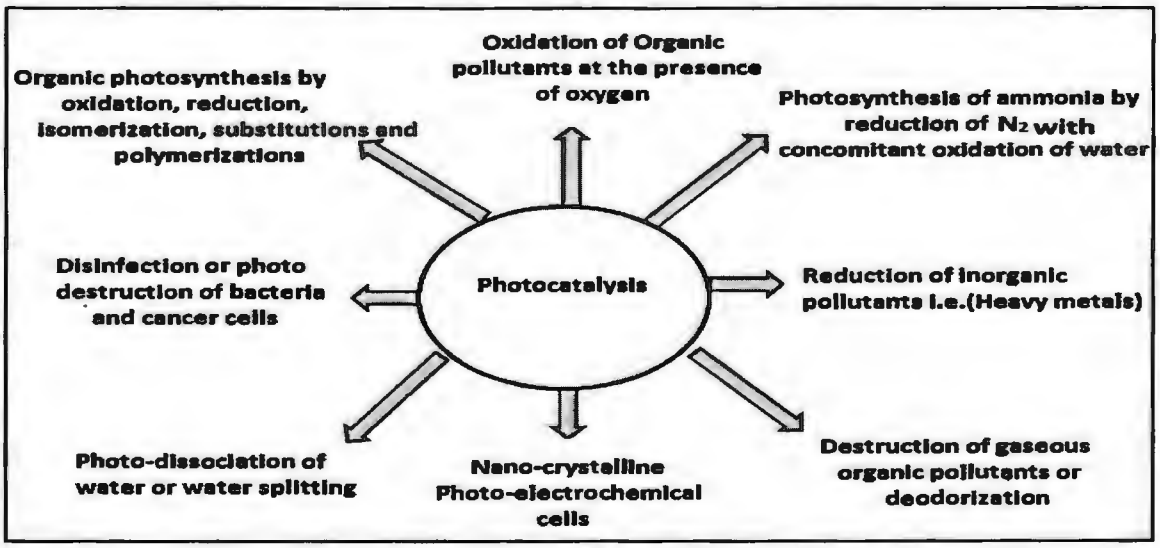


Figure 2.5: Applications of Photocatalysis

2.3.1. Mechanisms of Photocatalysis

The process of photocatalysis comprises of some basic chemical and physical steps, initiated by light, that have been discussed, in detail, by many researchers (Konstantinou and Albanis, 2003; Adesina, 2004; Tan *et al.*, 2011).

The electrons in the valence shells were stroked with the same energy or greater to their band gap. This excites the electrons of valence bands to move toward conduction band energy level (Eq. (1)). These electrons (e^-_{CB}) can recombine with the photo-generated electron-hole pairs (holes, h^+_{VB}) and decrease the efficiency of the photocatalosis (Eq. (2)). These electrons can also migrate on surface of the catalyst where particles are trapped by adsorbed oxygen, and result in formation of superoxide anion (O_2^-) thus increasing the speed of photocatalysis (Eq. (3)).

Initially the spaces created in the valence band (h^+_{VB}) are now stuck on the catalyst and promote the breakdown of H_2O or OH- that generates hydroxyl radicals (HO°) (Eqs. (4) and (5)) (A. Palominos *et al.*, 2009).

These HO° radicals are responsible for oxidation. At the end, the adsorbed organic matter is oxidized by liberating intermediates (Eq. (6)).

$$\text{Semiconductor} + h\nu \Longrightarrow e^-_{CB} + h^+_{VB} \quad (1)$$

$$e^-_{CB} + h^+_{VB} \Longrightarrow \text{semiconductor} + \text{heat} \quad (2)$$

$$e^-_{CB} + O_{2(adsorbed)} \Longrightarrow O_2^{-\circ} \quad (3)$$

$$h^+_{VB} + H_2O_{(adsorbed)} \quad H^+ + HO^\circ \quad (4)$$

$$h^+_{VB} + HO^-_{(adsorbed)} \quad HO^\circ \quad (5)$$

$$h^+_{VB} + \text{organic matter (OM)} \quad OM^{+\circ} \quad \text{oxidised products} \quad (6)$$

Following figure is displaying photocatalytic activity mechanism in Titania.

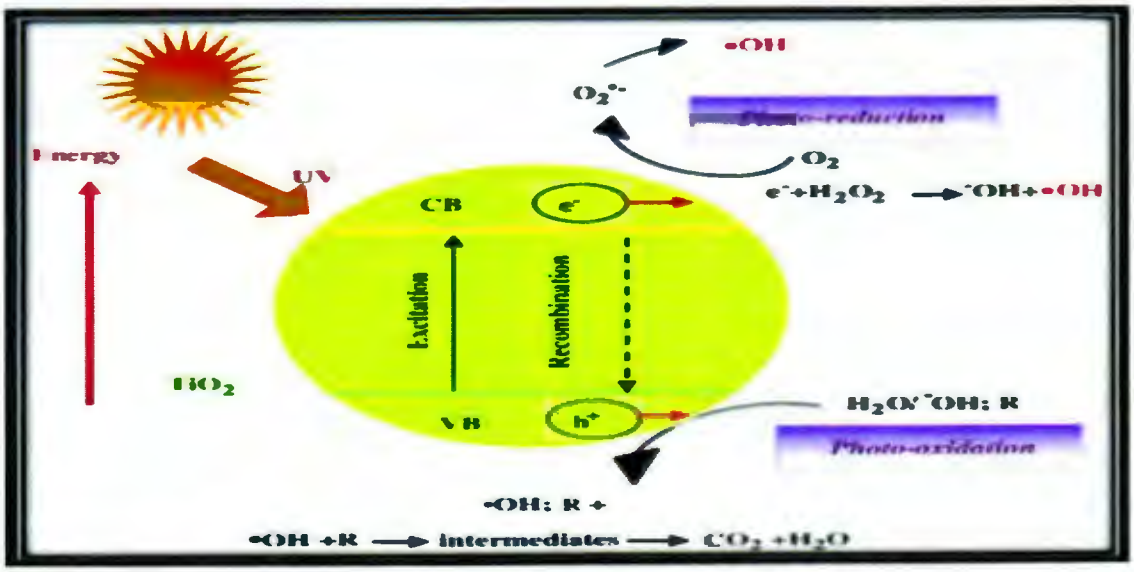


Figure 2.6: Photocatalytic Activity Mechanism in TiO_2 (Ahmed *et al.*, 2010)

years of development has made TiO_2 the most popular photocatalyst to resolve environmental problems (Wang *et al.*, 2014).

2.3.5. Polymorphs of TiO_2

Three polymorphs of TiO_2 are:

1) Anatase
2) Brookite
3) Rutile

Almost all studies have used Rutile and Anatase phase of TiO_2 in photocatalytic degradation processes. Of the two, the Anatase phase is the preferred polymorph having highest photo-catalytic activity (Beydoun *et al.*, 1999; Carp *et al.*, 2004).

Crystalline structure of titania is displayed in figure 2.7.

Figure 2.7: Crystal Structures of Polymorphs of TiO_2 (Danish *et al.*, 2013)

Some studies have claimed that mixtures of Anatse and Rutile phase give better results in term of photo-catalytic degradation of organic material (Giolli *et al.*, 2007; Sun *et al.*, 2008).

Table: 2.4 Required Wavelengths for Activation of Polymorphs of TiO_2

Polymorph	Band Gap Value	λ Photo-excitation (nm)
Rutile	3.2 eV	Upto 385
Anatase	3.02 eV	Upto 385
Brookite	2.96 eV	Upto 375

2.3.6. TiO_2 as a Photocatalyst

Photocatalytic activity of TiO_2 was first discovered by Fujishima and Honda in 1972 (Fujishima *et al.*, 1972). Redox process is induced on the surface of titania, commonly used as a photo catalyst due to generation of charge carriers (Kim *et al.*, 2007) (Benabbou *et al.*, 2007; Kumar and Raza, 2009). TiO_2 is renowned in the world of photo catalysis due to unique properties like;

- High photo excitation
- Nontoxic in nature
- Very stable
- Cost effective

2.3.7. TiO_2 Photocatalytic Mechanism

$TiO_2 + hv \longrightarrow TiO_2 + (e^- + h^+)$

Reaction involving conduction band

$$TiO_2 (e^-) + O_2 \longrightarrow TiO_2 + \bullet O_2^-$$

$$TiO_2 (e^-) + \bullet O_2^- + 2H^+ \longrightarrow TiO_2 + H_2O_2$$

$$TiO_2 (e\text{-}) + H_2O_2 \longrightarrow TiO_2 + \bullet OH + OH^-$$

$$\bullet O_2^{=} + H_2O_2 \longrightarrow \bullet OH + OH^- + O_2$$

$$\bullet O_2^{=} + H^+ \longrightarrow \bullet HO_2$$

$$TiO_2 (e^-) + \bullet HO_2 \longrightarrow TiO_2 + HO^{-2}$$

$$HO^{-2} + H^+ \longrightarrow H_2O_2$$

$$2\bullet HO_2 \longrightarrow H_2O_2 + O_2$$

Reaction involving valance band h+

$$TiO_2 (h^+) + H_2Oads \longrightarrow TiO_2 + \bullet OHads + H^+$$

$$TiO_2 (h+) + 2H_2Oads \longrightarrow TiO_2 + 2\,H^+ + H_2O_2$$

$$TiO_2 (h^+) + OH^-ads \longrightarrow TiO_2 + \bullet OHads$$

2.3.8. TiO_2 catalyst for wastewater of pharmaceuticals

Photocatalysis has been applied for water purification (Younas, *et al.*, 2014). Presently treatment of water and wastewater are done by old treatment technologies like chlorination, air stripping, ozonation, biodegradation, solidification and landfill and granulated activated carbon adsorption are used meanwhile latest technologies like membrane bioreactor and advance oxidation processes (AOPs), including photocatalysis and photolysis are also under research and trial practices. This latter has the advantage that it completely degrades, or breakdown, the organic pollutants in a single atempt and further treatment for the byproducts is not necessary. TiO_2 photocatalysis is therefore very suitable for removing many pollutants in both aqueous and gaseous waste stream (Bockelmann *et al.*, 1995; Alfano *et al.*, 2000; Zuo *et al.*, 2006; Xie *et al.*, 2011; Laokiat *et al.*, 1992).

Table: 2.5 Literature Review of Titania for Pharmaceutical Removal

Reference	Catalyst Used	Target Pharmaceutical
Ofiarska *et al.*, 2016	Pt–TiO_2	2 cytostatic drugs: ifosfamide and cyclophosphamide
Lai *et al.*, 2015	TiO_2	oxazaphosphorines ifosfamide (IFO), cyclophosphamide (CP) and trofosfamide (TRO)
Teixeira *et al.*, 2015	TiO_2 and ZnO	14 different pharmaceuticals
Zhang et al., 2015	P25-TiO_2/tetraethyl orthosilicate film	4 (NSAIDs): salicylic acid, ibuprofen, naproxen and diclofenac.
Pugazhenthiran *et al.*, 2013	TiO_2+Au	Ceftiofur sodium (antibiotic)

2.3.9. Technical Drawback of TiO_2 and its Solution

TiO_2 is the best among many photocatalysts but its band gap energy requirements restrict its use. TiO_2 requires 3.2 eV of energy (equivalent to a wavelength of 388 nm) in order to act as a photocatalyst; this energy can only be provided by longer incidence of UV light source making the process of photo catalysis slightly inconvenient (Linsebigler *et al.*, 1995). This difficulty has

been overcome by inducing low energy levels within the band gap of TiO_2 with the help of doping (Gaya and Abdullah, 2008).

2.3.10. TiO_2 Doping

Various elements can be used as dopants for TiO_2, including metals like iron, silver, manganese and copper or non-metallic dopants such as carbon, boron and nitrogen etc. (Arana *et al.*, 2004; Wu *et al.*, 2004; Hamal and Klabunde, 2007). Doping must be controlled carefully as high dopant concentrations may result in electron and hole recombination (Coleman *et al.*, 2005; Carp *et al.*, 2004).

2.3.10.1 AC doped TiO_2 for the treatment of wastewater

The combination of Tio_2 with some strong adsorbant was suggested in literature, like activated carbon. AC adsorb the pollutant and transfer it to TiO_2 for degradation, it also prolong the time period for separation of electrons and electron hole pair (Rioja *et al.*, 2012).

Thus in all supporting materials, granular activated carbons (ACs) found to be most attractive candidate because of its excellent adsorption performance, high porosity and financially affordable. It has been already studied in aqueous and gaseous phases for the photocatalysis of organic contaminants. TiO_2-AC produce a synergistic effect, as both share a common contact surface between both catalysts (Wang *et al.*, 2009). Table 2.6 is presenting the review of literature in which AC doped TiO_2 had been used by various researchers.

Table: 2.6 Literature Review of AC Doped Titania for Pharmaceutical Removal

Reference	Catalyst Used	Target Pollutant
Lin *et al.*, 2014	Carbon doped TiO_2	Ethylene
Wang *et al.*, 2009	Nano TiO_2 immobilzed on AC	Methyl orange
Muthirulan *et al.*, 2013	CAC-TiO_2 composite	Alizarin cyanine green dye
Li *et al.*, 2011	C-doped TiO_2	Methyl orange
Treschev *et al.*, 2008	Carbon containing TiO_2	Methylene blue and nitrogen monoxide
Wang *et al.*, 2007	TiO_2 films incorporated with	Methylene blue

	carbon	
Li *et al.*, 2005	Carbon-doped titania	Benzene

2.3.10.2 Tetracycline in Photocatalytic medium

Tetracycline is continuously importing to our environment and leading to the steady accumulation of it in aqueous and sedimental system. TC is soluble in water and hard to be evaporated from aqueous solution. Furthermore, TC couldn't be treated and removed by traditional water treatment technologies due to its antibiotic and water loving nature, and stable naphthacene ring structure, which is directly threatening our drinking water (Zhao *et al.*, 2014). Recent researches have shown that TC is not only fully oxidized by homogeneous and heterogeneous photocatalysis in very short period of time, but their antibacterial activity (AA) is also delayed by these photocatalysts (Palominos *et al.*, 2009). Following Table is showing review of previous studies about degradation of Tetracycline by using different photocatalysts.

Table: 2.7 Literature Review of AC Doped Titania for TC Removal

Reference	Catalyst Used	Target Pollutant
Shi *et al.*, 2016	Palygorskite-supported Cu_2O–TiO_2 composite	Tetracycline
Choina *et al.*, 2015	ZnO	Tetracycline
Zhang *et al.*, 2015	Amino-Fe (III)	Tetracycline
Mahamallik *et al.*, 2015	Highly porous MnO_2	Tetracycline
Zhao *et al.*, 2014	TiO_2/5A composite catalyst	Tetracycline
Guller and sarrioglu (2014)	Pumice stone	Tetracycline
Liu *et al.*, 2013	Microwave-assisted Cl-TiO_2	Tetracycline
Zhu *et al.*, 2013	Nano sized TiO_2	Tetracycline
Hao *et al.*, 2012	Mesoporous BiOI microspheres	Tetracycline hydrochloride
Gao *et al.*, 2012	Graphene oxide	Tetracycline
Chen *et al.*, 2011	polyvinylpyrrolidone (PVP-K30) modified	Tetracycline

	nanoscale zero valent iron	
Chang *et al.*, 2009	Palygorskite	Tetracycline
Palominos *et al.*, 2009	TiO_2 and ZnO	Tetracycline
Choi *et al.*, 2008	Coagulation and granular activated carbon	Seven classes of Tetracycline

Materials and Methods

MATERIALS AND METHODS

3.1 Materials and Chemicals

Titanium dioxide, Antanase (79.88 % pure, manufacturing: Kosdak listed company) was used as a source of Titania nano particles. Activated carbon used for the preparation of composite was obtained from BDH laboratory supplies. Sodium hydroxide (NaOH, made by merck) was used to adjust the pH of the solution 8-10. Tetracycline (TC, 98.0 % pure, Sigma).

All the nano catalysts were prepared in deionized water and Ethanol (BDH laboratory supplies, 99.8 % pure) was used for solutions preparation.

3.2 Tetracycline Stock Solution

Tetracycline stock solution (5 ppm) was produced by dissolving 0.02 g of tetracycline in 10 ml ethanol. The pH of the solution was adjusted to 8.7, using 0.1 M Sodium hydroxide. The solution was shaken well and then stored in dark.

3.3 Instrumentation

For the experimental work, a UV Lamp (18W), UV-Vis Spectrophotometer (T80 pg Instruments), pH-meter (CRISON Instruments), centrifuge (D37520 Sigma), drying oven (Thermo Fisher Scientific) and for stirring (ARE heating magnetic stirrer) were used.

3.3.1 UV/Vis Spectrophotometer

Spectrophotometry was used as a quantitative analytical technique by measuring the absorbance of Tetracycline solution before and after degradation reaction with catalyst. For this purpose, the tetracycline UV spectrum, in ethanol, was recorded to check the wavelength of maximum absorbance (λ *max*) and a calibration curve was developed.

3.3.1.2 Principle of Working

Spectrophotometry technique is widely used for the quantitative analysis of organic compounds. The molecules after exposure to UV-light absorb it. Due to absorption of light, electrons become excited and move to high energy orbitals. The instrument basically measures transmitted light

which is commonly known as transmittance. The absorbance is recorded by taking the difference of light transmitted before and after the sample solution is being exposed to light (Thomas and Burgess, 2007).

3.3.1.3 Beer-Lambert law

According to Beer-Lambert law, absorbance is directly proportional to the concentration of the sample and path length of the cell in which sample is taken (Hardesty and Attili, 2010).

$A = \varepsilon . c . L$

Where, A = absorbance

ε = absorption coefficient, specific to sample solution

c = concentration of sample solution

L = path length of cell

UV spectrophotometer was used to record the tetracycline spectra and then absorbance of tetracyline was measured at 410 nm.

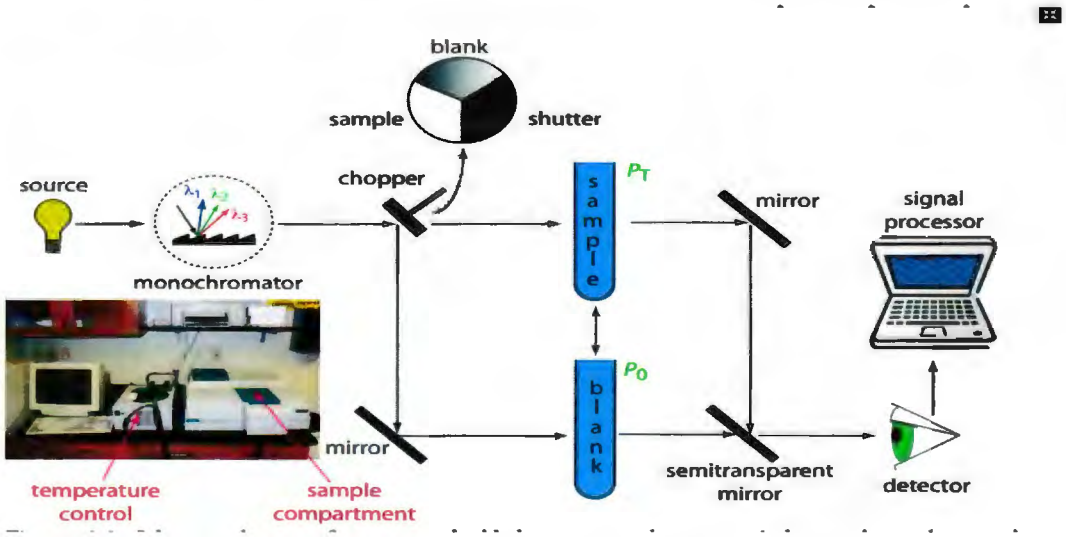


Figure 3.1: Schematic diagram of a scanning, double-beam spectrophotometer. A chopper directs the source's radiation, using a transparent window to pass radiation to the sample and a mirror to reflect radiation to the blank. The chopper's opaque surface serves as a shutter, which

allows for a constant adjustment of the spectrophotometer's 0% T. The photographic insert shows a typical instrument. The unit in the middle of the photo is a temperature control unit that allows the sample to be heated or cooled (Harvey, 2011).

3.4 Experimental Section

3.4.1 Synthesis of Pure TiO_2 Nanoparticles

Liquid Impregnation (LI) method was used for the synthesis of TiO_2 nanoparticles (Behnajady *et al.*, 2008). It involves following major steps:

Mixing: Slurry of TiO_2 nanoparticles was prepared in 300 ml water by mixing 5 g of TiO_2 GPR in a beaker and continuous stirring for 24 hours so that proper mixing of TiO_2 GPR with water could take place.

Settling: The solution was allowed to settle for another 24 hours so that proper settling of solution could take place.

Drying: After removing of the supernatant the solid material was placed in an oven for 12 hours at 105 ^{0}C so that water can be evaporated.

Calcination: After drying, dried material was crushed properly in ceramic mortar pastel. Finally crushed material was placed in china dishe and heated in a muffle furnace for 5 hours at 450 ^{0}C. The calcined nanoparticles were transferred to an airtight plastic bottle and stored in dark place.

3.4.2 Synthesis of TiO_2-AC nano composite

The AC-TiO_2 nano composite was prepared by using the same procedure of liquid impregnation. Different molar ratios of AC (1 %, 2% and 3% (w/w)) were taken and being added to the solution before adding Titania. Table 3.1 is showing the concentrations of the chemicals utilized.

Table 3.1: Concentrations of AC and TiO_2 for different molar ratios.

Sr. no	Molar ratio	TiO_2	AC
1	1% (w/w)	5 g	50 mg
2	2% (w/w)	5 g	100 mg
3	3% (w/w)	5 g	150 mg

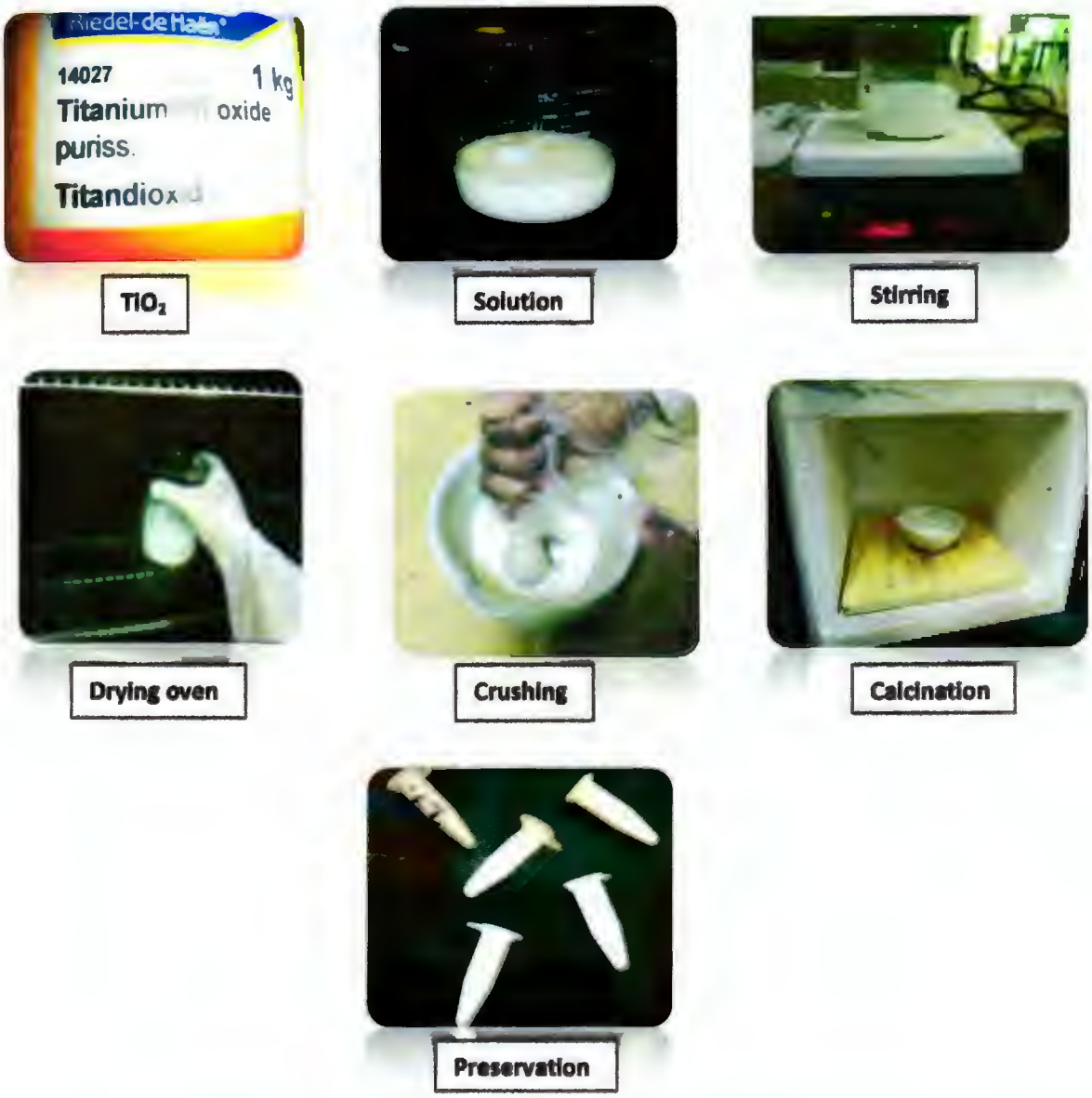


Figure 3.2: Steps involved for the Synthesis of Nanoparticles

3.4.3 Characterization of Nanoparticles

The nanoparticles synthesized were characterized using the following techniques.

3.4.3.1 Fourier Transform Infrared microscopy (FTIR)

FT-IR is Fourier Transform InfraRed, the favored technique of infrared spectroscopy. In IR spectroscopy, IR beam is thrown on the surface of a sample. A part of IR radiation is

absorbed by the sample and some of it shows transmittance (passed through). The result is obtained in the form of a spectrum representing the molecular absorption and transmission that would be like blue print of the sample. Like a blue print two molecular structures can't produce the same infrared spectrum. This makes infrared spectroscopy useful for molecular identification especially including several other types of analysis.

FTIR,

- Identifies unknown substances
- Determine the nature and quality of a molecule
- Find out the amount of elements in a mixture.

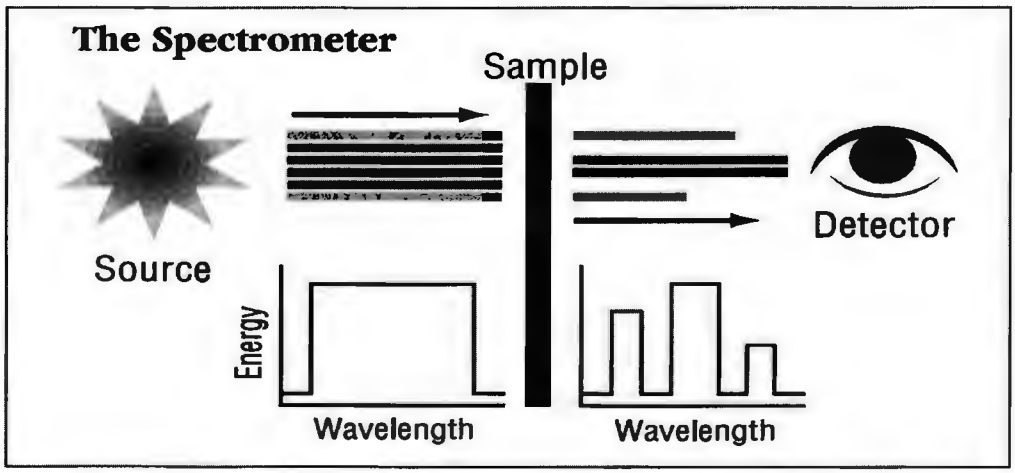


Figure 3.3: Diagram illustrating the basic principle of FTIR working (Thermo Nicolet Corporation, 2001)

Shimadzu brand model- IR tracer-100 was used for getting IR-spectra.

3.4.3.1 X-Ray Diffraction (XRD)

X-ray powder diffraction is a very fast and nondestructive analytical technique primarily used for phase identification of a crystalline material which can also provide data of unit cell dimensions. The material must be finely ground and homogenized for the analysis.

XRD can determine,

- The average spacing between rows and layers of atoms.
- Arrangement of a single atom or cluster.
- The crystalline structure of analysed material (whether known or unknown).
- Internal stress and size, shape of little crystal region

XRD studies of pure and TiO_2-AC composite were carried out by using XRD, JEOL JDX-II, X-ray diffractometer. Average crystalline size of nanoparticles was determined by using the Scherer formula ((Liu *et al.*, 2009: Younas *et al.*, 2014).

$L=K\lambda/\beta\cos\theta$

Where,

L= Average particle size

K=0.89, λ= 0.1542

θ = the diffraction angle of crystal phase

β= Full width of a diffraction line at one half of maximum intensity (FWHM) radian

3.4.3.2 Scanning Electron Microscopy (SEM)

Scanning Electron Microscope (SEM) is an influential technique to obtain largely magnified images by using a focused beam of electrons. The three-dimensional images of high-resolution produced by SEM provides information like;

- **Topography:** Its appearance; limit of detection is a few nanometers.
- **Morphology:** The form, size and order of the constituent particles from which that object is made, that are present on the sample surface or exposed by etching or grinding; limit of detection is a few nanometers.
- **Chemistry:** The elemental composition of the sample and the relative ratio of each element present, in areas ~ 1 μm in diameter.

- **Crystallography:** The degree of order and arrangement of different atoms present in that specimen. This is useful in case of particles composed of single crystalline form > 20 μm.
- **Orientation of grains:** These applications make SEM invaluable in a variety of science and industry applications. The SEM uses a beam of energetic electrons that are focused on a stage, where a solid sample is placed. When the incident electrons strike the sample, they release energetic electrons from the sample surface. The pattern of scattered electrons released by the sample surface gives information about its shape, size, texture and composition. Elemental and mineral information can be obtained from the x-rays emitted by the beneath of the sample.

SEM images were obtained using JEOL JSM-6460 scanning electron microscope at 10,000 magnifications.

3.4.4 Photocatalytic experiments

3.4.4.1 Adsorption and Photocatalysis of TC by TiO_2-AC composite

Photocatalytic experiments were performed in 200 mL beakers under irradiation of 18 W UV light lamp. A 25 mg TiO_2 nanomaterial and 1%, 2% and 3% of AC composites were added to 100 mL reagent ethanol (to give a 0.7 g/0.1L catalyst concentration).This water was spiked at 0.02 g/0.1L with TC for each catalyst. After 30 min adsorption time in the dark, each solution was stroked with the UV lamp for 90 min with continuous stirring. At the end of reaction, the samples were centrifuged at 12000 rpm for the time of 15 min in order to settle nanoparticles and separate them from the solutions (Rioja *et al.,* 2012). Finally, the products were analyzed by UV-vis spectrophotometer to determine the removal efficiency of each catalyst. Ethanol was used as solvent throughout the experiments. TC in ethanol solvent had following characteristics;

pH: 8.7, EC:8.27, TDC: 5.29

All the experiments were performed at room temperature.

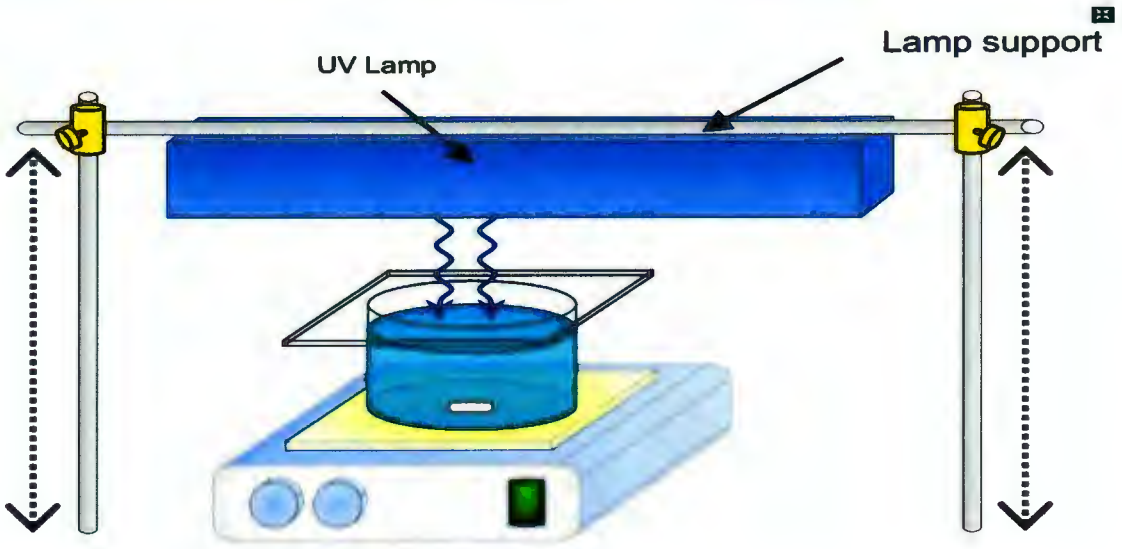


Figure 3.4: Experimental set-up of the suspended TiO_2-AC

3.4.4.2 Adsorption and Photocatalysis of TC by pure TNPs

Same experimental setup was used to perform photocatalysis of TC by pure TNPs. Volume of solvent (ethanol), concentration of catalyst (TiO_2 nano particle) and amount of TC was same as for composite experiment. Other physical factors like pH, temperature, time were constant.

3.4.4.3 Removal Efficiency

The removal efficiencies of pure and TiO_2-AC composites were calculated using the following equation.

$E = C_1\text{-}C_2/ C_1 \times 100\%$

Where C_1 is the initial and C_2 is the final equilibrium concentration of TC (mg/L) in the solution.

3.4.4.4 Effect of pH on Removal Efficiency

The process was done at different pH (4, 7, and 8.7) and removal efficiency at each pH was calculated.

Result and Discussion

RESULTS AND DISCUSSION

4.1 CHARACTERIZATION

4.1.1 Fourier Transform Infrared (FTIR)

FTIR shimadzu IR tracer-100 Model was used for characterization of nanoparticles. The FTIR patterns of the TiO_2-ACz are shown in figure 4.1-4.3. From the results of TiO_2-AC1, TiO_2-AC2, TiO_2-AC3 the peaks of similar intensities were at about 3292 and 943, 3388 and 924, 3332 and 924 cm^{-1} respectively, the reason in its explanation was vibration of stretched water molecules, including both OH^- and molecular water on the surface of Titania. In spectrum another band was located around 1643, 1646 and 1646 cm^{-1} (of all three composites) was reasonably attributed to the C–O–C bonds from carbonaceous organic materials created in the hydrothermal process. Characteristic peak at 367 cm^{-1} of the Ti–O bond stretching vibration was clearly observed, in all 3 composites. Broad absorption band in wavelength range from 367 to 664, 720 and 702 cm^{-1} was observed due to the quantum effect of the nanoparticle size (He *et al.*, 2013).

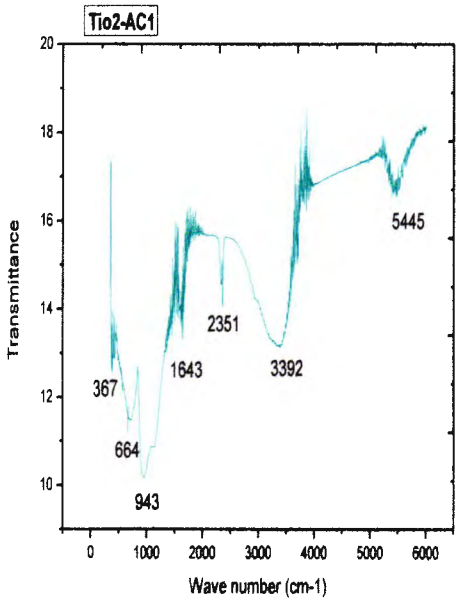


Figure 4.1: FTIR Spectra of TiO_2-AC1

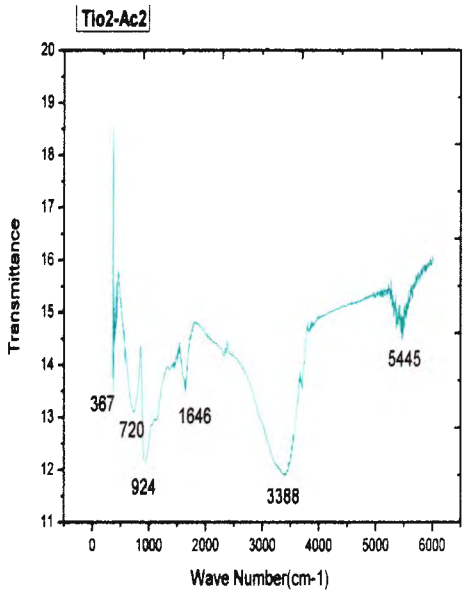


Figure 4.2: FTIR Spectra of TiO_2-AC2

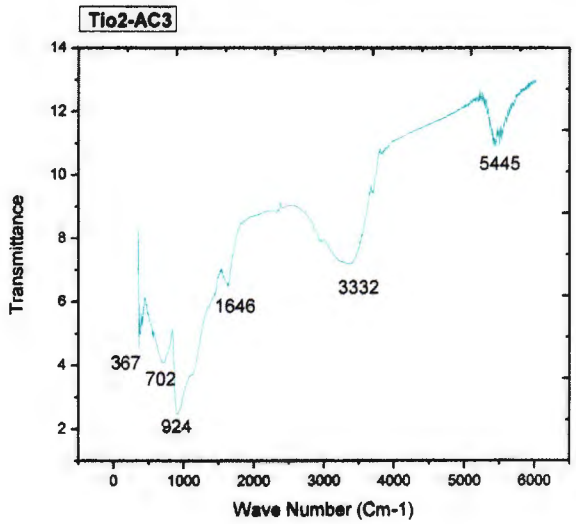


Figure 4.3: FTIR Spectra of TiO_2-AC3

4.1.2 X - Ray Diffraction (XRD) Analysis

The nanoparticles synthesized were analyzed by XRD, JEOL JDX-II, X-ray diffractometer, to find their crystal phase composition and crystallite size. The XRD patterns are shown in Fig.4.4-4.7. The pure and TiO_2-AC nanocomposites syntesized showed crystalline nature and the 2θ peaks arising at 25.27, 24.92, 24.99 and 25.04. The anatase give main peak in XRD at 2θ=25.25, while main peaks of brookite and rutile form appears at 2θ=30.8 and 2θ=27.4 respectively. It is clear from the XRD peaks that nanoparticles were in pure anatase phase and no rutile and brookite impurity was found. No characteristic peaks of the activated carbon (in case of composite) were found in the XRD patterns implying either the carbon was incorporated in the crystalline of TiO_2, or it was in very small quantity and highly dispersed. Peak position of TiO_2 was confirmed by the earlier work of Luo and his co-researchers (2015). The average sizes of the synthesized nanoparticles were in the range of 47-67 nm as given in Table.4.1 found from the strongest XRD peak (i.e. at 2θ=25.25) using Scherrer's formula. Table 4.1 is showing the achieved particle sizes of all the three composites and TiO_2.

Table 4.1: Average Particle Size of Nanoparticles

Nanoparticles	Average particle size	Lattice strain
Pure TiO_2	43.23nm	0.0038
TiO_2-AC 1%	55.35nm	0.0030
TiO_2-AC 2%	47.48nm	0.0035
TiO_2-AC 3%	66.48nm	0.0025

Average crystalline size of nanocomposites was determined by using the Scherer equation (Younas *et al.*, 2014).

$L=K\lambda/\beta\cos\theta$

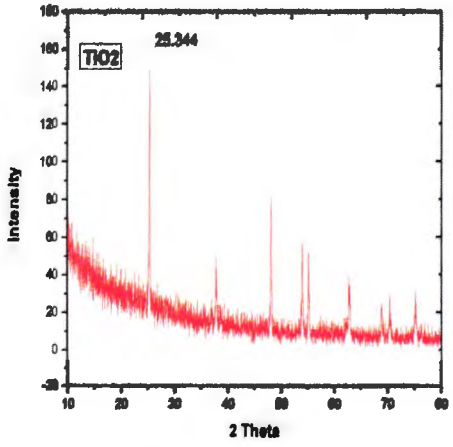
Where,

L= Average particle size

K=0.89, λ= 0.1542

θ = the diffraction angle of crystal phase

β= Full width of a diffraction line at one half of maximum intensity (FWHM) radian



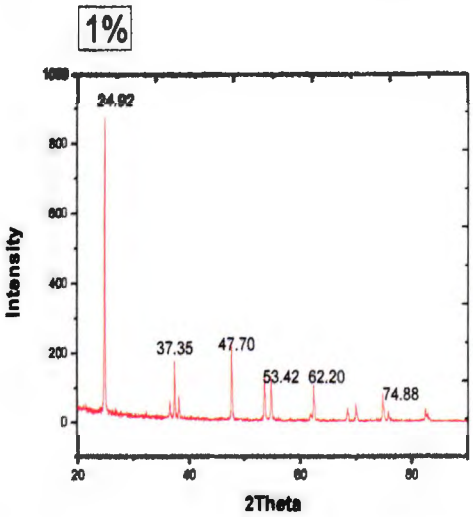
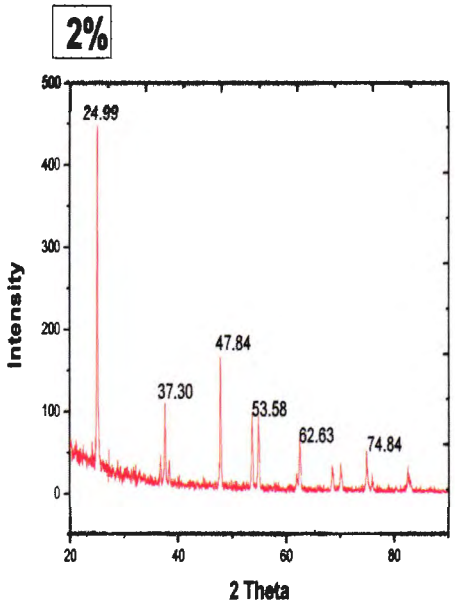


Figure 4.4: XRD pattern of pure nano TiO_2 **Figure 4.5: XRD pattern of TiO_2-AC1**



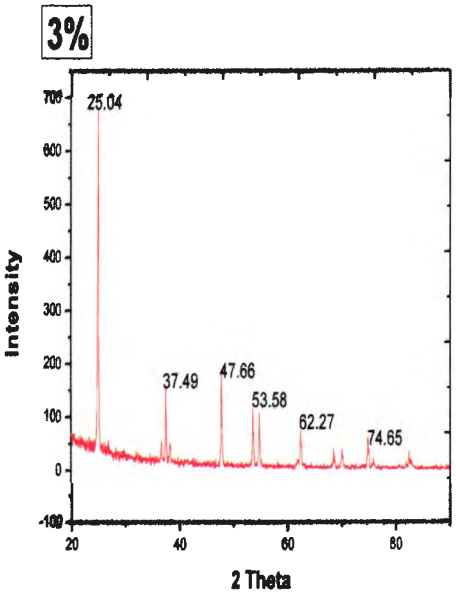


Figure 4.6: XRD pattern of TiO_2-AC2

Figure 4.7: XRD pattern of TiO_2-AC3

Nanoparticles Peaks of XRD results reveal that nanoparticles have Anatase TiO_2 crystalline structure which is chemically active phase of TiO_2 and support the catalytic process and materials (Rioja *et al.*, 2012).

4.1.3 Scanning Electron Microscopy (SEM)

The SEM images of pure and TiO_2-AC nanoparticles were obtained, at the magnification of 20,000, are shown in Figures 4.8- 4.11. Images of pure and TiO_2-AC nanoparticles confirmed the existence of porous, sponge like constitution, of intense roughness and complexity. Structure like this indicates the extended surface area which has been suggested to be suitable for photo catalytic degradation processes (Hameed *et al.*, 2014).

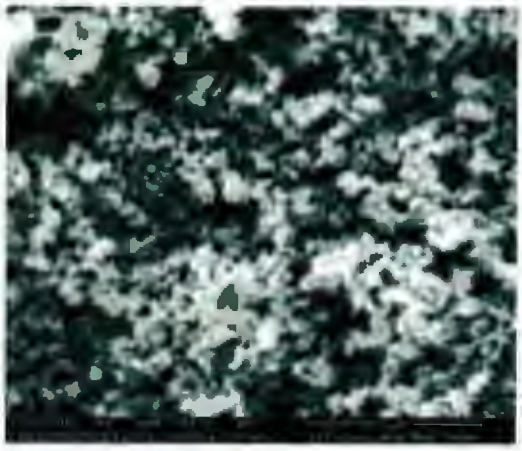


Figure 4.8: SEM Image of pure nano TiO_2

Figure 4.9: SEM Image of TiO_2-AC1

Figure 4.10: SEM Image of TiO_2-AC2

Figure 4.11: SEM Image of TiO_2-AC3

Pure AC had a clear surface but after comoposite formation with TiO_2, TiO_2 particles were well dispersed on its surface creating the nano sized particles. The size of TiO_2 particles shown in SEM images were larger than the size calculated by XRD images and possible explanation for this difference is that XRD was calculating the size of single crystalline while SEM pictures were agglomerates of many crystallites of TiO_2. Clusters were denser on the surface of pure TiO_2 rather than composites with AC. In the composites, TiO_2 was dispersed well on the high surface area of AC, this avoids agglomeration of TiO_2.

Least clusters were on TiO_2-AC3, as it had most molecular ratio of AC. On TiO_2-AC3, TiO_2 deposited in the porous structure of AC instead of just sticking to its surface. Photocatalysis is a light based reaction therefore, more TiO_2 on the surface of AC would trigger more catalytic performance (Wang *et al.*, 2009).

4.2 Tetracycline determination

Solution of ethanol containing tetracycline medicine was run through UV spectrophotometer (T80 pg Instruments) for full length scan as shown in Figure 4.12. The UV spectrum show maximum absorbance of 1.212 at wavelength (λ max) 410 nm. Which confirms the wavelength of tetracycline, further experimentation was done by using same wavelength.

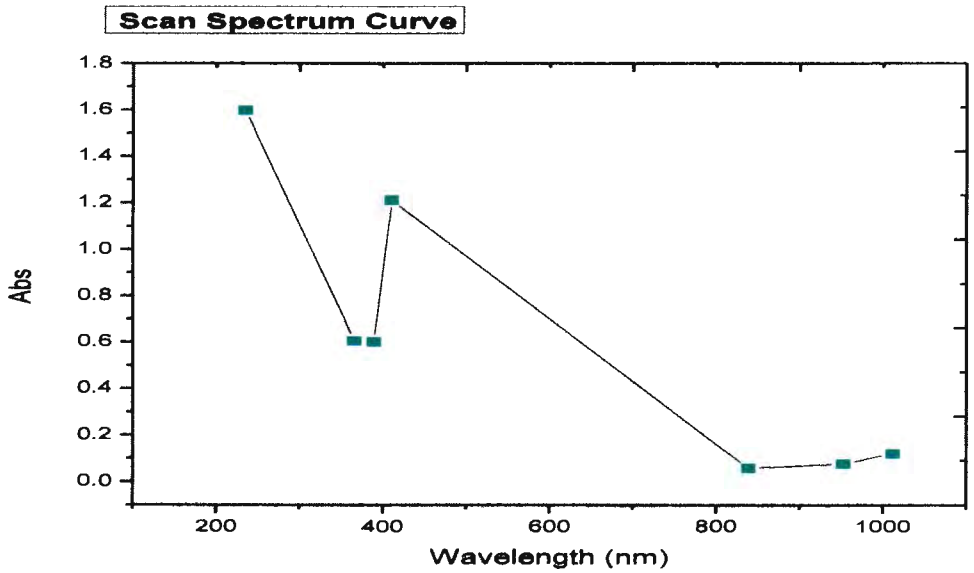


Figure 4.12: UV Absorbance Spectra of Tetracycline

To derive the following formula (Absorbance (y) = 0.032x + 0.048) calibration curve was developed through UV spectrophotometer using serial dilutions of the tetracycline stock solution of 5 ppm as curve is shown in Figure 4.13.

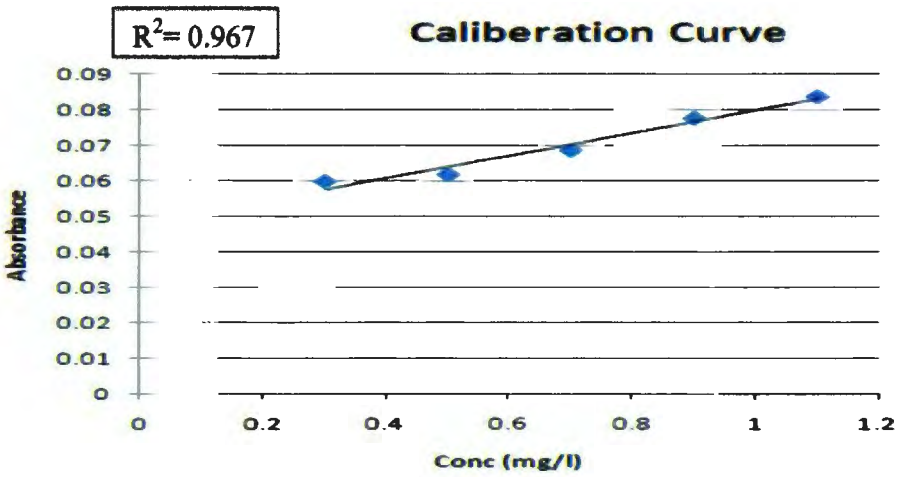


Figure 4.13: Calibration Curve (5 ppm) for Tetracycline in ethanol

4.3 Photocatalytic Experiment

Photocatalysis was carried out with pure and composite of TiO_2-AC of different molar ratios 1, 2 and 3% at room temperature. The removal efficiencies of pure and nano composite of TiO_2-AC were calculated by taking 1 ml of 5 ppm tetracycline solution into 100 ml reagent ethanol with varying values of catalyst dose, pH and time duration.

4.3.1 Effect of catalyst dose on removal efficiency

Table 4.2 is showing initial concentrations of tetracycline and composites of different molar ratios, absorption rates and removal efficiency of each composite. The results found after the treatment are shown in the Table 4.2.

Table 4.2: Removal efficiencies of TiO_2-AC nanoparticles used

TiO_2/AC Composite (molar ratio)	TC Initial Conc. (mg/0.1L)	Composite Conc. (mg/0.1L)	Initial Abs. Y_0	Final Abs. Y_f	Initial Conc. X_0	Final Conc. X_f	% Removal
1%	2.2	250	1.212	0.610	36.375	17.56	51.72
		500	1.212	0.623	36.375	14.07	61.31
		700	1.212	0.176	36.375	4	89

2%	2.2	250	1.212	0.552	36.375	15.75	56.70
		500	1.212	0.471	36.375	10.45	71.26
		700	1.212	0.89	36.375	1.281	96.48
3%	2.2	250	1.212	0.656	36.375	19.03	47.68
		500	1.212	0.564	36.375	12.67	65.17
		700	1.212	0.197	36.375	4.565	87.2

Different dose concentrations of catalysts were applied in the experiment to check the optimal concentration value for catalyst. 250 mg/0.1L, 500 mg/0.1L and 700 mg/0.1L were the varying amounts for 1, 2 and 3% catalysts as shown in figure 4.14. This is showing that highest degradation is achieved through TiO_2-AC2 at catalyst dose of 700 mg/0.1L.

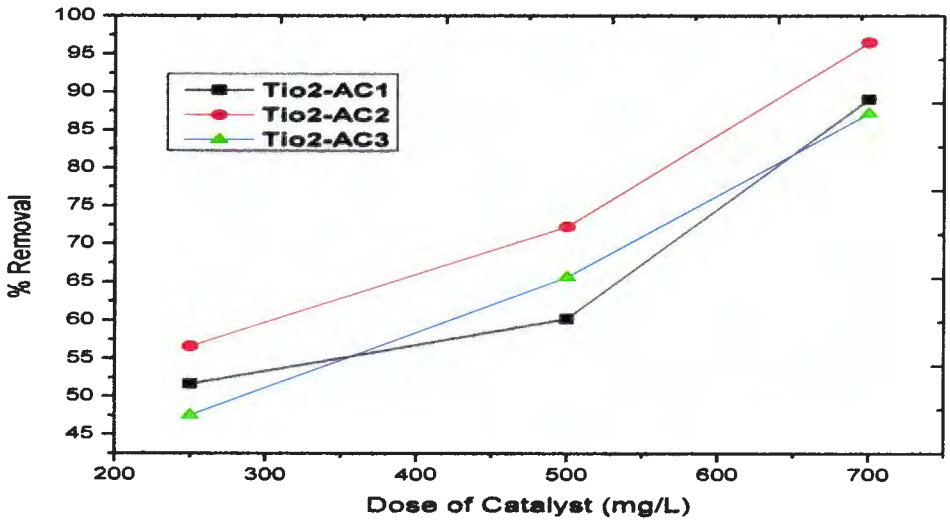


Figure 4.14: Effect of composites dosage on TC removal

Removal efficiency of pure and nanocomposites of TiO_2-ACz are given in Table 4.3.

Table 4.3: Removal Efficiencies of Catalysts

Nano particles	TiO_2	TiO_2-AC1	TiO_2-AC2	TiO_2-AC3
Removal Efficiency (%)	90	89	96.4	87.2

These results are comparable to the previously published results of Zhang and his co-researchers (2015) in which they proved that with the increase in the dose of catalyst, more removal efficiency could be achieved (Zhang *et al.*, 2015).

Tetracycline concentration was checked after every 30 minutes by measuring the absorbance of the solution, at 410 nm, and comparing the absorbance with the calibration curve. The best results were obtained when experiments were performed with TiO_2-AC2 in dark and under UV light at room temperature, after 120 min, tetracycline concentration was found to be below 0.89 which was initially 1.212 (measured by UV-Vis Spectrometer-T80 pg Instruments).

410 nm wavelength was set to measure the absorbance of each solution and calibration curve were drawn by plotting absorbance vs. concentration. The equation of the calibration curve was as follows:

Absorbance (y) = 0.032x + 0.048

Having correlation coefficient (R^2) 0.967.

This equation was used to find out the unknown concentrations of TC from the measured absorbance values.

The removal efficiency of TC was expressed using following equation:

$$\text{Removal efficiency} = \frac{C1-C2}{C1} \times 100\%$$

C_1 and C_2 in this equation are representing the concentrations of TC before and after photocatalytic reaction (Zhu *et al.*, 2013).

Results proved that the most efficient catalyst was TiO_2-AC with 2% molar ratio at 0.7g conc. which showed 96.48 % removal efficiency. Rioja *et al.*, (2012) worked on different medicines sulfamethoxazole (SMX), carbamazepine (CBZ) and clofibric acid (CA) at concentration of 0.5µg/mL and same catalyst with 2% molar ratio with 0.5g/L concentration was used to remove these drugs at the concentration of 0.0005g/L from the river water. They were succeeded in 56-80% removal of drugs by TiO_2-AC composite and results of bare TiO_2 were 61%.

4.3.2 Effect of pH on removal efficiency

The pH of the solution was one of the most essential affecting determinants on the removal efficiency of the catalyst (Jiao *et al.*, 2008). Thus adsorption of TC over TiO_2-AC 2% catalyst was tested at different pH by keeping all the other parameters constant. At 4, 7 and 8.7 pH the efficiency removal was 25, 53 and 87% respectively. In the previous work of R.A. Palominos and colleagues (2009), the effect of different pH values of the solution was also accessed for the removal of same drug over TiO_2 and ZnO. They concluded that with increase in pH, the removal efficiency increases. Most efficient degradation of TC was at 8.7 pH which can be explained by the logic of charges on both TC and TiO_2 catalyst. Both are positively charged in acidic conditions <4.3, negatively charged at 8.7 pH and produce repulsive effect. Results showed the best possible values for catalyst and pH were 0.7 g/0.1L and 8.7, respectively, as shown in the Table 4.2.

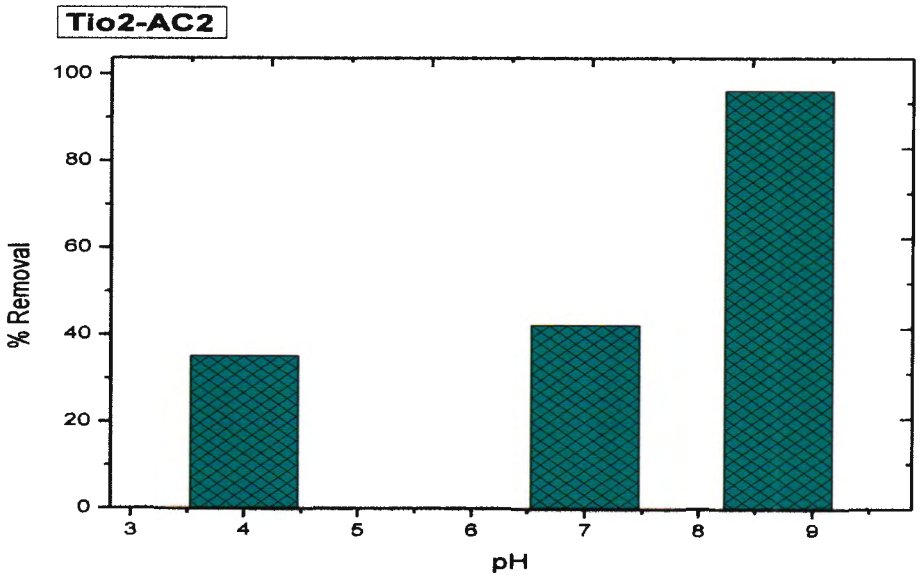


Figure 4.15: Effect of pH on Tetracycline Adsorption

4.3.3 Effect of Time duration on Degradation

Whole experiment was of 120 min, however after every 30 min adsorption of tetracycline was measured by taking 10 ml of working solution and its adsorption was measured by running the sample into UV spectrophotometer. During the first half hour (time from 0 min-30 min) drug

adsorption over composites was insignificant. After 60 min of reaction in the dark, the removal efficiencies were 21, 24 and 30% respectively for catalysts. On 90 min of reaction, removal efficiency increases to 45, 48 and 37% for 1, 2 and 3% respectively. Best results were achieved after 120 min of experiment for TiO_2-AC 2% catalyst. With increased contact time between pollutant and the catalyst, removal efficiency seemed to be increased in the literature as well (Muthirulan *et al.*, 2013).

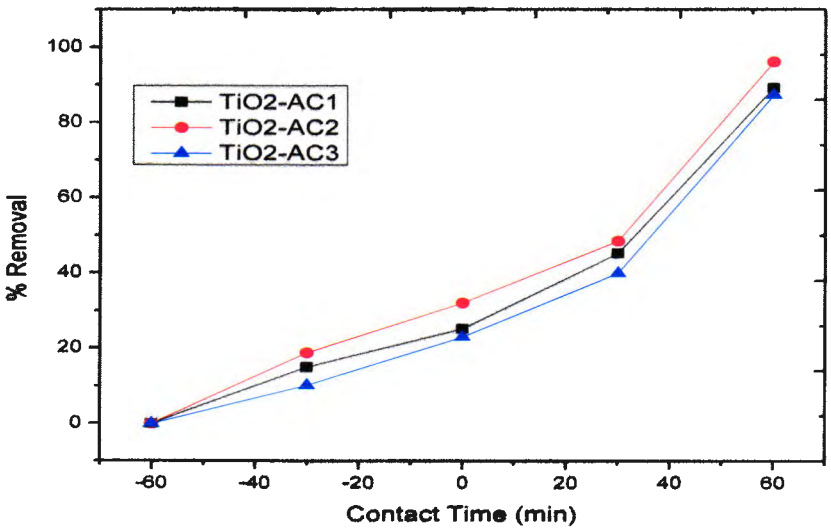


Figure 4.16: Effect of Contact time on degradation

4.3.4 Mechanism of TC degradation by TiO_2/AC

TiO_2-AC2 composite catalyst had better results for TC degradation as compared to bareTiO_2 and other composites TiO_2-AC1 and TiO_2-AC3 as shown in the Table 4.2. Possible explanation for this improvement could be the combined action of AC adsorption and TiO_2 photocatalysis. Suggested mechanism for the photocatalytic degradation of TC by TiO_2-AC is shown in Figure 4.17.

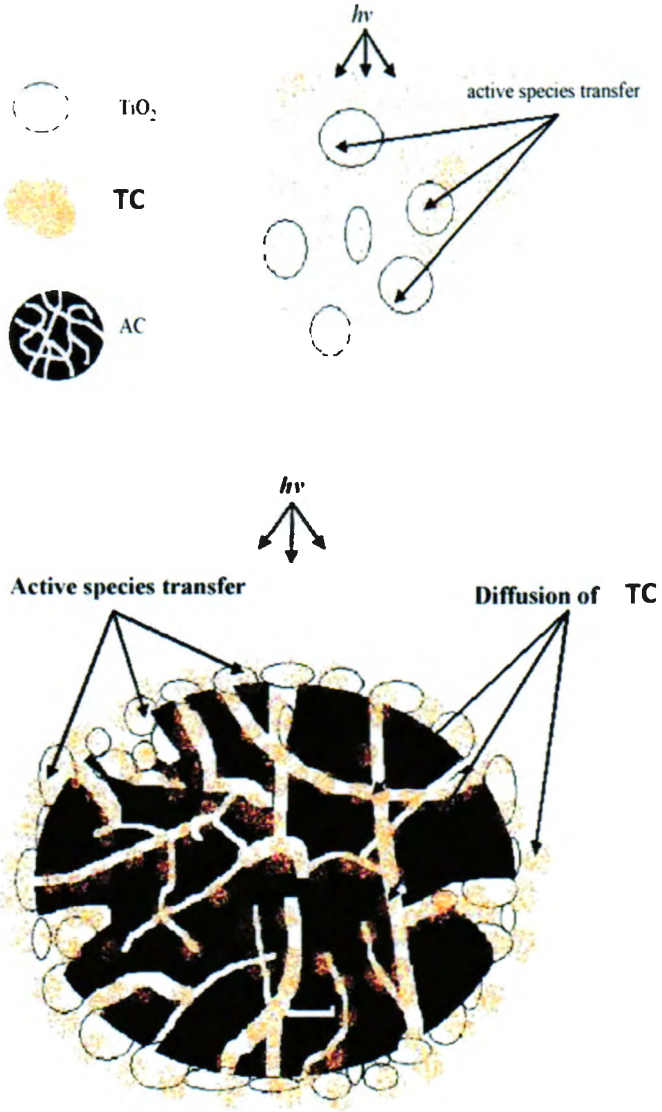


Figure 4.17: Mechanism of TC degradation by TiO_2 supported on AC (Wang *et al.*, 2009)

Activated carbon has large surface area with spongy structure, thus exhibiting high adsorption capacity for organic molecules before they undergo by the process of photocatalysis by TiO_2. In TiO_2-AC, TiO_2 was deposited on the AC structure, because of this deposition more TC was found on bare TiO_2 as compared to composite. The TC molecules deposited on TiO_2 surface decreased with an increase in degradation of TC by the catalyst. The TC molecules first adsorbed were now desorbed at AC surface, thus creating a adsorption-desorption mechanism. Therefore the removal of TC was a synergistic effect of TiO_2 and AC (Wang *et al.*, 2009).

4.4 Reuse of Catalyst

The reusability of TiO_2-AC2 nanoparticles had been checked by cycling experiments of TC degradation. Same 5ppm solution of TC was prepared and treated: the concentration of TC and used catalyst were 1mg and 0.7g/0.1L respectively. Same conditions including pH, temperature and contact time was provided for this experiment. The results are shown in the following Table 4.4.

Table 4.4: Removal Efficiencies of TiO_2-AC2 during 1st and 2nd cycle

TiO_2-AC2	TC Initial Conc. (mg/0.1L)	Composite Conc. (mg/0.1L)	Initial Abs. Y_0	Final Abs. Y_f	Initial Conc. X_0	Final Conc. X_f	Removal %
1st	2.2	700	1.212	0.089	36.375	1.281	96.48
2nd (reuse)				0.351		9.47	73.72

Results have shown that catalyst could be used for many successive cycles and favorable results would be achieved in a cost effective manner.

Conclusions & Recommendations

CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

This study showed that synthesized pure and nanocomposite of Titania and activated carbon showed excellent Tetracycline removal efficiency. The following conclusions can be drawn from this study.

- The synthesized pure and TiO_2-AC showed excellent Tetracycline removal efficiency (up to 96%).
- Combination of adsorption and photocatalysis is an effective treatment for Tetracycline removal, from wastewater, down to trace levels.
- Among composites TiO_2-AC2 gave the highest Tetracycline removal efficiency.
- The removal efficiency is dependent on pH, it is maximum at 8.7 pH and decrease by moving on acidic side.
- On the reuse of TiO_2-AC2 for the same solution of TC, 73.72% efficiency was achieved for once. So, it's a cost effective way to reuse the catalyst for three to four times.

5.2 RECOMMENDATIONS

The following are some recommendations for further studies

- As among composites TiO_2-AC2 was found highly efficient and only 1, 2 and 3% molar ratios of AC were tested, so the effect of further variation in AC percentage should be looked by upcoming researchers.
- Effect of any competing species in the wastewater on the Tetracycline removal efficiency of TiO_2-AC should be checked.
- Photocatalytic degradation of other antibiotics may be studied.
- The immobilization of Titania on other adsorbent should be checked.
- Determination of proper disposal method for the disposal of Tetracycline effluent.

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