Review of Literature

2.1. PHARMACEUTICAL INDUSTRIES IN INDIA

The Indian pharmaceutical sector manufactures the entire range of clinical products and wide range bulk drugs. A gradual progress of Indian pharmaceutical industry in the past ten years is presented in the Figure 2.1. More than 90-95% of the drugs manufactured in India are off-patent and specified on the World Health Organization’s (WHO) list of essential drugs. In course of availability of quality manpower and less production costs, India is expected to hold about ~33% total share of the increasing generic drug market.

![India's pharmaceutical industry on course for expansion](image)

**Fig. 2.1 The progress of Indian pharmaceutical industry (Global Insight, VCI; Forecast DB research)**

Indian pharmaceutical products are exported to various countries, including US, Canada, Japan, France, Latin America, Germany and few other developing countries. Whereas, importing of pharmaceuticals is restricted only to a few life-saving medicines like cardio-vascular, anti-cancer and anti-hypertension drugs.

India’s top leading pharmaceutical companies have demonstrated their capabilities in drug development through Research and Development (R&D) activities and therefore have become major contributors in the international pharma market. In 2003, several drug molecules were under development in Ranbaxy Laboratories, whose R&D expenditure cost was about US$52 million. Similarly, 11% of gross turnover was recorded for Dr. Reddy’s Laboratories R&D activities. A profitable trend of the pharma sector has made more number of Indian pharmaceutical companies to attain international approval standards from agencies like USFDA.
According to current estimates, 40% of the total production is exported, comprising 65% formulations and 45% bulk drugs. The Indian pharmaceutical market has ranked cephalosporins and combinations, broad spectrum penicillins and fluoroquinolones as the top three classes of therapeutics. As per a survey, the Indian cephalosporin market is estimated at 1000 crore for the year, 2002. Orchid Chemicals and Pharmaceuticals Ltd is the largest cephalosporin manufacturers in India with 12% share in the cephalosporin market world-wide. Aurobindo Pharma also produces different cephalosporins available in the market, including its first launch of a fourth generation injectable broad spectrum cephalosporin antibiotic, cefpirome in India (Bhattacharyya and Sen, 2006).

2.2. WASTES GENERATED IN PHARMACEUTICAL INDUSTRIES

Water is a critical ingredient in pharmaceutical manufacturing plants. Reliable and high-quality water supplies are needed for a range of purposes, including material processing, production and cooling operations. A wide range of products is made in the pharmaceutical manufacturing units, which typically requires large volumes of materials, chemicals and substances during process operations. Pharmaceutical manufacturing plants produce a diverse set of wastes during maintenance, manufacturing and housekeeping processes. In general, a pharmaceutical industry generates about 200 kg of waste stream per metric ton of active pharmaceutical ingredient (API) manufactured. A typical pharmaceutical waste stream contains process liquors, spent fermentation broths, solvents, spilled materials, equipment washwaters and used processing aids. Waste streams produced in these industries can be heavily laden with various contaminants, nutrients, toxins and organic content in substantial concentrations posing unique challenge during treatment processes (Gadipelly et al., 2014). Of these, particulate matter and volatile organic compounds (VOCs) released from the pharma industry contribute as major air pollutants. The pharmaceutical wastewaters are generally categorized as high strength organic effluents, which can be highly challenging during treatment with conventional wastewater treatment processes. Thus, the pharmaceutical industry is making headways for the disposal of hazardous waste substances, which are dangerous or potentially harmful to human health or the environment.

In the pharmaceutical manufacturing industries, waste is generated mainly at following stages during the production of pharmaceuticals (USEPA, 1998), (i) Water
formed from chemical reaction, (ii) Process solvent, the water used in the reaction process, (iii) Process stream washes, the water added to purify the stream, (iv) Product washes, water added to purify an intermediate, crude or final product, (v) Spent Acid/Caustic, the water released from the process during the separation, and (vi) Condensed steam, act as a sterilizing medium and aid in solvent recovery and wastewater treatment.

2.3. PHARMACEUTICAL WASTEWATER

An industrial batch process of pharmaceutical compounds synthesis contains a wide variety of active ingredients in wastewater. According to USEPA (1998), the composition of a typical pharmaceutical wastewater includes,

- Manufacturing process residues (organic chemical compounds)
- Halogenated/non-halogenated solids and sludges
- Sludge & tars
- Heavy metals
- The test animal remains
- Return pharmaceuticals
- Low-level radioactive wastes
- Biological products such as plasma derivatives, vaccines and serum components
- Contaminated gloves, clothings, filters, etc.
- Solid wastes (generally flammable, highly corrosive, reactive and ignitable)

2.4. SOURCES OF ANTIBIOTICS

In the past years, antibiotics used in human and veterinary medicine was widespread with an annual consumption of 100000-200000 tons, which could consequently lead to increased possibility of water contamination with these compounds (Xu et al., 2007). Human and veterinary antibiotic compounds have been detected in various matrices. These pollutants are being continuously discharged into various natural environment matrices as parent compounds, degradation products/metabolites or in both forms by a diverse set of input sources (Fig. 2.2).

Human antibiotics are released into the environment by excretion (urine and faeces), which enters the sewer network and subsequently reaches the wastewater treatment plants (WWTPs). When dispersed in the agricultural fields as fertilizer or
manure, it can contaminate the soil, surface and ground water by runoff or leaching (Díaz-Cruz et al., 2003; Kemper, 2008; Farré et al., 2008). Most of the conventional WWTPs are not actually designed to remove antibiotics, which are highly polar micropollutants (Xu et al., 2007). Therefore, they can be easily transported to surface and groundwater by leaching process. Eventually, the antibiotic contaminated surface waters can enter the water distribution systems and then to the drinking water treatment plants (DWTPs), which was also not equipped to remove these micropollutants.

Fig. 2.2 Different source points of antibiotics (Kummerer, 2003)

Wastewater from WWTPs is an another important source of point for aquatic exposure to toxic pharmaceutical compounds (Zuccato et al., 2010; Ottmar et al., 2010; Cardoso et al., 2014). Wastewater released from pharmaceutical production units has been thought of minor importance earlier. However, recent reports show that, concentrations of antibiotics could be up to several mg/L in effluents in some Asian countries (Bottoni et al., 2010; Khadka and Pokhrel, 2013).

Several hundreds of antibiotic compounds are used extensively in human and veterinary therapies to prevent (prophylaxis) or treat microbial infections (Kümmerer and Henninger, 2003; Monteiro and Boxall, 2010). There are currently about 250 different antibiotics registered for use in human and veterinary medicine (Kümmerer, 2003). Most of the international reports are based only on the estimates. However, the actual volume of antibiotic use in the private and public sectors is not known
(Kümmerer, 2009). Additionally, hospital wastewaters, illegal and uncontrolled drug disposal (Gomez et al., 2006; Emke et al., 2014) can also represent a significant source of antibiotic release into the environment.

Pharmaceuticals entering the environment from animal husbandry practices have also been considered as an important source as they are derived from spills of anaerobic manure lagoons, through run-offs from farms, by manure fertilization of farms, aquaculture discharges and dust. They follow the same pathways as human pharmaceuticals and finally reach the same environmental matrices, i.e., surface and ground waters, soil and air, thus contaminating these environmental matrices (Boxall et al., 2003; Boxall, 2004).

Pharmaceuticals are also found to enter the environment via soil to groundwater by various agricultural practices (Sabourinet et al., 2009; Lapworth et al., 2012). Sewage sludge (biosolids), manure (crop fertilizer) and irrigation with recycled water will entrain both human and veterinary pharmaceutical compounds into agricultural soil (Gottschall et al., 2012).

Direct discharge of veterinary antibiotics from aquaculture is another important source of pharmaceutical contamination (Comeau et al., 2008; Brozinski et al., 2013). Detection of antibiotics in coastal marine waters were also reported (Bottoni et al., 2010; McEneff et al., 2014).

2.5. OCCURRENCE

Antibiotics are generally metabolized by animals and humans. Depending on the class of the compounds, few are metabolized to 90% and a few may be only 10%. Hence, these metabolites are excreted into the wastewater and reach the sewage treatment plants (STPs), where they are only partially eliminated. The unmetabolized portion is excreted as an API. Approximately, 70% of the consumed antibiotics is excreted unchanged (Kümmerer and Henninger, 2003). If they are not properly eliminated during the removal process, they tend to pass through the sewage system and ultimately end up in the environment, mostly in the aqueous compartment. Residual amounts can also reach surface, ground waters or even sediments.

2.5.1. WASTE, SURFACE, GROUND, DRINKING AND SEA WATER

Several reports on occurrence of antibiotic compounds in aquatic ecosystems. Surface waters (Xu et al., 2007; Smith et al., 2007; Tamtam et al., 2008; Watkinson et al., 2009), groundwaters (Batt et al., 2006; Xu et al., 2007), sea waters (Xu et al.,
2007; Minh et al., 2009), drinking water (Watkinson et al., 2009; Yiruhan et al., 2010), WWTPs effluents (Seifrtová et al., 2008; Watkinson et al., 2009; Minh et al., 2009) and hospital wastewaters (Seifrtová et al., 2008; Watkinson et al., 2009).

2.5.2. SEWAGE SLUDGE AND SEDIMENTS

The occurrence of antibiotics in terrestrial matrices and biosolids (sludge) as a soil contaminant has also been reported (Kim and Carlson, 2006). The sludge produced during wastewater treatment processes is generally used as a soil amendment which may contaminate the soils, surface and ground waters through leaching process (Farré et al., 2008). The occurrence of high levels of antibiotics, viz., tetracyclines, sulphonamides, quinolones and macrolides at a total concentration ranging from 28 μg/Kg to 1051 μg/Kg in greenhouse soils and manure samples was reported by Li et al. (2015).

2.5.3. UPTAKE BY PLANTS

Some veterinary antibiotics have the ability to be transferred from soil to cultivated plants through the application of manure containing antibiotic residual compounds. Measurable quantities of antibiotic residues were detected in these soil after a few months. Some of these drugs were also reported to be taken up by some vegetables, such as lettuce leaves, carrot roots (tubers), green onions, cucumbers, cabbages, potatoes and corn (Kumar et al., 2005). The exposure route is important because they may elicit subtle toxicity over prolonged periods of continuous antibiotic exposure. Studies have shown that the presence of enrofloxacin at a concentration of 100 μg/L of in soil have contributed significant toxicity to the cotyledons, roots and leaves of several other cultivated plant species (Regitano and Leal, 2010).

2.6. ANTIBIOTICS: ENVIRONMENTAL CONCERN

The discovery of antibiotics is one of the major milestones in the history of medicine which has a profound effect on human life. The use of antibiotics began with the discovery of penicillin by Alexander Fleming in the 1940s. Microorganisms such as fungi, bacteria and actinomycetes can produce antibiotic. An antibiotic is a chemotherapeutic agent that abolishes or inhibits the microbial growth, such as bacteria, fungi, or protozoa without causing significant harm to the host or patient (Kummerer, 2009).

Antibiotics can be classified based on their chemical structure of the compound or mechanism of action. They are a diverse class of chemicals, divided into
various sub-groups, namely, β-lactams, tetracyclines, quinolones, sulphonamides, macrolides and others. Some important classes and groups of antibiotic compounds are shown in Table 2.1. They are complex molecules, which possess diverse functionalities within the same molecule. Hence, antibiotics can be cationic, anionic, neutral or zwitterionic at different pH conditions. Due to their diverse functionalities, their physico-chemical and biological properties such as photo reactivity, antibiotic activity, sorption behaviour and toxicity may change with different pH (Cunningham, 2008).

Table 2.1 Important classes and groups of antibiotic compounds.

<table>
<thead>
<tr>
<th>Class</th>
<th>Group</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-lactams</td>
<td>Penicillins</td>
<td>Phenoxyphenicillin, Amoxicillin</td>
</tr>
<tr>
<td></td>
<td>Cephalosporins</td>
<td>Cefoxitin, Cefdinir, Cefozopran</td>
</tr>
<tr>
<td></td>
<td>Carbapenems</td>
<td>Meropenam, Ertapenem</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td></td>
<td>Doxycycline, Oxytetracycline</td>
</tr>
<tr>
<td>Aminoglycosidases</td>
<td></td>
<td>Gentamycin, Neomycin</td>
</tr>
<tr>
<td>Macrolides</td>
<td></td>
<td>Erythromycin, Azithromycin</td>
</tr>
<tr>
<td>Glycopeptides</td>
<td></td>
<td>Vancomycin, Teicoplanin</td>
</tr>
<tr>
<td>Sulphonamides</td>
<td></td>
<td>Sulphamethoxazole, Sulfamethizole</td>
</tr>
<tr>
<td>Quinolones</td>
<td></td>
<td>Ciprofloxacin, Norfloxacin</td>
</tr>
</tbody>
</table>

Antibiotic residues present in the environment have prompted various research studies in academics and industries to address the effects of these toxic bioactive compounds (Lin et al., 2010). Many of the commonly used generic medicines viz., antibiotics, antihistamine, analgesics drugs, etc., are used in the same quantity as pesticides and other toxic organic micropollutants. However, antibiotics are not subjected to the same level of scrutiny for effects of possible environmental toxicity. Hence, the total spread and the concerns due to the presence of these antibiotic compounds in the environment are therefore mostly unknown or ill-defined.

The origin and principal discharge contamination routes of human and veterinary antibiotics into the environment are shown in the Figure 2.3. Following administration by humans, a huge portion of pharmaceuticals is excreted as API or unaltered which end up in WWTPs (Boxall, 2004). Recently, the extensive detection of these compounds and their residues in aquatic and terrestrial matrices has prompted significant regulatory and scientific concerns (Zuccato et al., 2010; Cardoso et al.,
The unaltered active ingredients of the pharmaceutical compounds are among the most widely studied components in terms of their distribution, absorption in cells and the biological effects on mammals (SRU, 2007).

![Diagram showing the origin and principal discharge routes of human and veterinary antibiotics](image)

**Fig. 2.3** Origin and principal discharge routes of human and veterinary antibiotics (Homem and Santos, 2011).

The development of antibiotic resistant pathogen and transfer of resistance gene of the microbial community is an emerging issue regarding public health and safety (WHO, 2001; Marti et al., 2013; Andersson and Hughes, 2014). They also have detrimental effects on natural microbial populations and their key functions (Wharfe et al., 2010; Alvarino et al., 2014).

Reports are available on the environmental antibiotic exposure causing adverse reproductive effects during the early stages of life in various aquatic organisms. A high mortality rate for nauplii and a significant decreased hatching rate for *Artemia sp.* cysts were reported due to the presence of antibiotics in the aqueous environment. Alteration of the pigment of *Artemia Salina* nauplii due to the presence of the antibiotic, flumequine resulted in a loss of fitness was demonstrated by Kummerer (2001). Toxic effects on the reproduction of *Daphnia magna*, a freshwater crustacean also demonstrated the serious impacts of various veterinary antibiotics on these aquatic organisms (Wollenberger et al., 2000). Antibiotics in the environment affecting the behaviour of aquatic organisms were reported by Frade et al. (2014).
2.6.1. CEPHALOSPORIN ANTIBIOTICS

The first isolation of cephalosporin compounds were from cultures of *Cephalosporium acremonium* from a sewer system in Sardania in the year, 1948 by Giuseppe Brotzu, an Italian scientist (Zhanel et al., 2014). Cephalosporins are considered as a highly important class of drugs that belongs to β-lactam group of antibiotics. They act as potent antimicrobial agents. They have maintained their glory among medicinal chemists for over 60 years. The general chemical structure of cephalosporin antibiotic is shown in Figure 2.4.

![Fig. 2.4 General chemical structure of cephalosporin antibiotics](image)

The cephalosporins consist of a ‘cepham’ nucleus, a dihydrothiazine two-ring (7-aminocephalosporanic acid, 7-ACA) system, fused to a β-lactam ring and varying side chain substituents at C₃ (R₂) and C₇ (acylamido, R₁) positions. The β-lactam ring and the substituents at C₃ and C₇ play an important role in deciding the biological activity of the antibiotic. The -COO group at C₄ position remains unsubstituted. The key group of acylamido (side chain) at C₇ position governs the hydrophilic/hydrophobic nature of the antibiotic compound (Van Krimpen et al., 1987).

Extensive research on cephalosporin drug molecules has produced four generations of cephalosporins, which are categorised based on the time of their discovery and anti-microbial properties (El-Shaboury et al., 2007). Ceftaroline and ceftobiprole, the fifth generation cephalosporin antibiotic has been launched and few other new drugs are under clinical evaluations. They are the derivatives of 7-ACA. Table 2.2 summarizes few examples of cephalosporin antibiotics of different generations, their activity and treatments for which they are used. They act effectively against Gram-negative organisms but show some reduced activity against Gram-positive organisms, and exhibit enhanced resistance to β-lactamases. However,
substitution of new molecules to the penam and cephem nuclei led to the synthesis of semi-synthetic cephalosporin antibiotics compounds with a higher activity against Gram-negative and positive microorganisms (García-Estrada and Martín, 2011).

**Table 2.2 Few examples of cephalosporin antibiotics**

<table>
<thead>
<tr>
<th>Generation</th>
<th>Derivatives (Brand names)</th>
<th>Description</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First</strong></td>
<td>Cefazolin (Ancef, Kefzol)</td>
<td><strong>Gram-positive:</strong> Has activity against penicillinase-producing, methicillin-susceptible, staphylococci and streptococci Gram-negative: Has moderate activity against <em>Proteus mirabilis</em>, <em>Escherichia coli</em>, and <em>Klebsiella pneumoniae</em></td>
<td>Upper and lower respiratory tract infections, Uncomplicated urinary tract infections</td>
</tr>
<tr>
<td></td>
<td>Cefadroxil (Duricef), Cephalexin (Keflex)</td>
<td><strong>Gram-positive:</strong> Has activity against penicillinase-producing, methicillin-susceptible, staphylococci and streptococci Gram-negative: Has moderate activity against <em>Proteus mirabilis</em>, <em>Escherichia coli</em>, and <em>Klebsiella pneumoniae</em></td>
<td>Upper and lower respiratory tract infections, Uncomplicated urinary tract infections</td>
</tr>
<tr>
<td><strong>Second</strong></td>
<td>Cefaclor (Keflor) Cefuroxime (Zinacef) Cefoxitin (Mefoxin)</td>
<td><strong>Gram-positive:</strong> Has less activity than first-generation <strong>Gram-negative:</strong> Has greater activity than first-generation</td>
<td>Upper respiratory tract infections, skin and soft tissue infections, Urinary tract infections</td>
</tr>
<tr>
<td><strong>Third</strong></td>
<td>Cefoperazone (Cefobid) Cefotaxime (Claforan) Cefdinir (Omnicef)</td>
<td><strong>Gram-positive:</strong> Has decreased activity against Gram-positive organisms. <strong>Gram-negative:</strong> Have a broad spectrum of activity and more increased activity than previous generations.</td>
<td>Gram negative mediated meningitis, complicated urinary tract infections and osteomyelitis</td>
</tr>
<tr>
<td><strong>Fourth</strong></td>
<td>Cefepime (Maxipime) Cefpirome (Cefrom) Cefozopran (Firstcin)</td>
<td><strong>Gram-positive:</strong> Have extended-spectrum activity as first-generation cephalosporins. <strong>Gram-negative:</strong> Exist as zwitterions that can penetrate the outer membrane of Gram-negative bacteria. They also have a greater resistance to β-lactamases than the third-generation cephalosporins.</td>
<td>Used in treating meningitis</td>
</tr>
<tr>
<td><strong>Fifth</strong></td>
<td>Ceftobiprole, Ceftaroline, Ceftolozane</td>
<td><strong>Gram-negative:</strong> Strongly active against <em>Pseudomonas</em> sp. and appears to be less susceptible to resistance development.</td>
<td>Complicated abdominal and urinary tract infections</td>
</tr>
</tbody>
</table>
2.6.2. MODE OF ACTION OF CEPHALOSPORINS

Cephalosporins antibiotics have the same mechanism of action as other β-lactam antibiotics, but are less susceptible to β-lactamases (Figure 2.5). They weaken the bacterial cell wall by covalently binding to the active site of penicillin-binding proteins (PBPs) thusly inhibiting the peptidoglycan biosynthesis during the final stage of the cell wall biosynthesis and ultimately leads to cell lysis.

Fig. 2.5 Mode of action of cephalosporins (Kong et al., 2010).

In addition, irreversible inhibition of the PBP enzymes, such as, PBP1 complex (transglycosylases), PBP3 (transpeptidases) and PBP4, PBP5, and PBP6 (carboxypeptidases) and the activation of cell wall hydrolases and autolysins by β-lactam antibiotics also assist in the cytolysis (Kong et al., 2010).

2.6.2.1. TOXICITY OF CEPHALOSPORINS

Cephalosporin antibiotics have a broad spectrum of antimicrobial activity and are the most extensively used human and veterinary antibiotics in many countries with their usage accounting for approximately 50–70% of the total antibiotics used by human (Kümmerer, 2009). Due to their widespread usage, cephalosporins are often being accumulated and become persistent in the environment producing harmful effects in both aquatic and terrestrial matrices. The hydrophilic and non-volatile nature of the antibiotic compounds prevents their removal from these matrices which tends to persist in the environment (Hernando et al., 2006). The physico-chemical characteristics of each antibiotic (e.g., size and shape, molecular structure, solubility and hydrophobicity) will decide their nature of distribution in various environmental matrices (Kemper, 2008).
The release of the cephalosporin antibiotic in the environment is considered most significant because of a huge percentage of antibiotic doses are excreted unchanged and can be bioactive in soil-water ecosystems. The increased toxicity of the functional group, 7-ACA of synthetic cephalosporins in aqueous medium were found to induce variable abnormal phenotypes during the organogenesis process (development of notochord, cranial, nerve, cardiovascular and abdomen) and pigment formation in zebrafish embryos (Zhang et al. 2013).

Cephalosporin resistant microorganisms were reported to cause serious problems of public health, leading to complications in treating pathologies and causes imbalances in microbial ecosystems (Subbiah et al., 2012; Kronenberg et al., 2013). So far, legal limits for antibiotics in food have been established as 4-1500 mg/kg for milk and 25-6000 mg/kg for the other foodstuffs of animal origin.

The wastewater discharged from cephalosporin production plants is highly bio-toxic, very harmful and hard to degrade. This will cause great harm to the human beings and the environment. The presence of cephalosporin at high concentrations in the environment leads to a very high levels of chemical oxygen demand (COD), which in turn increases the toxic strength of the effluent (Duan, 2009). Higher environmental toxicity in aqueous environment caused by photo-transformation or photo degradation of cephalosporin antibiotics was reported by Wang and Lin (2012).

2.7. REMEDIATION PROCESS

Various treatment processes are available for effective removal of antibiotics from water matrices.

2.7.1. CONVENTIONAL TREATMENTS

Biological methods, viz., filtration and coagulation/sedimentation/flocculation are the most generally used methods in conventional WWTPs (Stackelberg et al., 2007; Arikan, 2008).

Activated sludge technology is most extensively used biological methods in industrial WWTPs (Eckenfelder, 2000). This method employs activated sludge tanks operated in aerobic or anaerobic mode for the degradation of organic compounds with a constant monitoring of COD and temperature.

The filtration is the separation of the solids and suspended matter through a granular media (coal, sand, granular activated carbon, diatomaceous earth) by interstitial straining. The smaller particles are retained by electrostatic forces, weak
chemical bonds or adsorption (Eckenfelder, 2007). This process has a limitation of not degrading the pollutant and generating a new waste.

Flocculation/coagulation/sedimentation processes employ ceratin chemicals to enhance the settling out the solids through precipitation and sedimentation. The commonly used chemicals are alum, iron salts, lime and polymers (Eckenfelder, 2007). These methods require a subsequent treatment process to remove the coagulated form of the pollutants from the effluents. The efficiency of physico-chemical methods applied to sulphonamides, quinolones, macrolides, trimethoprim and quinoxaline derivatives showing a low removal of about 30% was reported (Stackelberg et al., 2007; Vieno et al., 2007). Due to the inefficiency of these treatments, other new alternative processes have emerged.

2.7.2. OXIDATION PROCESSES

2.7.2.1. CHLORINATION

Chlorine (Cl₂) is an inexpensive disinfectant that is widely used in drinking water treatment plants. It is also a strong oxidizing agent and act selectively based on chemical structure to break apart the chemical compounds. Oxidation using chlorine and chloramine has been demonstrated successfully in the removal of many organic compounds. However, only certain pharmaceutical compounds will be removed with high efficiency. Navalon et al. (2008) reported the complete oxidation of β-lactams (amoxicillin, cefadroxil and penicillin G) antibiotics using chlorine dioxide (ClO₂). Li et al. (2013a) also reported the cefazolin transformation using chlorine disinfection process. Adams et al. (2002) also reported on the effective removal (>90%) of trimethoprim, sulphonamides and carbadox by chlorine oxidation. The formation of highly toxic (carcinogenic) chlorinated by-products greatly limits this process (Stackelberg et al., 2007). Hence, this method has been replaced by advanced oxidation processes.

2.7.2.2. ADVANCED OXIDATION PROCESSES (AOPs)

AOPs are advanced oxidative processes works on the basis of generation of intermediate hydroxyl radicals (HO*), which are less selective and highly reactive than other oxidants. AOPs include ozonation, Fenton, photo-Fenton, photolysis, semiconductor photocatalysis and electrochemical processes.

2.7.2.2.1. OZONATION

Ozone is an equally strong oxidant and disinfectant, but more effective than chlorination in removing pharmaceuticals in wastewater (Westerhoff et al., 2005). The
organic compounds are oxidized or even mineralized to CO₂ and H₂O. But, the produced metabolites are potentially eco-toxic and practically remains unchanged in the environment (Dantas et al., 2008). Lower degradation rates of β-lactam antibiotics by this method has been reported, high operating costs and energy requirement (Britto and Rangel, 2008; Homem and Santos, 2011) limit its application to treat contaminated wastewater.

2.7.2.2.2. FENTON AND PHOTO-FENTON

This method employs Fenton’s reagent, (hydrogen peroxide and iron salt catalyst) which has strong oxidizing properties (Gan et al., 2009). This method has various advantages like, low-cost of the reagents, easy iron availability, non-toxic, and easy handling of hydrogen peroxide and eco-friendly. Though Fenton process produces good results in terms of degradation efficiency, photo-Fenton seems to be more efficient with respect to COD and TOC removal. However, photo-Fenton is generally not applied to wastewaters containing high organic (COD) content, such as hospital, municipal and pharmaceutical wastewaters.

2.7.2.2.3. PHOTOLYSIS

2.7.2.2.3.1. UV AND UV/H₂O₂ PHOTOLYSIS

UV light-based photolysis processes are promising alternatives for organic pollutant removal from contaminated wastewaters by direct or indirect photolysis (Trovó et al., 2009; Giokas and Vlessidis, 2007). Though direct and indirect photolysis reaction can occur simultaneously, indirect photolysis was reported to play a significant role in reducing the half-life of the pollutants (Giokas and Vlessidis, 2007). However, the success rate depends upon the intensity of the UV radiation, the dose of H₂O₂ and O₃ (Kummerer, 2009). A very low antibiotic removal rate of quinolones and sulphonamides were reported by Adams et al. (2002) and Arslan-Alaton et al. (2004). Limitations include, prolonged reaction times (Trovó et al., 2009) and applied only to wastewater containing photo-sensitive compounds with very low COD levels (e.g. drinking, river water).

2.7.2.2.3.2. SEMICONDUCTOR PHOTOLYSIS

The method involves the activation of a semiconductor catalyst (mostly TiO₂ due to its low cost, good performance and high stability) using artificial or sunlight. The process performance depends upon the pH, catalyst concentration, radiation intensity, wavelength and water matrix. Illuminated semiconductor particles catalysing wide range of redox reactions of both organic and inorganic compounds are
reported (Fujishima et al., 2007). A study on the degradation of β-lactam antibiotics using this technique was demonstrated which showed degradation above 50% along with a high removal of dissolved organic carbon (≈80%) (Elmolla and Chaudhuri, 2010). However, the industrial application of this process is restrained due to the difficulty of penetration of radiation and removal of catalyst, which can be time consuming and costly (Britto and Rangel, 2008) and also it can treat only the effluents with low loads of organic content. Moreover, this technique has not been practically implied in wastewater treatment because of its reduced electrical energy value per order (Homem and Santos, 2011).

2.7.2.2.4. ELECTROCHEMICAL PROCESSES

The electrochemical processes are applied as an efficient, cost-effective, versatile, easy and clean technology to remove toxic organic pollutants (Panizza and Cerisola, 2009) by the oxidation using anodes (e.g., Ti-based alloys, TiO₂, graphite, Ir or Ru oxides etc.) and an electrolyte. Degradation and removal of various antibiotics like tetracycline (Kitazono et al., 2012; Oturan et al., 2013), trimethoprim (Moreira et al., 2014) and oxacillin (Giraldo et al., 2015) from aqueous systems using this process have been reported. Despite the advantage of treating toxic wastewaters like pharmaceutical wastewater, the application of this method is limited because of its small flow rates and high operating costs (Mehrjouei et al., 2014).

2.7.3. ADSORPTION PROCESSES

Adsorption is a widely used process in the unit operations of downstream processing to remove various organic pollutants. Adsorption is a process of accumulation of matter (solid) from a gas or liquid phase onto an adsorbent surface. The process works on the tendency of the molecules (solid) in the liquid phase to adhere/adsorb onto a solid surface (Ahmed et al., 2015). The adsorption process can be divided into physical or chemical mode based on the nature of interactions. Physical adsorption exhibits weak van der Waals forces, whereas, chemical adsorption is relatively strong involving the electron transfer and formation of chemical bonds between adsorbate and adsorbent (Ruthven, 2000). The efficiency of the adsorption processes highly depends upon the adsorbate properties, type of adsorbent used and the wastewater composition (Aksu and Tunc, 2005). Several adsorptive materials viz., activated carbons, multi walled carbon nanotubes, ion exchange materials, natural clay materials (bentonite), hollow silica nanospheres, nanoparticles and nanocomposites are reported for antibiotic removal (Fakhri and
Behrouz, 2015). Some of these adsorbents require a previous activation treatment (such as thermal or chemical activation) called pre-treatment to increase their surface area and to improve the adsorption efficiency (Chergui et al., 2009). The removal of nitroimidazole and cephalaxin antibiotics from aqueous solutions using activated carbon as an adsorbent has been reported (Mendez-Diaz et al., 2010; Liu et al., 2011). The use of bentonite as an adsorbent to remove amoxicillin (Putra et al., 2009) and ampicillin (Rahardjo et al., 2011) have also been reported. Vasiliu et al. (2011) demonstrated the adsorption of cefotaxime using ion exchange resin. Fakri and Adami (2014) reported adsorption of cephalosporin antibiotics using MgO nanoparticles. However, all these adsorbents face a common disadvantage of high cost and difficulty in regeneration (Crisafulli et al., 2008). Therefore, increased research interests grew up for finding alternative adsorbents of low-cost derived from the waste materials or by-products of agricultural or industrial processes.

**2.7.3.1. BIOSORPTION**

Biosorption is an efficient, cost effective process, which uses live or dead biomass for remediation of pollutants. It is a safer treatment approach compared to most of the expensive and chemical adsorbents (Watkinson et al., 2009; Delgado et al., 2012). The unique surface properties of plants and microorganisms enable them to adsorb various pollutants from aqueous solutions. The cell wall and cellular components of microbial and plant biomass aid in the passive uptake of various pollutants by adsorption, micro-precipitation, ion exchange, complexation or chelation processes (Chergui et al., 2009). Both living and dead biomass can be used to remove hazardous chemicals, but maintaining a viable biomass during biosorption is always difficult, as it requires a continuous nutrient supply and intervention of toxic wastes towards microbial growth. Hence, the use of dead microbial cells in biosorption is considered more advantageous because the dead organisms remain unaffected by toxic wastes, do not require a continuous nutrient supply, can be reused and regenerated for many cycles (Aksu and Gonen, 2004). Biosorption has distinct advantages like, they do not produce dangerous (toxic) products, they are strongly selective and profitable in terms of cost during treatments of large volumes of wastewater (Chergui et al., 2009).

Biosorption has been widely applied to wastewater treatment processes over the years, particularly for the removal of antibiotics. Guo and Chen (2015) reported the removal of cephalosporin antibiotics using alga-activated sludge. Adsorptive
removal of sulphomethoxazole, carbezepine and caffeine using mixed microbial culture was studied by Vasiliadou et al. (2013). Compared to other processes discussed so far, adsorption can be applied to wastewaters containing either high concentration of antibiotic or high levels of organic content.

2.7.4. MEMBRANE PROCESSES

Membrane filtration involves the separation of contaminants from water, based on the molecular size and/or electrostatic interactions.

2.7.4.1. REVERSE OSMOSIS, NANO AND ULTRA-FILTERATION

Among, membrane filtration processes, reverse osmosis (RO) and nanofiltration (NF) methods had shown promising potential with the advantages of low investment, high product quality, easy scaling-up, etc. (Acero et al., 2010). These processes were found to remove over 80% of all target pollutants and hence can serve as an important water resource for reuse in industries, agriculture, urban areas, aquifer recharges, etc. (Shanmuganathan et al., 2015). Both RO and NF technology face certain limitations like, they are highly energy intensive, poor removal of organic compounds, suffer severe membrane deterioration/fouling, disposal of the concentrate at the end of the process containing a wide range of organic contaminants, refractory chemicals (PCPs) and soluble microbial products which would inevitably lead to serious pollution in the ecosystem (Malaeb and Ayoub, 2011; Justo et al., 2013). An efficient removal of >87% was achieved by combining NF with ozone-based AOPs during the removal of the four antibiotics, ofloxacin, norfloxacin, azithromycin and roxithromycin from aqueous medium. Similar studies on application of RO, NF and ultrafiltration for antibiotic removal were reported by various other researchers too (Adams et al., 2002; Kosutic et al., 2007; Radjenovic et al., 2008).

In recent decades, forward osmosis (FO), a new membrane process has been developed which is driven by a difference in osmotic pressure across the membrane with advantages of high quality permeate, no need of hydraulic pressures and low membrane fouling propensity. They are exclusively designed for industrial wastewater treatment, reclamation of impaired water, desalination of seawater etc. (Linares et al., 2014; Kwan et al., 2015). Removal of trimethoprim, sulfamethoxazole, roxithromycin and norfloxacin antibiotics using FO technology have been reported by Liu et al. (2015).
2.8. MICROBIAL DEGRADATION OF ANTIBIOTICS

Microorganisms present in the environment (indigenous microbes) are large communities of unexplored reservoir of vast genetic diversity and metabolic capability. They play a pivotal role in the fundamental ecological processes of biogeochemical cycling and organic contaminant degradation (xenobiotics, pharmaceuticals, etc.,) (Lahti and Oikarai, 2011; Rodarte-Morales et al., 2011). They form an important class of degraders and provide metabolites as nutrients to other organisms, thus maintaining a normal flow in the food web (Megharaj et al., 2011). Bioremediation is a process by which the contaminants are destroyed/degraded or reduced to harmless contaminants by exploiting the catalytic capabilities of the living (indigenous) microbes. This process offers various advantages such as, implying low-technology techniques, relatively less cost, highly accepted by the public and can be carried out on site (Vidali, 2001). Though biological WWTPs are not generally designed to remove trace organic compounds, continuous monitoring efforts and controlled laboratory experiments have demonstrated the effective attenuation of various pharmaceuticals. pH, oxygen, moisture, nutrient availability, absence of alternative carbon and nitrogen source and the presence of a acclimatized microbial population are shown to be the prime factors required for the degradation of antibiotics (Wang et al., 2006; Gartiser et al., 2007).

2.8.1. AEROBIC DEGRADATION

2.8.1.1. BACTERIAL DEGRADATION

Numerous studies on indigenous bacteria exhibiting their natural resistance/degradation capability against various toxic compounds including antibiotics are available in the literature. Zhang and Dick (2014) investigated the penicillin and neomycin degradation by nineteen bacterial soil isolates of *Proteobacteria* sp. and *Bacteroidetes* sp. and found them utilizing the antibiotics as the sole carbon sources for their metabolic needs. Degradation of erythromycin and ciprofloxacin antibiotics using bacteria and fungi from aquatic environment was investigated by Nnenna et al., (2011). The biodegradability of clinically important antibiotics viz., cephalosporins, penicillins, lincosamides, macrolides, sulphonamides aminoglycosides, quinolones etc., using indigenous bacteria was studied in a closed bottle test (Alexy et al., 2004). Penicillin G, amoxicillin, imipenam and nystatin antibiotics were found to be readily biodegradable as reported by Gartisier et al.
A study on aquaculture sediments showed bacterial mineralization of erythromycin A (Kim et al., 2004). Degradation of various derivatives of cephalosporin antibiotics using bacteria has also been reported (Gartiser et al., 2007; Jiang et al., 2010; Krishnan et al., 2012). The effective treatment of pharmaceutical wastewater containing antibiotics using Pseudomonas putida and Pseudomonas aeruginosa has been studied (Mansour et al., 2012; Njoku et al., 2013). There are reports on the use of various biological reactor to treat wastewater from cephalosporin production unit (Duan, 2009). The treatment of volatile organic compounds (VOCs) present in pharmaceutical wastewater by submerged aerated biological filter was reported by Priya and Ligy Philip (2015).

2.8.1.2. FUNGAL DEGRADATION

Fungi are robust microorganisms which act as symbionts, decomposers, and pathogens all ecosystems (Anastasi et al., 2013). Mycoremediation have certain advantages over other microbial treatments like, their extreme tolerance to pollutants, easy penetration and rapid colonization. These promising abilities have attracted the interest of many researchers for a couple of decades. Complete biodegradation of fluoroquinolones (norfloxacin, ofloxacin and ciprofloxacin) by a group of white rot fungi (Panus tigrinus, Irpex lacteus, Trametes versicolor, Dichomitus squalens and Pleurotus ostreatus) in 10-14 days has been reported (Gros et al., 2014; Čvančarová et al., 2015).

2.8.1.3. DEGRADATION BY YEAST

Different from other microbial processes, yeast treatment processes exhibit unique characteristics of highly adaptive, faster growth, lower production costs, resist unfavourable environments, higher pollutant removal rates, no need of excess nutrient supplements and very efficient in treating high strength pharmaceutical wastewaters (Yang et al., 2008; Yang and Zheng, 2014). Yeasts were found to show high tolerance to low pH, organic content, salinity, antibiotic concentration, and the presence of sterilizers (Wang et al., 2011). Based on the origin, they have different pollutant removal abilities (Yang et al., 2013a). It can metabolize a broad range of carbon substrates (sugars, pentoses, biopolymers, alcohols, hydrocarbons, organic and fatty acids), nitrogen substrates (urea, ammonia) and inorganic salts (containing sulphates and phosphates) (Bekatorou et al., 2006).

Industrial wastewaters often have a highly acidic pH (<5) contributing to pH toxicity (Zheng et al., 2010). The yeast treatment process, improving the pH levels of
highly acidic industrial wastewaters to neutral after treatment was reported (Arnold et al., 2000; Yang et al., 2008). This cuts down treatment costs and facilitates subsequent activated sludge processes (ASPs).

Indigenous yeast isolates were reported to produce more biomass and reduce COD levels more effectively than the microbes of other origin (Arnold et al., 2000). Efficient COD reduction in the pharmaceutical wastewater by 10 environmentally isolated yeast strains of *Pichia anomala* was reported (Recek and Raspor, 1999; Recek et al., 2002). A similar study on effective reduction of both BOD and COD levels in pharmaceutical wastewater using *S. cerevisae* has also been reported (Njoku et al., 2013). Addition of yeast and yeast enzyme was reported to assist the remediation of pharmaceutical wastewater (Uwadiae et al., 2011).

Therefore, yeast treatment processes appeared to be more suitable and cost-effective for the treatment of high strength pharmaceutical wastewaters.

### 2.8.2. ANAEROBIC DEGRADATION

The decomposition/degradation of organic and inorganic matter by microbes in the absence of molecular oxygen is termed as anaerobic process. The anaerobic process involves step-wise biological conversion of organic matter to various end products including methane (CH\(_4\)) and carbon dioxide (CO\(_2\)). This process offers several advantages over other treatment methods, viz., efficient, needs less space, easy scaling, simple operation and maintenance, energy production (CH\(_4\)) and low sludge generation (Seghezzo et al., 1998). Carballa et al. (2007) reported the use of mesophilic anaerobic digestion using stirred tank reactor (STR) for 30 days to degrade roxithromycin and sulfamethoxazole. Chelliapan et al. (2011) demonstrated the use of anaerobic packed bed reactor and concluded that, it can be used effectively as an option for pre-treating the pharmaceutical wastewater. Similarly, Saravanane et al. (2010) reported treatment of cephalosporin containing pharmaceutical wastewater using a bioaugmented up-flow anaerobic fluidized bed reactor. The use of facultative and obligate anaerobes to degrade ceftiofur antibiotic was also reported (Wagner et al., 2011). A recent study on the use of bioaugmented anaerobic membrane bioreactor employing 2 dominant methanogens, *Methanolobus* and *Methanosaeta* to treat penicillin containing pharmaceutical wastewater was reported (Ng et al., 2015).

However, anaerobic processes have certain disadvantages too. Pathogens used in the process are only partially removed, requires post-treatment to remove unused nutrients, slow growth rate of methanogens requiring a long start-up period than
aerobic treatments and unpleasant odour due to the production of hydrogen sulphide (Seghezzo et al., 1998).

2.9. EFFECTS OF ENVIRONMENTAL FACTORS ON DEGRADATION OF ANTIBIOTICS

The degradation of antibiotics is driven by various environmental factors such as physico-chemical characteristics, type of soil, prevailing climatic conditions, microbial activity, etc. (Gros et al., 2014). Direct sunlight plays a key role in the photo-degradation of antibiotics. Reduced light (sunlight) intensity or when the antibiotics are protected in a sludge or slurry, improper or reduced photodegradation of tetracyclines, fluoroquinolones and sulphonamides was observed (Sturini et al., 2015). Photodegradation of cephalosporin antibiotics in natural waters resulted in higher toxicity was observed (Wang and Lin, 2012). Higher antibiotic residues were observed in groundwater during the spring season than in the winter, which proved the influence of seasonal variation of antibiotic concentration (Yao et al., 2010). Another important factor is soil, which influences the major antibiotic degradation by a variety of microbial enzymatic reactions (Al-Ahmad et al., 1999). Increased biodegradation of antibiotic in soils was seen in manure/sludge containing high numbers of microbes (Ingerslev and Halling-Sørensen, 2001). Additionally, other environmental factors viz., pH, temperature, bioavailability, substrate concentration, time of exposure, other nutrient availability and microbial enzymatic abilities have a major impact on the microbial growth and their degrading capabilities (Elcey and Kunhi, 2010). However, according to Nnenna et al. (2011), pH did not have any significant effect on the degradation of antibiotics. Similar results were reported for macrolide and amphenicol antibiotics, which remained stable and showed no observable hydrolysis under ambient environmental conditions of pH and temperature (Mitchell et al., 2015). This reason may be due to the presence of ionizable functional groups, due to which they exist as a zwitterion at environmentally relevant pH values (Kemper, 2008).

2.10. ANTIBIOTICS DEGRADATION PATHWAYS

Since antibiotics are introduced from various source points mostly via water (pharmaceutical wastewater) into the environment, hydrolysis can be a common and an important degradation option. Very few studies have been conducted to assess the biodegradation pathway of antibiotics. Nägele and Moritz, (2005) demonstrated the
acid degradation of amoxicillin, a β-lactam antibiotic. They also elucidated a degradation pathway of amoxicillin (Figure 2.6). Related studies on different β-lactam antibiotic degradation by various other methods have also been reported. Amoxicillin (Xu et al., 2011; Dimitrakopoulou et al., 2012), monocyclic β-lactam antibiotics (Braschi et al., 2013), oxacillin (Giraldo et al., 2015), cephalosporin derivatives (Okamoto et al., 1996b; Li et al., 2013a; Fabbri et al., 2015) have been reported by various researchers.

Fig. 2.6 Degradation pathway of amoxicillin (Nägele and Moritz, 2005).

Wang and Lin (2012) reported the direct photolytic degradation of cephalosporin derivatives, viz., cephradine (CFD), cephalixin (CFX), cefazolin (CFZ), cefotaxime (CTX) and cephapirin (CFP). A possible CFP degradation pathway by direct photolysis along with its degraded products are shown in Figure 2.7. The degraded products showed an intact cephem ring structure, which confirmed the incomplete degradation of the parent compounds by photolysis method.
Studies on aerobic biodegradation of various structurally related sulfonamide antibiotics using *Achromobacter denitrificans* PR1 and *Microbacterium* sp. strain BR1 was reported (Ricken et al., 2013; Reis et al., 2014). Another recent study on sulfonamide catabolism using resting and crude cell extracts of *Microbacterium* sp. strain BR1 was reported (Ricken et al., 2015). They have also elucidated a degradation pathway of sulfamethoxazole by *Microbacterium* sp. strain BR1 as shown in Figure 2.8.

**Fig. 2.7** Predicted direct photolysis products and degradation pathway of cephapirin (CFP) (Wang and Lin, 2012).

**Fig 2.8** Proposed degradation pathway of sulfamethoxazole by *Microbacterium* sp. strain BR1 (Ricken et al., 2015).
The study of analysis of degradation pathway has contributed to understand the complete behaviour of the microbial community towards antibacterial compounds and its capability in inducing degradative enzymes to achieve a possible degradation of the compound. So far, no pathway has been proposed where yeast has played a role as degrader of antibiotics.

2.11. ROLE OF ENZYMES ON ANTIBIOTIC DEGRADATION

Enzymes play a vital role in catalyzing most of the biochemical reactions by enhancing the rate of a reaction and govern the chemical changes, which are proportional to the amount of enzyme. Antibiotics undergo degradation by means of various mechanisms, which include hydrolysis, group transfer and redox mechanisms (Wright, 2005). Out of these, hydrolysis is a clinically important degradation mode and mostly applied to many antibiotic compounds particularly β-lactam antibiotics.

2.11.1. EXTENDED SPECTRUM β-LACTAMASES (ESBL)

Extended spectrum β-lactamases (ESBLs) enzymes are produced by certain microorganisms, especially, gram-negative bacilli. They attack on the oxyimino group of third generation cephalosporins thusly inactivating the β-lactam antibiotics (Paterson and Robert, 2005). They are more efficient than the simple parent β-lactamases because of their ability to hydrolyze a wide spectrum of β-lactam antibiotics and hence, they are referred as “extended-spectrum” β-lactamases. The breaking of the β-lactam bond will decide the rate-determining step of the degradation reaction. Extended spectrum β-lactamases (ESBLs) are categorised under group 2 of β-lactamases enzymes (Shah et al., 2004). The intestinal microbiota species of the cattle produces a variety of β-lactamases that can readily transfer between animals in a herd (Tragesser et al., 2006).

Ceftiofur degradation by β-lactamases produced by *Bacillus* sp. *Roseomonas* sp. and *Azospirillum* sp. has been reported by Rafii et al. (2009). *E. coli* and *K. pneumoniae* are recognised as the main ESBL-producing bacterial members by various researchers (Song et al., 2009; Ashrafian et al., 2013). β-lactamases group represents one of the major enzyme in cephalosporin hydrolysis (Brites et al., 2013). The role of the enzyme β-lactamases towards the degradation of cephalosporin antibiotics was reported by various researchers (Spencer et al., 2001; Wagner et al., 2011).
2.11.2. AMYLASES

Amylases are the most important enzymes with high industrial significance which can be derived from microbes, plants and animals (Aiyer, 2005). They catalyse the hydrolysis of glucosidic linkages of polysaccharide moiety. Application of amylases requires unique property with respect to pH, temperature, specificity and stability (Mc Tigue et al., 1995). The application of yeast enzyme, amylase aiding the remediation of pharmaceutical wastewater was reported by Uwadiae et al. (2011).

2.11.3. CYTOCHROME P450

Cytochrome P450 (Cyt P450) is heme b containing monooxygenase enzymes, which play a significant role in the drug metabolism (Guengerich, 1999). Cyt P450 and NADPH reductase enzymes were reported to catalyse wide range of reactions of drug metabolism and in the synthesis of fine chemicals (Guengerich, 1999; Niwa et al., 2004; Urlacher and Eiben, 2006).

2.11.4. LACCASE

Laccases are multi-copper oxidases produced by bacteria and fungi. It can oxidize a wide range of pollutants, including pharmaceuticals and pesticides, requiring only oxygen as a co-substrate (Yang et al., 2013b). The potentiality of laccase-mediator systems (LMS) towards the removal and detoxification of antibiotic, sulfamethoxazole and herbicide, isoproturon was reported (Margot et al., 2015). The degradation of ciprofloxacin and norfloxacin antibiotics by laccase enzyme produced by a white-rot fungus, *Trametes versicolor* was reported (Prieto et al., 2011). Application of immobilized fungal laccases in various matrices towards effluent treatment has been well established (Claus et al., 2003).

2.11.5. MANGANESE PEROXIDASE

Manganese peroxidase (MnP), the extracellular haem protein enzymes possess the catalytic properties of both oxidase and peroxidase (Xu et al., 2013). Crude manganese peroxidase produced by a white rot fungus, *Phanerochaete chrysosporium* served as a highly efficient biocatalyst during degradation of two widely used antibiotics, tetracycline and oxytetracycline (Wen et al., 2010).
2.12. ROLE OF IMMOBILIZATION AND BIOFILM IN ANTIBIOTIC REMOVAL

2.12.1. BIOFILM FORMATION

Application of freely suspended biomass (such as bacteria, yeast, fungi and algae) has been always not suitable for degradation processes because of certain practical disadvantages like, low mechanical strength, small particle size and possibility of clogging of reactors during treatment (Godjevargova et al., 2004). Hence, a biofilm, which is the structured microbial aggregates enclosed in self-produced gelatinous extracellular polysaccharides (EPS) can be applied for wastewater treatment.

EPS exudates are composed of structurally branched homo and hetero polymers, which help in protection, structural integrity, nutrient utilization, environmental resistance and cell-cell communication (Stoodley et al., 2002; Simoes et al., 2010). In recent years, biofilm based WWTPs has attracted many researchers due to its simplicity, easy operation, reliability, high adaptability to influent loading, minimal sludge production and high pollutant removal efficiency (Huang et al., 2014; Whiteley and Lee, 2015). The biodegradation of three active pharmaceutical compounds, viz., ibuprofen, clofibric acid and diclofenac was investigated in a pilot sewage plant employing biofilm reactors as model systems from sewage (municipal) water (Zwiener and Frimmel, 2003).

2.12.2. IMMOBILIZATION

Microbial cells immobilized in a suitable matrix have proven to show improved tolerance towards a variety of recalcitrant and toxic compounds (Sarma and Pakshirajan, 2011). Immobilization is a common term which describes a cell entrapment or particle attachment using suitable matrices (Lopez et al., 1997). There are four principle methods of immobilization viz., entrapment, covalent binding, adsorption and membrane confinement (Costa et al., 2011). Immobilized microbial cells have the following advantages in comparison with freely suspended cells, like immobilization can achieve high microbial population density in a limited volume, low substrate inhibition, high degradation efficiency, toxicity to microbes can be overcome due to diffusion constraints, improved microbial performances, enhanced operational stability, can afford environmental protection against adverse conditions, can remain viable for long periods, can be reused, reduces treatment costs (Kadakol et
Wastewater treatment using immobilized microbial cells is receiving increasing interest in recent years (Tong et al., 2013; Wang et al., 2012). There are many reports on the use of immobilized microorganisms and microbial biofilms for the remediation of wastewater containing organic compounds (Kim et al., 2012; Park et al., 2012). The application of immobilized laccase isolated from *Trametes versicolor* showed successful depletion of tetracycline antibiotic from aqueous solutions carried out in an enzymatic membrane reactor (De Cazes et al., 2014).

### 2.12.2.1. IMMobilization Matrices

Selection of a suitable immobilization matrix is very crucial because it will reflect on the overall efficiency of the treatment process. Fundamental properties of a matrix include, a) physical properties (shape, strength, density, degree of porosity), b) chemical properties (hydrophilicity, inertness), c) stability properties (storage, regeneration, cell viability), d) resistance properties (pH, temperature, solvents, cell defense), e) reaction properties (catalytic productivity, mass transfer, flow rate), f) safety concerns and g) economic aspects (Bickerstaff, 1997). Natural (e.g., sodium alginate, chitosan, carboxymethylcellulose, agar etc.), synthetic matrices (e.g. polyvinyl chloride, polyvinyl alcohol, polyurethane etc.) and inorganic matrices (e.g. Zeolites, ceramide, activated carbon, betonite etc.) are used for immobilization. However, natural matrices were found to show highest cell survival rate (Leenen et al., 1996).

#### 2.12.2.1.1. SODIUM ALGINATE

Sodium alginate, the sodium salt of alginic acid is a linear polysaccharide derived from marine brown algae and seaweed (Penman and Sanderson, 1972). Alginate has superior properties like cyto-compatibility, compatible to biodegradation, chemical versatility and solgel transition properties which enable them to serve as a good immobilization matrix (Pawar and Edgar, 2012). Application of sodium alginate along with chitosan were reported to be effective in treating wastewater containing toxic metals and dyes (Qin et al., 2007).

#### 2.12.2.1.2. CARBOXYMETHYLCELLULOSE (CMC)

Carboxymethylcellulose (CMC) or cellulose gum, a natural polymer has been proved an excellent support matrix for immobilization experiments. Various studies were reported based on the application of CMC towards sorption and degradation of
many organic compounds (Wang et al., 2008). CMC is the most widely investigated natural biopolymer in bioremediation research studies (Scott, 1987).

2.12.2.1.3. CHITOSAN

Chitosan is extracted from a cheap, low cost biomaterial like the exoskeleton of crustaceans (crabs, shrimps, lobsters and krills) and from the seafood processing industrial wastes (Hirano et al., 1990). They serve as a biocompatible, non-toxic and economically attractive source with potential industrial applications (Paul, 2000; Muzzarelli and Muzzarelli, 2005). The multidimensional properties of chitosan enable them to be easily modified as immobilization matrix for immobilizing cells and enzymes (Dash et al., 2011). Recent developments of chitosan and chitosan modified biopolymer materials have proven their applications in the field wastewater treatment (Crini, 2005).

2.12.2.1.4. AGAR

Agar (agar-agar) is derived from the species of red algal genera, Gelidium, Gracilaria and Pterocladia. They exhibit a wide range of functional properties depending on the source from it is derived and the extraction protocols (Painter, 1983). It has a strong gelling capability due to its structural composition of polysaccharide molecules. It is highly acid resistant and show no protein reactivity. Moreover, use of this matrix works very economical as compared with other matrices (Prakash and Jaiswal, 2011).

2.12.2.1.5. POLYVINYL ALCOHOL (PVA)

PVA is a synthetic and hydrophilic polymer which has excellent physical stabilities to serve as an encapsulation matrix. Among synthetic carriers, PVA is most preferred because it is non-toxic, non-carcinogenic (Zhang et al., 2007). It also has relatively good tensile stress, impact strength, high water affinity, wear resistance, good biocompatibility, process ability, minimal cell and protein adhesion and excellent electrical insulation properties (Yujian et al., 2006; Zhang et al., 2007). Research investigations have proved PVA as an effective alternative for domestic and advanced WWTPs (Chen et al., 1998; Chang et al., 2005). The wide application of PVA in pharmaceutical and biomedical applications has been known for decades (De Merlis and Schoneker, 2003; Paradossi et al., 2003).

2.12.2.2. IMMOBILIZATION USING HYBRID MATRICES

Hybrid matrix immobilization with different polymeric matrices has distinctive advantages such as, high or improved degradation efficiency, reduced cell
leakage, prolonged shelf-life (Mollaei et al., 2010). Various polymers can be mixed with sodium alginate to form a hybrid matrix with modified properties. Among them, PVA proved to blend well with alginate with a strong interaction between the two components (Russo et al., 2001). PVA/alginate matrix as a promising material for has found various applications in life sciences as reported by many researchers (Yujian et al., 2006; Zhang et al., 2007; Cho et al., 2009; El-Naas et al., 2013). Innovative applications of PVA and PVA-based hydrogels/hybrids in encapsulating biomolecules in biomaterials field has been reported (Dos Reis et al., 2006). Application of PVA- alginate immobilized beads towards degradation and removal of various xenobiotic compounds has also been reported by a few researchers (Tsai et al., 2013; Huang et al., 2015).

2.13. NANO-AIDED DEGRADATION OF ANTIBIOTICS

In recent years, nanotechnology has revolutionized the environmental field with many novel strategies to address various pollution problems (Singh and Tripathi, 2007; Klabunde et al., 2010). Nanoparticles offer ultra-high selectivity, sensitivity, detection, removal and remediation of micropollutants including pharmaceutical compounds (Watlington, 2005; Pratt, 2014).

Many nanoparticles have been reported for hazardous and ground water remediation. Among those, zero-valent iron (n-Fe\(^0\)) and magnesium oxide (n-MgO) nanoparticles have gained increasing research interests in the remediation of antibiotic containing wastewaters (Ghauch et al., 2009; Moussavi et al., 2010; Moussavi et al., 2014).

n-Fe\(^0\) is of low cost, less toxic and reduce electrical conductivity (EC) and total dissolved solids levels (TDS) unlike ferric and ferrous salts, which generally increase the TDS and EC after the treatment process (Deng et al., 2013). Because of its super-magnetic property, they enable easy recovery and reuse of the nanocatalyst, thusby making the degradation process more economical and eco-friendly (Ansari et al., 2009; Li et al., 2013b). Similarly, MgO nanoparticles act by adsorption or direct penetration into the cell membrane approach to remove the contaminants from the polluted environment (Shi et al., 2010; Moussavi et al., 2014). The retrieval of n-MgO from treated wastewater can be achieved by adding commercially available surfactants, which may prevent the secondary (nano) pollution onto the further stages of the WWTPs (Bhawana and Fulekar, 2012).
Studies on the successful application of n-Fe$^0$ and n-MgO towards the degradation of organic environmental pollutants, including β-lactam antibiotics have been reported (Ghauch et al., 2009; Deng et al., 2013; Fakri and Adami, 2014). Similar studies on the use of iron nanoparticles towards other antibiotics such as sulphonamide (Shi et al., 2014) and levofloxacin (Wei et al., 2015) degradation is also reported.

The strategy of integrating the metal nanoparticles with biological systems has a great impact in many fields, including environmental remediation (Thatai et al., 2014; Li et al., 2013b). The surface characteristics such as, selectivity and the sensitivity of the nanoparticles play a vital role in deciding their applications. Appropriate functionalization of the nanoparticles surface was found to improve their properties in terms of selectivity, application characteristics, exhibiting of enhanced reactivities towards contaminants, which corresponds with the surface-to-volume ratios (Pratt, 2014). Integration of nanoparticles and biological system has proved to show enhanced efficiencies than the individual systems, which might be due to the combined effect of the both the system, in which, the nanoparticles would have stimulated the biological system (Li et al., 2013b; Moussavi et al., 2014). Few researchers have reported the role of nanoparticles (due to their size and surface area) towards improved microbial enzyme levels and activities during integrated approaches (Petkar et al., 2006; Macario et al., 2013; Besteti et al., 2014).

2.14. REMOVAL OF ANTIBIOTICS FROM PHARMACEUTICAL WASTEWATER USING PACKED BED COLUMN

Wastewaters produced from pharmaceutical industries pose several problems for successful biological treatment. These pharmaceutical wastewaters contain high levels of soluble organics and suspended solids, which are recalcitrant in nature (Chelliappan et al., 2011). Implementation of traditional treatment techniques requires costly operational units and continuous supply of chemicals, which becomes highly impracticable, uneconomical and also lead to further environmental damage (Homem and Santos, 2011). Hence, an easy, efficient, economic and eco-friendly methods are necessary for the designing of a sustainable effluent/wastewater treatment facility. Though batch systems show desired results, it can be operated only in small scale, whereas, continuous systems (packed columns) are much preferred.
industrial scale treatments and also it will provide a real perception into the applicability of pollutant removal.

Packed bed column is a set-up containing a cylindrical column packed with appropriate treatment material. The packing material can be biomass (biological treatment), sand, granular activated carbon (GAC), plastics, reticulated foam polymers, quartz, granites, stones etc. It can be packed in two forms (loose or modular). As far as biological packing materials are concerned, biomass can be living (biofilm) or dead (adsorption). However, maintaining a living biomass during the process will be a costly affair, as it requires a steady supply of nutrients. The use of dead biomass in powdered form also has certain limitations, like, small particle size, separation difficulties, loss of regeneration capacity and low strength. To solve these problems, dead biomass can be immobilized on a suitable polymeric or biopolymeric matrix which acts as supporting material (Aksu and Gonen, 2004; Hong et al., 2013).

The packed bed reactors can be fed through down-flow or up-flow mode operated at both aerobic and anaerobic options (Young, 1991; Kennedy and Droste, 1991). Packed bed offers remarkable benefits by providing an inert inlet region for developing dense aggregates of biomass (bio-film formation), and thus preventing them from easy wash outs. This greater amount of retained inoculum will facilitate shorter start-up time-period during the treatment process. Several chemical engineering unit operations such as adsorption, absorption, extraction and distillation can be carried out in packed columns (Girish and Murty, 2013; Kulkarni, 2014).

A packed bed column process is very effective as it is subjected to cyclic sorption/desorption operation to make the maximum use of the packed material. Various studies on antibiotic removal from aqueous solutions suggesting the efficiency of adsorption as an effective treatment has been reported (Putra et al., 2009; Kim et al., 2010a; Chen and Huang, 2010; Ahmed et al., 2015).

Photocatalytic degradation of wastewater containing antibiotics (atenolol, ibuprofen and carbamazepine) was demonstrated using alginate as a packing material in packed bed photo reactor experiments (Hapeshi et al., 2010; Harikumar et al., 2011; Georgaki et al., 2014; Sarkar et al., 2015). Anaerobic pre-treatment of pharmaceutical wastewater using packed bed reactor was also reported (Chelliappan et al., 2011). There are reports on the removal of antibiotics, flumequine (Sotelo et al., 2013) and amoxicillin (Yaghmaeian et al., 2014) by fixed bed column reactors too.