International Academic Institute for Science and Technology

International Academic Journal of Science and Engineering
Vol. 3, No. 1, 2016, pp. 174-185.
ISSN 2454-3896

International Academic Journal of Science and Engineering

www.iaiest.com

Qualitative evaluation of antibiotics in WWTP and review of some antibiotics removal methods

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Abstract

In recent years the increasing use of pharmaceuticals and personal-care products (PPCPs), especially antibiotics, has become a particular concern because of their undesirable potential ecological and human health effects. A large part of the antibiotics consumed ends up in wastewater, and in the wastewater the antibiotics may exert selective pressure for or maintain resistance among microorganisms. This study aimed to survey a total four common human and veterinary antibiotics, including amoxicillin, Penicillin, Cephalexin, and Azithromycin based on SPE-LC-MS-MS technology in a wastewater treatment plant at one of equipped wastewater treatment plant (WWTP) in Isfahan province. Samples were taken from the influent and effluent of a WWTP. The samples were prepared using solid-phase extraction (SPE). Also some antibiotics removal methods reviewed that these methods can be put into practice in Iran. In conclusion, It can be said except Azithromycin, other antibiotics were detected in the input and output of WWTP and perhaps after passing through various stages of WWTP, their concentration were low or may exert selective pressure for or maintain resistance among microorganisms and Azithromycin, not detected in input and output of WWPT. Also in Compare of methods together, can be said that ACs, for eliminating of important most used antibiotics in Iran, (amoxicillin and cephalexin) is a good choice and has a high removal efficiency and easier Operating condition than other methods. By usage of ACs method in WWTPs, we can prevent the entry of antibiotics in the environment and water resources in Iran.

Keywords: Qualitative evaluation, Antibiotics, WWTP, removal methods.

Introduction

Among all the pharmaceutical drugs that cause contamination of the environment, antibiotics occupy an important place due to their high consumption rates in both veterinary and human medicine. Antibiotics are the most important groups of PPCPs (pharmaceuticals and personal-care products) being used in medicine, veterinary medicine, farming and aquaculture for the prevention and treatment of diseases(Kümmerer 2008). The term "antibiotic" has been expanded for antibacterial, antiviral, antifungal and antitumour compounds. Most of these substances have a microbial origin, but they may be also semi synthetic or totally synthetic. Antibiotics can be divided into several classes, according to different criteria: spectrum, mechanism of action or chemical structure (Marzo and Dal Bo 1998; Bannister 2000). After intake, pharmaceutically active compounds undergo metabolic processes in organisms. Treating living cells as bio-organisms, effect of antibiotics on living cells after intake can be investigated applying molecular dynamics simulations which is proven to be a promising tool in providing details of bio-chemical processes (Damirchi et al 2013, Shamloo et al. 2014). Significant fractions of the parent compound are excreted in unmetabolized form or as metabolites (active or inactive) into raw sewage and wastewater treatment systems (Cha, Yang, and Carlson 2006) Municipal sewage treatment plant effluents are discharged to water bodies or reused for irrigation, and produced bio solids are reused in agriculture as a soil amendment or disposed to landfill(Jelić et al. 2012).

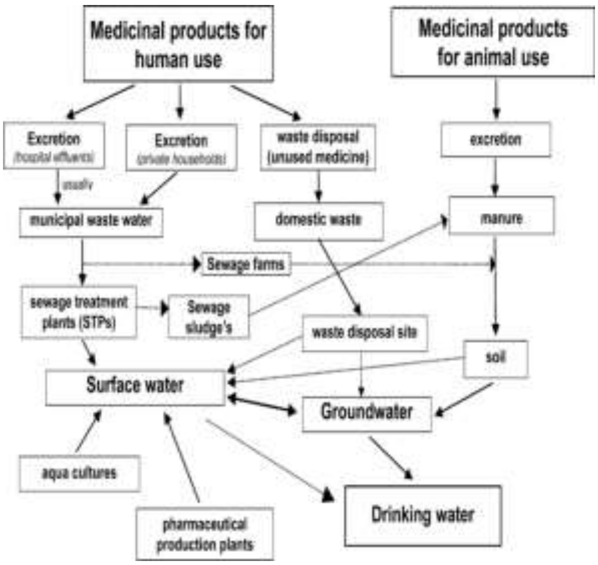

Fig1. Reviews of the entrance possibility of pharmaceutical compounds in wastewater and surface water and groundwater (Heberer 2002).

For years scientists have been aware of the potential problems of antibiotics being present in wastewater, and the research of engineering professor Olya Keen is showing that treatments to clean wastewater may actually be creating new antibiotics and further contributing to the development of antibiotic resistance in the environment (Keen 2015). Based on published data, the rate of antibiotic prescription in developed countries (e.g.15.3% in the USA) is much more than developing countries varying from 17.5% in

Lebanon to 60% in Jordan (Safaeian L 2011). In Iran, about 37.3% of urban households are connected to 136 sewage treatment plant(National water and wastewater engineering Co 2008)) . There is no national program or regulation for the collection of unused or expired drugs. However, it seems that many households dispose their drugs into toilets or garbage bins which ultimately end up in water bodies. The first reported case of water contamination by antibiotics was in England in 1982, when Watts et al, detected macrolides, tetracyclines and sulphonamides in a river at concentrations of 1 mg/L (Sarmah, Meyer, and Boxall 2006) [10]. After this case, several studies about the occurrence of antibiotic residues in aquatic ecosystems have been reported: WWTPs effluents(Brown et al. 2006; Cha, Yang, and Carlson 2006; Watkinson et al. 2009), and hospital wastewaters(Lindberg et al. 2004; Seifrtová et al. 2008).

Lifetime exposure to contaminants such as trace metals and other chemicals in the environment through ingestion, inhalation and dermal contact can pose risk to human health (Moses and Etuk 2015). As no comprehensive study in fate of antibiotics in WWTPS in Iran and their excessive usage (table 2) and entry to wastewater, this study aims to evaluate of fate of antibiotics in one of WWPT in Iran (case study Isfahan province) , and review of some removal methods of them that have ability to run in Iran.

Materials and methods

This study was conducted in three stages including; Consumption of most important antibiotics in Iran, fate of antibiotics in WWTP (case study of Isfahan WWTP) and review of some removal methods of them. In stage two, samples were taken from the influent and effluent of a WWPT. The samples were prepared using solid-phase extraction (SPE). There are different absorbents to extract antibiotics from aqueous matrices, operation solid phase extraction (SPE), approximately matched by PA 1694 (Agency 2007). It has been proved that the pH and extracted material are most important for Antibiotic treatment by solid phase extraction process (Gao et al. 2012). Due to some technical and economic limitations and lack of data retrieval (Recovery) and matrix effect, the presence of antibiotics were evaluated qualitatively.

Result

Consumption of most important antibiotics in Iran

In many countries the risk and effects of pharmaceuticals in the environment is known and some countries have regulations to limit their effects on the environment (EU 2006). Based on the research study, compared to three European countries (Denmark, Sweden, and Norway), the antibiotic consumption in Iran is several times higher (table 1).

Table1: Consumption of some antibiotics in Iran and three European countries (No./1000 habitation year) (Ansari 2014).

Pharmaceutical	Denmark	Sweden	Norway	Iran
Penicillin	7.3	8	7.5	76.25
Cephalosporins	< 0.05	-	0.36	2.71
Trimethoprim Sulfamethoxazole	0.8	0.9	1.45	8.95
Aminoglicozide	< 0.05	<0.1	0.5	60

despite the high consumption rate of PPCPs in Iran (Mohme 2011) there is no comprehensive study on the risk and amount of PPCPs or antibiotics in WWTPs.

Table2 -The 7 most used Antibiotics in Iran in 2011(Alighardashi. A 2014).

No	Active compound	CAS - no	An amount active compound used (Kg/year)	An amount active compound used(g/capita/year)
1	Amoxicillin	026787-78-0	387.09*	5.02
2	Cephalexin	015686-71-2	109.22	1.41
3	Co-Amoxiclav:		48.10	0.62
	Amoxicillin	026787-78-0	32.07	0.41
	Clavulanic Acid	058001-44-8	16.03	0.2
4	Penicillin V	000087-08-1	24.13	0.31
	Penicillin G	001538-09-6	14.76	0.19
5	Co-Trimoxazole:		22.96	0.29
	Trimethoprim	738-70-5	3.19	0.04
	Sulfamethoxazole	723-46-6	19.14	0.24
6	Azithromycin	83905-01-5	17.65	0.22
7	Erythromycin	000114-07-8	10.4	0.13

* including the value in Co-Amoxiclav.

Qualitative measured data

In this section (in table3), the qualitative measured of antibiotics in the influent and effluent of WWTP of Isfahan are visible that is one of equipped WWPT in Isfahan**.**

Table3- the qualitative measured of antibiotics in influent and effluent of WWTP

compound	Measured at the entrance of WWTP	Measured at the end of WWTP
Amoxicillin	+	+
Cephalexin	+	+
Penicillin V	+	+
Azithromycin	-	-

+ Detected, - Undetected

Adsorptive processes for antibiotic removal

The term adsorption is the accumulation of matter from a gas or liquid phase to the surface of an adsorbent, which could involve physical and/or chemical adsorption. Although adsorption is a well-known process, in the past decades the study of this technology for antibiotic removal has not been extensively explored. The most widely reported adsorbents for antibiotic removal include ACs, CNTs and bentonite. In general, electrostatic interaction (cation and anion attractions), hydrophobic effect (hydrophobic interaction), hydrogen bonds, partition into un-carbonized fractions, pore filling, and other processes (surface precipitation, π–π interactions) are the main mechanism for the adsorption of antibiotics onto the carbon-based adsorbents such as AC and BC(Tan et al. 2015). Adsorption phenomenon of contaminants involves four steps: (i) solute transportation in the bulk, (ii) film diffusion of adsorbate, (iii) pores diffusion of adsorbate diffusion, (iv) adsorption — interaction between adsorbate and porous structure. The potentially different and diverse adsorption mechanisms by which antibiotics bind to carbon materials (BC, CNT and AC) are shown in Fig. 2. The internal properties of adsorptive materials play a major role in the adsorption of organic compounds as those materials have heterogeneous surface area due to co-existing carbonized and non-carbonized fractions, which possess different adsorption mechanisms. In general, bentonite has different mechanisms for adsorptive removal of contaminants. For carboxyl and amine groups the protonation took place as follows but phenol groups were not protonated on amoxicillin (Andreozzi et al. 2005). In the following we will investigate the ACs, CNTs and bentonite methods.

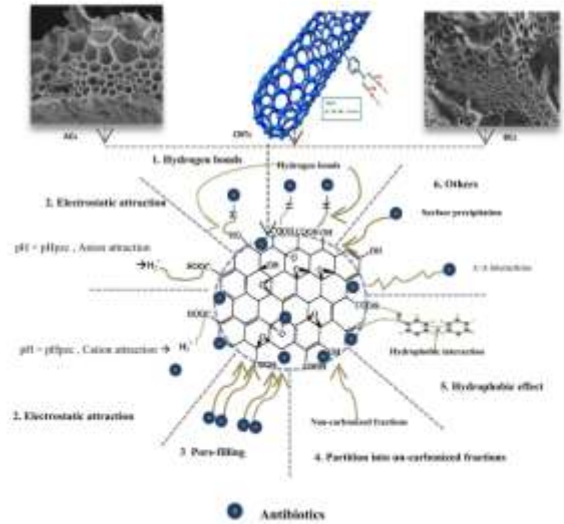

Fig2- Adsorption mechanism during the adsorption of antibiotics by carbonaceous materials (Ahmed et al. 2015).

ACs

ACs have been widely used to remove organic contaminants from water and wastewater in industrial scale applications and more recently in removing pharmaceuticals from sewage effluent (Grover et al. 2011). High degree of micro porosity, well developed surface area, and high adsorption capacity are the key features of ACs (both granular and powdered) that make them suitable as adsorbent for the removal of organic contaminants. During batch adsorption of nitroimidazoles on AC, it was found that the pH of the mediumand the electrolyte concentration did not influence the adsorption process (Rivera-Utrilla et al. 2009).

Table4- Summary of the antibiotic removal by AC adsorbents applied in the treatment of contaminated water.

Antibiotic class/compound	Adsorbate concentration (mg/L)	Operating condition	Removal (%)	Reference
β -Lactams/amoxicillin	300	pH 2–7, 0.1–3.5 g AC, 30 °C	95	(Putra et al. 2009)
Penicillins/penicillin G	20	pH 2, 45 °C, 0.4 g/L AC, time 8 h	74–88	(Pouretedal and Sadegh 2014)
Tetracycline	50, 100, 300	Batch, time 120 h, pH 3.4–11		Torres-) Pérez, Gérente,

				and Andrès (2012)
Cephalexin	20	pH 2, 45 °C, 0.4 g/L AC, time = 8 h	74–88	Pouretedal and Sadegh (2014)
Sulfamethoxazole		Time 1.5–3 min	100	Torres-Pérez, Gérente, and Andrès 2012)

CNTs

Engineered CNTs (single and multiwall) have recently shown great promise for many remediation applications including pharmaceutical chemicals such as antibiotics since the discovery of CNTs in 1991 (Singh 2014). CNTs contain cylindrical layered graphite sheets with a characteristically large surface area which have high van der Waals index. The benzenoid rings of graphite sheets have high polarizability due to the presence of sp2-hybridized carbon atoms. These properties of CNTs make them super hydrophobic materials that can interact with aromatic pollutants by π–π coupling stacking (Long 2001) The removal of antibiotics such as sulphonamides, lincomycine, and amoxicillin by CNTs has been studied in fixed bed columns or batch mode under a broad range of conditions, and great removal success (80% to N90%) has been reported (Mohammadi et al. 2015).

Table 5- Summary of antibiotic removal process by CNTs applied in the treatment of contaminated water.

Antibiotic class/compound	**Adsorbate concentration (mg/L)**	**Operating condition**	**Removal (%)**	**Reference**
β-Lactams/amoxicillin	80	Batch: 0.1–0.2 g MWCNT, pH 4.6, time 120 h	86.5	Mohammadi et al. 2015)
Sulfamethoxazole	200	Fixed bed column: pH 3.0–9.0, time 2 h	96	Tian et al. (2013)

Lincosamides/lincomycin	12000	Batch: 20 °C, pH 6.0, time 100 h	>90	Kim, Hwang, and Sharma 2014

Clay mineral (bentonite)

Clay mineral e.g., bentonite is an adsorbent of aluminum phyllosilicate, which has high surface area and pore volume. Bentonite can act as an adsorbent for the remediation of antibiotics from water and wastewater. Up to now, antibiotic removal by bentonite has not been widely explored, only a few literature reports have been found. In batch mode, the adsorption of ciprofloxacin from aqueous solution on bentonite was found to be very high with 99% removal efficiency, with a contact time of 30 min at pH 4.5 (Genç 2013) As shown in table 6 for the removal of ciprofloxacin on using bentonite, AC, zeolite and pumice, it was observed that bentonite achieved the highest removal capacity. During amoxicillin removal from real wastewater on bentonite and AC, bentonite efficiency (88%) was found to be less than using AC (95%); and neither adsorbent can completely remove amoxicillin due to the sorption competition from other substances which were also present in the wastewater (Putra et al. 2009).

Table 6 - Summary of antibiotic removal by clay material (bentonite) applied in the treatment of contaminated water.

Antibiotic class/compound	**Adsorbate concentration (mg/L)**	**Operating condition**	**Removal (%)**	**Reference**
β-Lactams/amoxicillin	300	pH 2–7, 0.1–3.5 g adsorbent, 30 °C	88	Putra et al. (2009)
Flouroquinolones/ciprofloxacin (CIP)	50-500	Batch: contact time 30 min, pH 4.5, 2.5 g/L bentonite	99	Genç (2013)

Conclusion

A large part of the antibiotics consumed ends up in wastewater, especially In Iran that according to table2 and table 3, consumption of antibiotics is very more than some other countries. Wastewater treatment plants have been suggested to play a role in the dissemination and development of antibiotic resistant bacteria. Easy access and uncontrolled usage of antibiotics cause discharge of antibiotics to wastewaters and consequently diminish the drugs' effectiveness. High concentration of antibiotic and diversity in wastewater of hospital in comparison with urban wastewater causes to transfer resistant agents between bacteria and increased the multiple resistances. The presence of the antibiotics was reported qualitatively and according to table 3, It can be said except Azithromycin, other antibiotics were detected in the input and output of WWTP and perhaps after passing through various stages of WWTP, their concentration were low or may exert selective pressure for or maintain resistance among microorganisms. Azithromycin has not been detected in input and output of WWPT. Also in compare Tables 4, 5 and 6 together, can be said that ACs method for eliminating of most used antibiotics in Iran (amoxicillin and cephalexin), according to table2, has a high removal efficiency and easier operating condition than other methods. By implementation of the ACs method in WWTPS, we can prevent the entry of antibiotics in the environment and water resources in Iran. Finally, the findings of this study may not be directly related to the public health but, showed the important of broader study of antibiotics in aqueous environments, including wastewater treatment plants in Iran.

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